Summaries of Completed

MISA Projects

March 2008

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1. Aquaculture Innovation

1.1 Reduction of Pacific oyster mortality by improving farming & processing techniques in South Australia.

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
R&D-5645	2003/208	Li, Xiaoxu	01-Dec-2002	30-Dec-2006	364,200

Outcomes Achieved to Date

The research on stress assays conducted in this project has provided a tool to determine best practice techniques in oyster farming. Once adopted, this should result in an improvement in the performance of oyster farms. A 1% reduction in oyster mortalities would save approximately $200,000 per annum for the South Australian oyster industry. In addition, this project has contributed to a better understanding of the frequency, location and possible causes of Pacific oyster mortalities in South Australia and on disease agents that occur in oysters farmed in South Australian waters. This information will be used to help reduce and manage future mortalities. Finally, the survey of oyster processors has provided valuable information regarding the quality of South Australian oysters and the most important traits with respect to their marketing. This has highlighted to the industry where improvements need to be made to increase the saleability of oysters and to expend into new markets.

Non-Technical Summary

Ever since mortalities in Pacific oysters (*Crassostrea gigas*) were first recorded in South Australia (SA) about 10 years ago, oyster farmers have continually refined farming methods in an attempt to increase oyster survival, although limited improvements have been made. In some regions unusual mortalities occurred nearly every year prior to 2002, especially during the summer periods when ambient temperatures have remained high for several days. The purpose of this project was to investigate oyster mortalities and to minimise their occurrence by developing low stress management strategies for the oyster industry.

In April 2005 it was decided at a Project Steering Committee meeting (comprising SARDI and SAORC representatives, but subsequently involving FRDC) that the project should be terminated due to:

- no unusual oyster mortalities having occurred in South Australia during the two years since the project's commencement in June 2003;
- further refinement of the stress assays would be required prior to their application; and
- oyster industry representatives pursuing a best practice husbandry manual for the farming of Pacific oysters in SA waters through other means (industry bench mark workshops).

At that time objectives 1, 3, 5 and 8 had been achieved:

1. Design and conduct an industry survey to assess the location, time and prevalence of oyster mortality.
3. Design and conduct an oyster health survey to investigate the prevalence of oyster pathogens in stock in SA coastal waters.
5. Identify quantitatively the stress levels created by different equipment and different farming and processing activities.
8. Communicate advice to farmers.

Objectives 2, 4, 6, 7 and 9 were not achieved and were subsequently cancelled:

2. Design and conduct on farm experiments to investigate the correlation between oyster mortalities and environmental and biological factors.
4. Reduce the number of possible causative factors by analysing the data from the industry survey, on farm experiments and oyster health survey.
6. Identify quantitatively and qualitatively the likely pathogens responsible for oyster mortality.
7. Develop best practice advice for farmers.
9. Develop a best practice husbandry manual for the farming of Pacific oysters in SA water.

The 1st project objective was to design and conduct an industry survey to assess the location, time and prevalence of oyster mortalities during the last five years from 1998 to 2003. Two industry surveys were conducted to gather information relating to mortality, health and quality of oysters. The farm-based survey found that farmers from Coffin Bay and Cowell had not experienced any unusual mortality events whilst some Denial and Smoky Bay farmers did report oyster mortalities. Some farmers reported higher mortalities in family lines compared to standard commercial stock. Many farmers thought that mortality occurred at the spat stage. The percentage of spat farmers expected to reach saleable size ranged from 50 to 95%. Most farmers thought that low mortality occurred in adult stock with estimated losses at each grade ranging between 1-2%. From the survey it was apparent that most farmers attributed mortalities to stock management and stress related issues, including grading, air exposure, high air and water temperatures and the reproductive cycle of the oysters. The survey also revealed that most farmers did not keep detailed records that allowed determination of when and where oyster mortalities were occurring.

The main issues raised from the processor/wholesaler survey were spawning oysters during summer, mudworm infestation and non-uniformity in oyster size and condition. Five out of eight processors/wholesalers stated that meat to shell ratio was the most important quality trait. The rest ranked uniform shell size as the most important quality trait. Four processors/wholesalers reported having observed abnormalities in oysters they had received during summer months. Two processors/wholesalers observed oysters with a dark or black spot in the white of the meat and an unusual smell.

The 3rd objective of the project was to design and conduct an oyster health survey to investigate the prevalence of oyster pathogens in stock in SA coastal waters. A shellfish health survey was conducted by the PIRSA Aquaculture group. A total of 2,238 Pacific oysters from 16 sites in SA were collected for assessment. The pathological findings of the survey were:

- the detection of microcell-like cells in vesicular connective tissue;
- the abnormal hypertrophied nuclei with marginated chromatin in vesicular connective tissue cells;
- atrophy of digestive tubules;
- a viral gametocytic hypertrophy-like lesion in the gonad;
- metaplastic changes of the digestive tubule epithelium (a lesion distinct from tubule atrophy);
- diapedesis through the gut epithelium;
- parasites and symbionts; and
- rickettsia-like organisms.

The health survey has helped to produce a pathology-based overview of the disease agents that currently occur in SA. This information will further help the SA oyster industry to determine the relationship between oyster mortalities and pathogens.

The 5th project objective was to quantitatively measure the stress levels created by different oyster farming equipment and different farming and processing activities. Three techniques were developed and evaluated to measure stress as part of six student projects (five Masters projects from Dalian Fisheries University, China, and one Honours project from Flinders University, South Australia). The three techniques evaluated were: 1) measurement of catecholamine levels in oyster blood, 2) measurement of phenoloxidase activity in the blood and 3) assessment of lysosomal membrane integrity using neutral red staining. Results from laboratory and field experiments showed, based on the methodology used, that the phenoloxidase assay was not a reliable method for detecting stress responses in Pacific oysters. Measurement of catecholamine activity was suitable for single stressors while the neutral red retention assay could be applied to both single and multiple stressor analysis.

In this study the major factors identified in the industry survey as contributing to oyster mortalities or poor performance were assessed using either the neutral red retention (NRR) assay, catecholamine analysis or both. Significant differences were found when animals were subjected to:

- slow and rapid temperature changes;
- exposure to different air temperatures for different durations;
- recovery after different air exposure treatments;
- starvation or food deprivation;
- gonad development;
- spawning and post spawning recovery;

- grading for different durations;
- grading with different types of graders; and
- recovery after grading.

In addition, significant differences were also found between animals of different sizes in response to some of the above-mentioned treatments using the NRR assay.

It is therefore reasonable to assume that these assays could be used as a tool to evaluate the stresses induced by different farming practices and thus identify those which result in the least stress, leading to an improvement in oyster performance on farms.

The 8th project objective was to communicate advice to farmers. Results that could be used to improve the oyster farming practices were immediately circulated to the Project Steering Committee and the South Australian Oyster Research Council (SAORC). The oyster growers were directly informed through four articles in the "SAORC Newsletters" and two presentations at SAORC annual meetings. Five articles in scientific journals have also been published at this time.

1.2 Experimental production of tetraploid oysters for use as broodstock for commercial hatchery production of triploids

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
	1994/081	Li, Xiaoxu	*	*	*

*Some IP issues remain. Project has commercially in confidence information.

Outcomes Achieved to Date
Tetraploid oyster embryos were induced by most methods used in this study; including hydraulic pressure shock, thermal shock, chemical treatment and combined chemical treatment. Tetraploid larvae and spat were produced by the combined chemical method developed in this study.

Non-Technical Summary
Triploids (3n) are organisms with three sets of chromosomes, while the normal diploid (2n) organisms have only two sets. Over the past two decades, triploids have been studied in more than 20 species of molluscs. The main interests of those studies have primarily focused on their sterility and improved growth rates. These are mainly due to that in the normal diploid commercial stocks the animals expend considerable energy on gametogenesis and become watery in the summer months making them less desirable in the market. In Pacific oysters, for example, they release approximately 50% of their body mass during spawning, affecting their meat quality and acceptance by consumers for an extended period of time. In a sterile stock, on the other hand, they could partially redirect this energy to growth and maintain their meat quality during these periods, allowing them to be marketed year round. Triploids, in most species studied so

far, experience significantly higher growth rates than their diploid siblings. In bivalve species, triploids have showed 10 to 80% faster growth than their diploid siblings. However, the expression of the triploid advantage in growth can be influenced by genetic and environmental factors. In addition, production of triploids from normal diploid stock directly is technically difficult in most bivalve species; often resulting in inconsistent percentage of triploid and high larval mortality in the first few days. The commercial use of triploids may, therefore, ultimately depend on the development of tetraploids, which can produce 100 % pure triploids simply by mating with normal diploids.

The establishment of tetraploid breeding stock however, is still a major challenge in most molluscan species. Prior to 1997, tetraploid bivalve spats have only been produced in three species: mussels (17.2% in one month old); Manila clam (3 spat detected); and Pacific oysters (67% in juveniles). The tetraploid mussels and clams were induced using gametes from diploid males and females and the tetraploid Pacific oysters were produced using eggs from triploid females and sperm from diploid males.

The main objectives of this project were to evaluate and develop techniques for the production of tetraploid broodstock in Pacific oysters and to investigate the potential to produce triploids by crossing tetraploids with diploids. Throughout this project (March 1995 to February 1998), most techniques developed to induce tetraploids from diploid stock in fish and shellfish, have been attempted; new techniques were investigated; and tetraploid spats were produced. The techniques published by Guo and Allen (1994) were not tried because no mature triploid broodstock were available in South Australia.

The first objective “the experimental production of tetraploid (4n) oyster embryos, larvae and spat” was achieved. Tetraploid embryos were induced by most methods used in this study; including, 1) electrofusion of cells in two cell stage embryos; 2) thermal, and heat + caffeine treatments, to inhibit first mitotic division; 3) hydraulic pressure treatments to prevent first mitotic division; 4) Cytochalasin B (CB) inhibition of first polar body formation or both polar body formations; 5) CB and 6-dimethylaminopurine (6-DMAP) inhibition of first mitotic division; and 6) combined chemicals treatment. The majority of tetraploid embryos produced in this study (29 % or more) were produced by the hydraulic pressure treatment and the combined chemical methods. Flow cytometric analysis was used to identify tetraploid spat. Analysis from experiments in the second (23 February 1996) and third (25 February 1997) year of the project identified 1 tetraploid in 28 and 79 spat tested respectively. In general, tetraploid levels in embryos were initially high, however, these were not stable; as embryos developed, the ploidy levels decreased.

The second objective “on-growing of tetraploid oyster spats to adulthood and reproductive capability” was partially achieved. In the third year of the project, March 1997, approximately 2 million, eight-day-old larvae (8 % tetraploids) and 150 twelve month old spats (4% tetraploids, as analysed by flow cytometry; n = 28), were sent to the South Australian Oyster Hatchery for grow-out. A sub-sample from the larval batch was also reared at Flinders University. The spat (12 months old) from this batch were

sampled towards the end of the third year of the project (January 1998) using flow cytometric analysis. Non-destructive ploidy assessment of spat is not possible and therefore precise estimates of the proportion of tetraploids in the spat being grown out on oyster farms are not available. The percentage of tetraploids growing out on farms can only be estimated based on the samples analysed at Flinders University. At the time of writing the initial report draft, August 1999, the reproductive capability of oysters grown out at the South Australian Oyster Hatchery had not been obtained, however they are being maintained and checked periodically for indications of sexual maturity.

During the second year of the project (July 1997), the industry was consulted about the direction of oyster research at the South Australian Oyster Growers Association (SAOGA) Field Day, Smoky Bay. At this time the industry indicated that grower demand for triploid oysters had fallen considerably in the light of poor growth rates and meat quality of chemically produced triploid oysters on commercial leases. As a result, plans for continued research into production of tetraploid broodstock were abandoned.

The last objective “hybridization of diploid gametes (from tetraploid broodstock) with 1n gametes (from “normal” diploid broodstock) to produce triploid embryos, larvae and spats,” was not achieved due to non-completion of the second objective.

Unfortunately, the ploidy levels and the performance of the stocks sent to the South Australian Oyster Hatchery for grow-out were not collected. If enough tetraploids were produced and both male and female existed in the tetraploid stock, the breeding tetraploid line could be established by mating between them. In United States the second generation Pacific oyster tetraploids have already been established by mating the tetraploids produced using the eggs from triploid females.

Although very low percentages of tetraploid Pacific oyster spat were induced in some experiments, the success of this study also indicates that the development of zygotes, produced by fertilising eggs from diploid females with sperm from diploid males, could tolerate tetraploid genome in their cells.

2. Biosecurity

2.1 Development of gene probes for introduced marine pest species (*Musculista senhousia, Corbula gibba, Sabella spallanzanii*).

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
SVE-4036	RD04/0197	Westphalen, Grant	10-Jun-2005	30-Jun-2006	227,273

Executive Summary
There is an identified need for marine pest monitoring to detect new incursions of species already present in Australia, and to detect exotic species not yet recorded in Australia. Current methods of identification rely on specialist taxonomic skills. These skills are not always readily available, and routine surveillance may generate more samples than specialist laboratories can handle.

DNA techniques allow rapid and definitive identification of target taxa. They are useful as an adjunct to traditional morphological identification, as well as a tool for rapid and high-throughput monitoring. One of the potential strengths of this technology is that it allows identification of multiple taxa in a single sample. However, there are only a small number of target taxa for which DNA tests have been developed. A concerted effort has been made to develop DNA based assays for a number of priority marine pests.

In this study, real-time PCR tests have been designed for three taxa of introduced marine pests- *Sabella spallanzanii, Corbula gibba* and *Musculista senhousia.* These tests have been demonstrated to be specific for the target taxa.

This study demonstrates that the real-time PCR tests can be used to quantify the organisms in phytoplankton tow samples and sediments. This will make them useful for implementation in routine monitoring programs.

Musculista senhousia (Asian date mussel) is an introduced species, which can invade benthic communities to the detriment of native species. It has been recorded in southern and western Australia. Although *Musculista senhousia* is the only species of this genus occurring in Australia, the mytilid family is large and diverse (21 genera and 71 species). *M. senhousia* has a rapid growth rate and high fecundity, reaching adult size in 9 months. It is a very adaptive species, surviving in inter-tidal to sub-tidal habitats, tolerating low salinities.

Corbula gibba (European clam) is one of 16 species of corbulids that occur in Australia, all of which belong to the *Corbula* genus. The latter is divided into four subgenera, of which the *C. gibba* group (Varicorbula) is represented by only one other species (*C. rotalis* from QLD). *Corbula gibba* is native to the eastern Atlantic but has been recorded in Victoria and Tasmania. It was first recorded in Port Phillip Bay in 1987 where it is now abundant, with densities of up to 250 clams/ m^2. It is considered a pest because of its

rapid growth rate and its ability to adapt to a diversity of environments, thereby competing with native species.

Sabella spallanzanii (fanworm) is the only large (> 300 mm) sabellid known to occur in Australia (Clapin and Evans 1995). The *Sabella* genus is itself not common in Australia, although other members of the parent family, Sabellidae, are well represented. While European populations of *Sabella spallanzanii* exhibit substantial genetic variability, the Australian branch of the species is relatively more stable (18 – 20% less variable). The fanworms colonise hard substrates such as pilings and rocks- they can then reach high population densities and are inedible to fish. They present a hazard because they alter turbulence and may impact severely on benthic denitrification. They were first recorded in Port Phillip Bay in 1988 and have also established in SA and WA. They appear to be transferred on the hulls of dredges and other vessels.

2.2 *Caulerpa taxifolia* – 2007 Survey of current distribution and high risk areas.

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
SLA-4115	RD06/0330	Westphalen, Grant	30-Jan-2006	28-Apr-2006	51,000

Executive Summary

The distribution of the marine pest, *Caulerpa taxifolia,* has substantially expanded such that the alga cannot be eradicated from South Australia with current technology. Ongoing monitoring of its distribution has become critical to the management of the alga. The current distribution of *C. taxifolia* is still contained within the Port River area, and is found in much higher densities in areas impacted by thermal effluent. Isolated colonies of the alga have been located further downstream than in previous surveys, although there appears to be fewer colonies, possibly due to the winter senescence of the alga. There is no evidence that the alga has been translocated to boat ramps along the Adelaide metropolitan coastline.

2.3 Hazard assessment of marine pests in relation to Australian aquaculture industries.

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
SVE-4006	RD04/0204	Westphalen, Grant	30-Apr-2005	30-Jun-2006	155,633

Executive Summary

Marine pests are known to be introduced or translocated by a variety of anthropogenic and natural vectors, including ballast water from commercial shipping, biofouling on a wide range of vessels, aquaculture operations, aquarium imports, marine debris and

ocean current movements. The continuing encroachment of marine pests into new areas threatens biodiversity, natural resources, aquatic and human health, as well as specific industries dependent on the marine environment. This document outlines the results of an investigation into the hazards posed by Australian aquaculture as a vector for marine pests. However, it should be noted that aquaculture in Australia does not pose a significant threat of introducing new marine pests from overseas. Rather it is the potential for secondary transfer of marine pests that already occur in Australian waters that requires assessment.

A hazard analysis was conducted on representative marine aquaculture sectors in Australia following the Infection Modes and Effects Analysis (IMEA) method developed by Hayes in 2002. For this approach it must be recognised that hazard is not the same as risk and it is important to bear in mind that a hazard assessment is not the same as risk assessment. Hazard should be viewed as a latent capacity to engender harm, whereas risk is about the chances of that harm occurring. A consequence of this difference is that an entity may pose a substantial hazard, but at the same time present limited (or no) risk.

Five industry sectors were selected as representative case studies for the industry, including; edible oysters, pearl oysters, mussels, salmonids and southern bluefin tuna. Sectors were chosen according to the geographical range, production volume and economic value and to obtain a cross-section of a range of farming methods. The IMEA method involved four steps:

1. Breakdown of industry operations and infrastructure to specific components at the operator level
2. Determining which of eight of infection modes that could occur relative to each component.
3. Rating each component/infection mode combination from 1 – 10 according to;
    a. Environmental suitability,
    b. Likelihood of establishment, and
    c. Likelihood of detection.
4. Calculation and evaluation of the Risk Priority Number (RPN) for each combination (0-1000).

Breakdowns of practices in each sector, identification of infection modes and scoring of each combination was undertaken through a series of meetings with industry representatives. Average RPNs for each sector were calculated and listed in order from highest to lowest scores. The top 50% of component/ infection mode combinations were employed in the interpretation of the relative level of hazard each sector may pose in terms of marine pest transfer. These interpretations were offset against the best available information on the regulatory framework in which each sector operates

In sectors where they occur, interstate transfers of stock are generally more stringently controlled than intrastate relocations and it is probably the latter that offer the greatest marine pest relocation hazard. This study has found the identification of gaps in

relocation protocols difficult, as many sectors have state by state internal relocation guidelines that may be very similar, but nonetheless are a potential cause for confusion. The development of a single document for sector specific transfer protocols that covers all states would improve both awareness of issues elsewhere and the identification of gaps.

In most sectors, the measures in place to combat disease (depuration, emersion, freshwater/brine washes, scrutiny) are also likely to be effective barriers to marine pests. However, a higher level of pest risk awareness in some sectors/regions has led to creation of some pest specific protocols. This specifically relates to the mussel industry in Victoria, with measures developed by operators targeting the threat posed by the Pacific seastar (*Asterias amurensis*). There is a high level of awareness within the pearl oyster sector following the outbreak of black-striped mussel (*Mytilopsis sallei*) in Darwin Harbour. Finally, oyster farmers in NSW have measures in place to limit the spread of Pacific oysters (*Crassostrea gigas*). While these approaches are targeted to particular pest species in particular regions, they are likely to be similarly effective against other pests.

Intrastate movements of equipment and infrastructure present generally greater marine pest hazards than stock. Within edible oysters, sticks, rafts and racks present the largest marine pest hazard, while for mussels and pearl oysters, anchors, buoys and longlines presented a relatively high hazard. In the salmon and southern Bluefin tuna sectors, seacage infrastructure (nets, rings, ropes and anchors) present the largest marine pest relocation hazard. External and internal fouling and refuge infections present the majority of higher hazard infection modes, although others occur in specific instances (e.g. wood borers on sticks used in NSW oyster farming).

It must be remembered that the identification of a hazard does not necessarily mean that the risk of marine pest relocation exists, only that there is a potential.

The most effective control over marine pest risks would appear to be through development of pest management protocols for particular areas, similar to those operating in the Victorian mussel sector. Similarly, salmon farming in Tasmania is already divided into three management zones, albeit targeted to hygiene, but the effectiveness of this approach to pest risk management is worthy of further investigation. For sectors with a geographically small range, such as southern Bluefin tuna, the development of pest management protocols is currently unwarranted, but a greater awareness of marine pest risks and the need for development of protocols that cater for expansion of the sector to new areas is required.

2.4 Temperature and salinity tolerances of priority marine pests.

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
SVE-4115A	RD06/0751	Westphalen, Grant	01-May-2006	28-Jul-2006	14,055

Executive Summary
In planning and undertaking surveys for marine pests in Australia as part of the National System for the Prevention and Management of Marine Pest Incursions, an understanding of temperature and salinity tolerances of the pest species on the current priority list would be useful so as to focus efforts on those species that can survive in a particular area. The purpose of the present report was thus to undertake a literature review and collate the available data on temperature and salinity maxima and minima for priority marine pests. Information on the environmental cues and periods for reproduction were included where such data would be readily obtained.

Temperature and salinity maxima and minima for a total of 50 different species were considered, with summaries including an estimate of the reliability of the data (see table below). Information on many pest species was sparse or absent. For species with no directly applicable information, data were extrapolated from known their distribution and/or inferred from other members of the same genus. Neither of these approaches are considered ideal, and data reliability in these instances is given as low (L). In addition, the scientific rigour of many sources was open to question, and this information was also given a low (L) reliability index.

Data for each taxon were summarised to cover the temperature and salinity range encompassed by different strains within a species, across different congeners, and home ranges employed as proxies where direct information was lacking (Grey Background – table below).

Tolerance information was incomplete for four species, *Charybdis japonica, Megabalanus tintinnabulum, Hydroides sanctaecrucis* and *Watersipora arcuata*. Data for species related to these might be employed as a proxy, although retaining this group in all surveys would be preferable. For the purposes of marine pest surveys in ports and harbours, a precautionary approach would dictate that priority pests in the low reliability category should be considered. Further, many pests (particularly the micro- and some macroalgae; Blue – table below) can occur as different strains which, when combined, have broad environmental tolerances and should be included in all surveys. It is also recommended that all microalgae (dinoflagellates and diatoms) be retained.

3. Ecosystem Services

3.1 Assessment of the implications of interactions between fur seals and sea lions and the southern rock lobster and gillnet sector of the Southern and Eastern Scalefish and Shark Fishery (SESSF) in South Australia.

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
R&D-4050	2005/077	Goldsworthy, Simon	01-Jul-2005	28-Feb-2006	19,999

Outcomes Achieved to Date
This report provides the most comprehensive appraisal of the risk posed by bycatch to subpopulations of Australian sea lions and New Zealand fur seals, by the SA rock lobster and gillnet sector SESSF fisheries. Further it has identified the research required to ensure that SA rock lobster and the gillnet sector SESSF fisheries are managed according to ESD principles, and that interactions with seals are measured, assessed and mitigated. Adoption of these recommendations will lead to the development and adoption by industry and management of mitigation options to reduce seal bycatch. This will ensure that outstanding ESD recommendations detailed in fishery ESD assessments and the mitigation of the key threatening process identified in the Australian sea lion Draft Recovery Plan are addressed, leading to the recovery and potential future delisting of the species.

Non-Technical Summary
Recent Commonwealth Department of the Environment and the Heritage (DEH) Ecological Sustainable Development (ESD) assessments of the South Australian (SA) rock lobster (SARLF) and southern and eastern scalefish and shark fishery (SESSF) identified interactions with protected species (particularly seals), as one of the key bycatch issues. The issues are most relevant to SA waters where the majority of Australia's New Zealand fur seal(NZFS) and endemic and *threatened* Australian sea lion (ASL) populations are located, and where un-quantified interactions between seals and the SARLF and gillnet sector of the SESSF fisheries are known to occur. Recommendations from fishery ESD assessments, fishery Bycatch Action Plans, and a recently drafted Recovery Plan for the ASL, have all identified the importance of assessing and mitigating interactions between seals and commercial fisheries. This study provides a desk-top risk-assessment of seal fisheries interactions in the SARLF and gillnet sector SESSF in SA and adjacent waters, and makes recommendations on future research and management responses. A review of the PIRSA and AFMA fishery logbooks identified the major constraint to the assessment of bycatch risk to seal subpopulations was the absence of quantitative data on bycatch rates in both the gillnet sector SESSF and SARLF. Anecdotal evidence and entanglement data suggest there has been significant underreporting of seal interactions in these fisheries.

In SA there are 38 ASL subpopulations that produce around 2,674 pups, with the total population size estimated at about 10,900. However, most pup production (67%) occurs

at 6 sites, hence the median pup production is very low (25.5 pups), with the majority of sites producing small numbers of pups (60% produce <30 pups per season). Not surprisingly, population viability analysis (PVA) on ASL subpopulations reinforced the recent listing of the ASL as a *threatened* species, by confirming that large numbers of subpopulations with low pup production are vulnerable to extinction. PVA simulations suggested that in absence of anthropogenic mortality, a number of ASL subpopulations will go *quasi-extinct* (<10 females), but in the face of small (1-2 additional females/year) but sustained anthropogenic mortality (eg. from fishery bycatch), most other small subpopulations will become *quasi-extinct* and negative growth will become a feature of even the largest subpopulations. There is apparent depletion (i.e. very low pup production) of a large number of subpopulations that may be indicative of widespread subpopulation declines in the species. That such declines may be ongoing and attributable to anthropogenic mortality (ie. fishery bycatch) is a hypothesis that requires urgent attention.

In contrast to ASL, there are relatively few NZFS subpopulations (13) in SA, but the total pup production is considerably greater (17,622), with an estimated total population size of around 83,800. Populations are increasing, and PVA identified that most subpopulations were not-threatened. The risk of bycatch to both seal species in the gillnet SESSF and SARLF were assessed based on estimates of interaction probabilities. These were a function of the extent to which historic fishing effort and seal foraging effort (based on foraging distribution and population models) overlap in space and time. ASL demonstrated the highest risk of significant depletion and quasi-extinction as a result of fishery bycatch. In contrast, the risk to NZFS subpopulations is very low. By combining PVA outcomes with bycatch scenarios based on interaction probabilities, this study identified the subpopulations, regions and marine fishing areas (MFAs) most at-risk from seal bycatch. Bycatch from the gillnet SESSF is most likely to provide the greatest risk to ASL, because of almost complete spatial overlap in fishing effort with ASL foraging effort, it is a year-round fishery with relatively high fishing effort that can potentially target all ASL age-classes. The impact from SA RLF is likely to be less because there is less overlap in fishing effort with ASL foraging effort, fishing is restricted to seven months of the year (November-May), and bycatch is likely to be restricted to pups and juvenile seals. However, the potential additive and interactive impacts posed by combined bycatch in these fisheries could be significant, especially for ASL.

Results from this study suggest the two fisheries investigated lend themselves to different mitigation approaches to addressing seal bycatch issues. In the gillnet SESSF, gear modification options are limited, but spatial management of fishing effort may provide a range of risk-reduction options to management, but would need to be coupled with independent observer bycatch data to demonstrate and justify the benefits from different closure options. In contrast, there are significant options for gear modification in the SARLF, with pot protection devices already used in some parts of the fishery. Quantitative testing of these and alternate protection measures (as is taking place in the WA WRLF), and industry wide adoption of best-mitigation practices may eliminate seal bycatch in this fishery, without the need for an expansive and costly independent

observer program. Recommendations for future research are made, that should result in the successful mitigation of seal bycatch issues, and as a consequence address the recommendations of the fishery ESD, Bycatch Action Plan, ASL Recovery Plan and assist in the recovery of the *threatened* ASL.

3.2 Assessment of pup production and maternal investment strategies of the Australian sea lion *Neophoca cinerea* at Dangerous Reef in the 2006-07 breeding season.

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
SVE-5453G	RD06/0402	Goldsworthy, Simon	01-Jun-2006	28-Feb-2007	8,500

Executive Summary
Australian sea lion pup abundance was determined for the Dangerous Reef population during the 2006-07 breeding season. Using mark-recapture methods (including cumulative mortality), pup production was estimated to be 708 (95% CL 632-779). The exponential rate of increase in pup production from 1999 until 2006-07, based on maximum live-pup counts plus cumulative mortality and mark-recapture methods, ranged between 6.7-9.9% per breeding season, or 4.4-6.4% per year. This provides further evidence of strong positive growth in the population, which has been occurring since 2000.

A study to characterise the maternal strategies of Australian sea lions at Dangerous Reef was also undertaken. The parameters of maternal investment assessed included female mass, the duration of foraging trips and shore attendance bouts, the percentage time that females spent ashore, the birth mass and growth rates of pups to 100 days of age, and the lipid content of milk. These were compared to results from an earlier study undertaken at Seal Bay. No differences were detected in the mass of adult females, or the duration of foraging trips (~1.8 days) between sites. However, the duration of attendance bouts was significantly longer at Seal Bay (1.6 days vs. 0.9 days), and as a consequence, females at Seal Bay were ashore for a greater percentage of time (48% vs. 32%). Although the estimated birth mass of pups at Dangerous Reef was greater than that at Seal Bay, the growth rates of pups at Seal Bay were 27% higher than at Dangerous Reef. Consistent with this, the estimated milk-lipid content for females at Seal Bay was about 27% greater than that estimated for Dangerous Reef females. Based on these results, conditions for foraging and for raising pups would appear more optimal at Seal Bay than at Dangerous Reef, although there is uncertainty in this conclusion due to difference in the timing of the studies.

Available data suggest a marked difference in the respective population trends of the Australian sea lion populations at Dangerous Reef and Seal Bay. The Dangerous Reef population is currently increasing by about 5% per year, while Seal Bay population has been declining by about 1% per year, for at least 20 years. Comparison of the maternal

strategies at each site are contrary to expectations based on the differing population trajectories, suggesting that differences in population trajectories are not related to differences in foraging conditions at each site. There is a growing consensus that the most likely cause for the lack of recovery in Australian sea lion populations is principally due to anthropogenic related mortality, especially from fishery bycatch. The recent pronounced increase in pup abundance at Dangerous Reef has occurred coincidentally with the cessation of shark fishing in Spencer Gulf in 2001. This provides circumstantial evidence that positive growth has followed a reduction in anthropogenic mortality for this population. In contrast, demersal gillnet fishing effort remains significant in waters adjacent to the Seal Bay population, and entanglement rates in monofilament gillnets and a declines in pup abundance suggests that mortality related to fishery bycatch may be significant in this population.

3.3 A population monitoring and research program to assist management of the Australian sea lion population at Seal Bay Conservation Park, Kangaroo Island

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
SVE-5453H	RD03/0183	Goldsworthy, Simon	17-Jul-2006	31-Aug-2006	8,000

Executive Summary
Seals are the premier tourism attraction on Kangaroo Island and they underpin a regional multimillion dollar tourism industry. Its centrepiece is the Australian sea lion population at Seal Bay Conservation Park.

Monitoring of the trends in abundance of the population has been undertaken by the Department for Environment and Heritage (DEH) for over 30 years. Analyses of these data and the methodological basis of the surveys were not reviewed until recently, and determined that the population had been in decline for at least 20 years. This decline is continuing.

This report aims to provide stakeholders' with details of the monitoring and research program required to support the sustainable use of the Australian sea lion population at Seal Bay on Kangaroo Island. The report covers five topics including: a historical summary of research and monitoring of the sea lion colony; an evaluation of the current status of the population; a detailed appraisal of the ongoing management needs of the sea lion population in terms of population status, trends and demography, as well as targeted research programs; what an ongoing population management program should comprise of and what it would cost; and potential funding sources to support such a program.

The report recommends that sustainable use of the Australian sea lion population at Seal Bay should underpin the broader management objectives of the Seal Bay

Conservation Park if it is to remain a sustainable tourism destination. A population monitoring and research program should be implemented that includes: 1) long-term monitoring of pup production, pup mortality and vital demographic rates, and 2) targeted projects that address specific data gaps and management needs. This program requires strong scientific leadership and management to ensure that results and methodologies are regularly reported upon and reviewed. Access restrictions, which presently impede monitoring and research activities at Seal Bay need to be reviewed.

For the population monitoring and research program to be effective, long-term recurrent funding will need to be secured. Options for cost-recovery from visitor fees and tourism operator licence fees should be investigated because alternate sources of funding are unlikely to be adequate to meet the real costs of population monitoring and research. Donations and sponsorships options should be investigated to supplement the costs of population monitoring and targeted research programs.

3.4 A catalogue of shallow-water sponges from the Investigator Islands, South Australia.

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
SVE-5453I	RD03/0183	Currie, David	20-May-2006	30-Apr-2007	4,575

Executive Summary
A multidisciplinary expedition to the Investigator Islands was conducted in May 2006 to examine the natural history of the islands. As part of the expedition the diversity of marine sponges was investigated for the first time. Sponges were collected by SCUBA divers from the fringing rocky reefs of 6 islands: Flinders, Pearson, Dorothee, Ward and Topgallant.

This document catalogues the sponge collection, with all sponges identified to genus. Voucher specimens, photographs and specimen slides are held at the Museum of South Australia, Adelaide. The investigation was time limited and the collection of sponges is by no means comprehensive, but is a preliminary investigation of the sponge diversity of the Islands.

Of the 101 sponges collected, there were 71 species; 10 in the Class Calcarea and 61 in the class Demospongiae. Four sponges from the Investigator Islands that were already lodged at the South Australian Museum are included in our list. Many of the sponges had evidence of algal or cyanobacterial symbionts, reflecting the clear shallow-water conditions of the sites. There was little overlap between this collection and an earlier collection from the Great Australian Bight Marine Park – Benthic Protection Park, with only 3 sponges in common.

3.5 Foraging ecology and diet analysis of Australian sea lions

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
SVE-4108		Goldsworthy, Simon	1-Jan-06	1-Dec-06	81,763

Executive Summary
Recent Commonwealth Department of the Environment and the Heritage (DEH) Ecological Sustainable Development (ESD) assessments of the South Australian (SA) rock lobster (SARLF) and southern and eastern scalefish and shark fishery (SESSF) identified interactions with protected species (particularly seals), as one of the key bycatch issues. The issues are most relevant to SA waters where *threatened* Australian sea lion (ASL) populations are located, and where un-quantified interactions between seals and the SARLF and gillnet sector of the SESSF fisheries are known to occur. Recommendations from fishery ESD assessments, fishery Bycatch Action Plans, and a recently drafted Recovery Plan for the ASL, have all identified the importance of assessing and mitigating interactions between seals and commercial fisheries. This study provides a desk-top risk-assessment of seal fisheries interactions in the SARLF and gillnet sector SESSF in SA and adjacent waters, and makes recommendations on future research and management responses. A review of the PIRSA and AFMA fishery logbooks identified the major constraint to the assessment of bycatch risk to seal subpopulations was the absence of quantitative data on bycatch rates in both the gillnet sector SESSF and SARLF. Anecdotal evidence and entanglement data suggest there has been significant underreporting of seal interactions in these fisheries.

In SA there are 38 ASL subpopulations that produce around 2,674 pups, with the total population size estimated at about 10,900. However, most pup production (67%) occurs at 6 sites, hence the median pup production is very low (25.5 pups), with the majority of sites producing small numbers of pups (60% produce <30 pups per season). Population viability analysis (PVA) on ASL subpopulations reinforced the recent listing of the ASL as a *threatened* species, by confirming that large numbers of subpopulations with low pup production are vulnerable to extinction. PVA simulations suggested that in absence of anthropogenic mortality, a number of ASL subpopulations will go *quasi-extinct* (ie the number of adult females is too low to ensure population persistence; <10 females), but in the face of small (1-2 additional females/year) but sustained anthropogenic mortality (eg. from fishery bycatch), most other small subpopulations will become *quasi-extinct* and negative growth will become a feature of even the largest subpopulations. There is apparent depletion (ie. very low pup production) of a large number of subpopulations that may be indicative of widespread subpopulation declines in the species. That such declines may be ongoing and attributable to anthropogenic mortality (ie. fishery bycatch) is a hypothesis that requires urgent attention.

The risk of bycatch in the gillnet SESSF and SARLF were assessed based on estimates of interaction probabilities. These were a function of the extent to which historic fishing effort and seal foraging effort (based on foraging distribution and population models)

overlap in space and time. ASL demonstrated a high risk of significant depletion and quasi-extinction as a result of fishery bycatch. By combining PVA outcomes with bycatch scenarios based on interaction probabilities, this study identified the subpopulations, regions and marine fishing areas (MFAs) most at-risk from seal bycatch.

Bycatch from the gillnet SESSF is most likely to provide the greatest risk to ASL, because of almost complete spatial overlap in fishing effort with ASL foraging effort, it is a year-round fishery with relatively high fishing effort that can potentially interact with all ASL age-classes. The impact from SARLF is likely to be less because there is less overlap in fishing effort with ASL foraging effort, fishing is restricted to seven months of the year (November-May) and bycatch is likely to be restricted to pups and juvenile seals. However, the potential additive and interactive impacts posed by combined bycatch in these fisheries could be significant. Results from this study suggest the two fisheries investigated lend themselves to different mitigation approaches to addressing seal bycatch issues. In the gillnet SESSF, gear modification options are limited, but spatial management of fishing effort may provide a range of risk-reduction options, but would need to be coupled with independent observer bycatch data to demonstrate and justify the benefits from different closure options. In contrast, there are significant options for gear modification in the SARLF, with pot-protection devices already used in some parts of the fishery. Quantitative testing of these and alternate protection measures (as is taking place in the WA WRLF), and industry wide adoption of best-mitigation practices may eliminate seal bycatch in this fishery, without the need for an expansive and costly independent observer program. Recommendations for future research are made, that should result in the successful mitigation of seal bycatch issues and as a consequence address the recommendations of the fishery ESD, Bycatch Action Plan, ASL Recovery Plan and assist in the recovery of the *threatened* ASL.

Enhanced spatial tools for risk assessment will be required if spatial management of fishing effort is to become a management strategy for mitigating ASL bycatch in the demersal gillnet fishery. Such tools would provide a simple mechanism for policy makers and managers to evaluate the benefits and costs of different spatial allocations of fishing effort, in terms of increasing or decreasing: 1) risk to sea lion subpopulations and 2) fishery catches. However, further development of such tools are required, because current models are limited by the absence of data on the foraging movements of sea lions in some high-risk regions, as well as the absence of accurate fishing effort data.

Further satellite tracking of ASLs at subpopulations identified as high-risk was undertaken as part of this study, to improve the accuracy of spatial foraging models. This pilot study demonstrated an approach to refine assessments of sea lion interactions with commercial fisheries, where quantitative data on interactions are not available. This approach may enhance the spatial information on which mitigation options and decisions about spatial management of fisheries are based. Importantly, the pilot study determined that colony specific information on sea lion foraging effort could be used to refine the spatial management of fisheries when using fishing effort data that

was summarised into 1 x 1 degree boxes (Marine Fishing Areas). In 2006, demersal gillnet fishers were required to record the latitude/longitude positions of each net-set, and from July 2007, all vessels will be fitted with satellite-linked vessel monitoring systems that will significantly improve the resolution of fishing effort. Following these improvements to fishing effort data sets, it is recommended that bycatch probabilities be re-estimated with colony-specific information on seal lion foraging effort and used to model the benefits of different spatial-management scenarios that could include area-closures, and reductions or redistributions of fishing effort.

The diet of the ASL is currently poorly understood, which hampers our understanding of their key prey species, habitats and trophic interactions with fisheries. Traditional faecal analysis techniques have proven ineffective in ASL because most prey remains are completely digested. We conducted a feeding trial on captive ASL to determine whether faecal DNA analysis could be used to quantify sea lion diet. This study demonstrated that analysis of faecal DNA can detect the presence of sea lion prey DNA, despite no identifiable prey hard parts being recovered from the same scats. The results from our experiments also demonstrate that DNA extracted from ASL scats is highly degraded, because we could not detect prey DNA using molecular primers more than 100 base pairs in length. Quantitative PCR indicated that the amount of mtDNA amplified from scats was related to the amount of novel prey ingested. Quantitative estimates determined the difference between periods of high consumption of novel prey and low consumption of a particular prey type, but did not pick up smaller differences in consumption rates. Interestingly, mtDNA quantitative estimates were significantly higher for squid than shark from periods when the seal was fed in equal proportions. These differences may be related to the concentration of DNA in the different tissue types of each species.

3.6 Developing population monitoring protocols for Australian sea lions

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
SVE-4112		Goldsworthy, Simon	1-Jan-06	1-Dec-06	44,000

Executive Summary

Australian sea lions (ASL) were listed as a threatened species under the EPBC Act in February 2005. Information on the size and status of most subpopulations is poor and significantly hampers developing appropriate management strategies for the species. Many aspects of the species' breeding biology and ecology are unique among otariids (fur seals and sea lions) and make accurate assessment of pup production challenging. Unlike other otariids where pupping seasons are short, with all pups being easily recognisable and available for sighting at the end of the breeding season, in Australian sea lion populations the breeding season can extend for up to eight months. As such, a significant portion of the pups may have fully moulted, dispersed or died by the end of the pupping season.

Traditionally direct counting methods have been used to determine ASL pup production, however, these are prone to underestimation due to sightability and availability biases, and only provide point estimates of numbers with no confidence limits. Recently, mark-recapture methods using the Petersen estimate have improved estimates of ASL pup production at some large colonies, and have addressed some of the under estimation caused by sightability biases. However, dispersal and unaccounted mortality (availability biases) may still cause significant underestimation of actual pup production, and immigration has been observed in some small colonies.

We developed and tested the appropriateness of two new methods for estimating pup production in ASL subpopulations. The first utilised individual resight histories of pups and Cormack-Jolly-Seber (CJS) models in conjunction with standard mark-recapture methods to improve estimates of pup production for large ASL subpopulations (>40 pups). The second developed a cumulative mark and count (CMC) method for improving estimates of pup production in small ASL subpopulations (<40 pups).

CJS methods were trialled at Olive Island and produced pup production estimates that were greater than those based on direct counting and on mark-recapture (Petersen estimate) methods. Pup mortality during the study period was estimated to range from 15-52. As recovered mortalities numbered 34 in total, ground surveys may have underestimated pup mortality by up to 35%. There was no evidence for permanent emigration, suggesting that the most important source of error in mark-recapture procedures at Olive Island were due to unaccounted mortality. The best estimate of pup production for the 2006 season at Olive Island based on CJS methods was 205 (range 193-256). This was 1.37 times the estimate based on direct counting methods (150 pups), but was similar to the result (1.03 times larger) obtained from the Petersen estimate (mean 197, range 191-203). However, an adjusted Petersen estimate (adding the mortality range 34-52) produced the same estimate as the CJS approach (206, range 191-223).

CJS analyses suggested no significant pup production occurred beyond the second session, only three months into the breeding season. This was contrary to observations of the presence of perinatal mothers and new born pups up to session 5 (19 June), increases in pup abundance between sessions 2-5 based on the sum of the number of tagged pups, dead pups and unmarked pups, and the Petersen estimate, and on evidence of a 5-7 months breeding season elsewhere. The reasons for disparities in methods are currently unclear, but should be addressed in the future.

The cumulative mark and count (CMC) method trialled at a small colony (Seal Slide, Kangaroo Island), supported the observation that not all pups are available for counting during ground surveys, and produced a consistent (repeatable) estimate on two occasions (10 pups). The surveys would have benefited from greater numbers of pups being marked over more sessions. The development of both CJS and CMC methods has advanced the methods of monitoring for both large and small ASL colonies.

Distance analysis among ASL subpopulations identified 11 metapopulations in the species, seven of them were in South Australia (SA). Among SA metapopulations, only four provided sites where accurate, repeatable, cost effective and logistically feasible surveys could be undertaken. Within each of these, one large (>40 pups) and one small (<40 pups) site were selected (8 in total) as regionally representative sites to form the basis for ongoing surveys. Ongoing surveys at these sites would provide critical data on the status and trends in abundance within metapopulations to support management of the species across its range.

Refining of CJS and CMC methods (including trialling at WA subpopulations and identification of regionally representative subpopulations) is recommended so that national standards in subpopulation monitoring protocols can be developed. Resources need to be secured and coordinated to support ongoing assessment of key representative sites across the range of the species, to provide critical information on subpopulation status and trends, and to measure any recovery in the species.

3.7 What is causing the decline of little penguins (*Eudyptula minor*) on Granite Island, South Australia?

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
SVE-5453F	RD06/0401	Goldsworthy, Simon	01-Feb-2006	30-Nov-2006	4,700

Executive Summary
The number of little penguins at Granite Island has been declining since the early 1990s. Large numbers of tourists visit Granite Island to view the penguins and the island is inhabited by non-native black rats (*Rattus rattus*) and water rats (*Hydromys chrysogastes*) whereas nearby West Island does not have any tourists nor land-based predators. Data on the little penguin population size, breeding success, diet composition, foraging behaviour and predation by both rats and New Zealand fur seals (*Arctocephalus forsteri*) were collected at Granite and West Islands to determine the likely onshore or offshore factors that may be causing the decline in the Granite Island population. In 2006, the percentage of chicks that fledged was significantly less at Granite Island (37%) compared to West Island (54%). Although there was little overlap (9%) in the foraging areas used by penguins at each site, there were no significant differences in prey species consumed or the mass of stomach contents at Granite (45.0 ± 33.1 g) (mean ± SD) and West Islands (46.0 ± 35.0 g). Both sites showed a similar delayed onset of breeding in 2006 and population surveys in 2006 indicated that both sites might be in decline, suggesting that tourism and predation by rats are not solely responsible for the decline in the Granite Island population. New Zealand fur seals consumed adult penguins at both sites, indicating that they may be causing part of the decline at Granite and West Islands and accordingly, ongoing monitoring of fur seal diet is required.

4. Product Quality & Value Adding

4.1 Optimising at-sea post-harvest handling procedures for the Australian sardine (*Sardinops sagax*).

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
R&D-5506	2002/236	Carragher, John	01-Jul-2002	31-Jan-2005	261,534

Outcomes Achieved to Date

The research documented in this report has contributed to the development of the value-adding component of the South Australian sardine (*Sardinops sagax*) fishery by providing information on the key factors affecting sardine quality from harvest through to the factory. The research showed that although there were differences in the rate at which sardine quality deteriorated between different times of year, different fishing boats, different on-board holding tanks, and the start and end of each net haul, the main effect was the detrimental consequence of not chilling the fish as quickly as possible after capture. Emphasis was placed on the ability to chill fish rapidly to maximize sardine quality via an appropriate low fish load, hold water:volume ratio, more effective circulation of chilling water and/or the opportunities for new flo-ice technologies. This information, together with an audit of vessel hygiene and operating practices, has been used by industry to improve processes and practices to maintain sardine quality such that several more vessels and value-adding ventures are now operating in Port Lincoln than when the project started. Indications are that these ventures are supplying value-added sardines to the substantial domestic commercial and recreational fishing bait markets and thereby replacing some imported baitfish. The option of being able to value-add to a percentage of the sardine catch is of economic and social benefit to the fishers and factory owners in Port Lincoln.

Non-Technical Summary

The sardine (*Sardinops sagax*) fishery based in Port Lincoln, South Australia expanded to ~26,000 tonnes in 2006. Most of the catch is used as feed for the established tuna farming operations that are co-located at Port Lincoln. The tuna aquaculture industry is willing and able to absorb the total output from the sardine fishery but at a low price per kg. Some of the sardine fishers have sought to increase their earnings by supplying value-added sardine products – recreational and commercial fishing bait, and/or sardines for human consumption. The main issue these fishers face in striving to supply these markets is deterioration in the physical integrity of the sardines. This deterioration shortens the window during which fish are suitable for value adding, this is further compounded by the limited capacity of the on-land freezing infrastructure to process a significant proportion of the catch. The aims of this project were to (a) understand the pre-harvest, harvest and post-harvest factors that are responsible for the rapid rate of deterioration in the physical integrity of sardines, (b) identify the domestic market status and opportunities for value-added sardines, and (c) undertake a cost-benefit analysis of implementing the recommended changes that would improve sardine quality.

An audit of harvest practices on several boats interested in undertaking value adding revealed a number of shortcomings that were likely to contribute to deterioration in product quality. The main issues were related to holding tank hygiene and the effective circulation of refrigerated water in fish filled holds. This information was used by some of the boats to make modifications to holding tank cooling functionality and improvements to holding tank hygiene practices.

A desktop and survey approach was used to identify the nature of the current domestic markets for frozen bait sardines and fresh, frozen and processed sardines for human consumption. Following this status report there was an assessment of where Port Lincoln sardine products could capture some of this market. The short-medium term opportunities were assessed as being in the bait markets and the fresh fish markets in Sydney and Melbourne. The marketing advantages of Port Lincoln sardines as bait would include the 'clean and green' perception of the fishery, as well as the perception that this is a 'natural food' for the main target species. These advantages, however, will not overcome an inferior quality product or a significant price premium. For the human-consumption market the price premium is guaranteed for fresh product providing the quality is high; however, any further processing to butterfly fillets and/or canned product would require substantial investment in processing technology, and the financial returns may not be justified in the short-medium term.

An investigation was carried out on the biological and ecological factors affecting rates of deterioration in sardine quality in the immediate post-harvest period (i.e. net to processing factory) using a quality index (QI) and physico chemical measurements. One of the major issues to be addressed in the study was post mortem belly burst, thought to result from visceral autolysis accentuated by consumption of particular prey species (i.e. copepods). However, although a degree of visceral post-mortem autolysis was detected, very few fish were found with burst bellies therefore it is suggested that this issue is not a major constraint on the progress of value-adding in the fishery. There was only one significant instance of burst stomachs/bellies within the field sample. There may be a relationship between spawning fish and susceptibility to stomach/belly burst as this was also the only time ripe gonads were observed during the fieldwork. Postmortem autolysis was more commonly recorded and is likely to have been caused by digestive enzyme activity, significantly contributing to the observed increase in sardine QI with time.

The QI method proved useful in sardine quality assessment, with inclusion of ten of the seventeen parameters suggested in the literature. Four of these (i.e. gill colour, eye clarity, body appearance and body stiffness) accounted for 76% of the overall QI. It was evident that the QI would provide a framework for standardisation of the quality of fish going to market, a necessity in the process of value adding. The setting of the QI acceptability threshold would involve market acceptability trials including taste testing, microbiological studies to determine safe levels of bacteria on/in the product, and work to determine safe rancidity levels and should be included as a essential element of any further value adding work on this species.

Methods are suggested for maintaining sardine quality through the onboard storage process to allow entry of the product into premium markets. These include more rapid water temperature reduction using more efficient, dedicated, on-board value-adding tanks, and reduction in the quantity of fish loaded into tanks to maintain water circulation and facilitate cooling. The practice of topping up nearly-full, cold tanks with the later shots containing relatively warm fish should be limited as this practice causes a temperature spike and probably accelerates deterioration of fish already in the tank. Fish should also be iced between unloading at the jetty and arrival at the processing factory, and jetty-to factory transport bins chilled using an efficient cooling medium such as flow ice.

A cost-benefit analysis was undertaken of various options including investment in small scale (processing 50 to 100 tpa) and large scale (processing 200 to 400 t.p.a) equipment for improved post-harvest handling and processing of sardines. The analysis was conducted from an individual licence holder (single boat) perspective using a model based on financial data provided by all active licence holders.

The results of the analysis showed that investment in post harvest handling equipment can generate positive returns to the fishery. However, the outcomes are sensitive to the premiums available for human consumption sardines, the quantity processed (which will be determined by the volume that the licence holder can market) and the initial cost of the equipment and its installation. For small scale equipment, for example, the prices (landed beach price equivalent) at which the investment would yield a breakeven return ranged from $1.46/kg for 50 tpa throughput down to $1.15/kg for 100 tpa throughput. For large scale investment, the breakeven prices ranged from $1.26/kg for 200 tpa throughput down to $1.06/kg for 400 tpa throughput.

While the analysis demonstrates the potential returns that can be generated by investment in post harvest handing of sardines, it also highlights the critical role that marketing will play in securing those returns. Successful marketing of the product will be essential to achieve both the price and the volume necessary to generate positive returns to licence holder investment.

4.2 Nutritional profiles of baitfish 3: effects of harvest and post-harvest processes on quality of local baitfish for feeding SBT.

ID Number	Project Number	Manager	Start Date	End Date	Total Project Funds ($)
SVE-4034	2004/211	Carragher, John	01-Jun-2005	01-Nov-2006	305,109

Outcomes Achieved to Date

The research documented in this report demonstrates the effects of postharvest practices on the nutritional quality of locally-caught Australian sardines (*Sardinops sagax*) and of redbait (*Emmelichthys nitidus nitidus*) sourced from Tasmania. The

research showed that there were substantial losses in key elements of the nutritional profile (i.e. vitamins and nucleotides), concomitant with increases in rancidity and loss of freshness. Greatest losses in quality and freshness occurred during post-harvest transport and freezing, and thawing of baitfish, prior to feeding to SBT. Losses during extended frozen storage can be substantial.

This information will be used by suppliers to improve post-harvest treatment of baitfish to optimise quality. The tuna industry will use the information to improve the efficiency and effectiveness of their tuna-feeding strategy to optimise SBT growth performance, health, flesh quality and return at the market.

Non-Technical Summary
Past SBT Aquaculture Subprogram-Aquafin CRC projects have sought to determine the nutritional profiles of the 23 or so different baitfish species that are used by tuna farming companies to feed to southern bluefin tuna (SBT). These studies have shown inter- and intra-species differences in nutritional parameters including amino acid and fatty acid ratios, crude protein, crude fat, ash and energy, and indicators of biochemical quality, such as free fatty acids and peroxide levels. Whilst differences in these characteristics between species are expected, differences between different batches of the same species can indicate either seasonal or regional effects, and/or the impacts of different harvest and post-harvest practices on baitfish quality. For example, data were collected from 34 different batches of local sardines supplied to the tuna farms. Whilst some proximate parameters such as protein vary only slightly (10% or less), the level of fat changes over 6-fold, and that of peroxides over 10-fold between batches.

Not only does this indicate that it is important to know when to harvest the sardines in order to maximise their nutritional value (i.e. when fat levels are high before spawning), it also shows the effects of oxidative processes (e.g. lack of antioxidants, prolonged processing and/or storage times, inadequate storage temperatures) on the quality of the nutrients (especially the fats) in the baitfish. In this regard there was a significant positive correlation (R2 = 0.61) between storage time and peroxide concentration, suggesting that the storage conditions in Port Lincoln (South Australia) can lead to high peroxide levels in the local sardines (Ellis and Rough, 2005).

Tissue integrity and health of the SBT is damaged by oxidative processes and protected by antioxidant vitamins. These protective antioxidants have to be supplied to tuna in their feed. High peroxide values are of concern because they indicate low levels of antioxidant vitamins in baitfish, and that the tuna consuming the baitfish are taking in a high burden of reactive oxygen species that can lead to further antioxidant depletion and peroxidation of the structural and functional compounds. Both mechanisms (i.e. low vitamins or high oxidative burden) could, in turn affect metabolic processes, and impact on flesh quality characteristics. It will be important to be aware of the potential deleterious effects of long-term feeding of nutritionally poor-quality baitfish on the health and flesh quality of tuna.

Postharvest deterioration in Australian sardine (*Sardinops sagax*) nutritional quality was followed from the jetty through short (4-6 weeks) and long (3-6 months) term storage trials (Objective 1) to subsequent thawing (Objective 2) for feeding to SBT. Samples were taken from fishing vessels at the jetty and during and after storage and thawing trials. Redbait (*Emmelichthys nitidus nitidus*) were sampled directly after capture onboard the fishing vessel, at the Triabunna (Tasmania) factory, then at the Port Lincoln factory/commercial freezer during and after storage and thawing trials.

The results of this study are as follows:

- Existing harvest and post-harvest practices have a significant impact on the nutritional quality of baitfish fed to SBT in seacages. Vitamins and nucleotides decline to low levels, rancidity increases and fish freshness declines significantly, to the point where the baitfish becomes of questionable utility, particularly as a source of antioxidants.
- Freezing method affected the rate of vitamin E loss. IQF sardines stored at -20oC lost vitamin E at a higher rate than those which were block frozen, possibly because individually frozen fish are more likely to be exposed to air than those in a solid block.
- Baitfish, particularly sardines, should be thawed in seawater, not air, to minimise vitamin losses. Vitamin E losses were actually least in freshwater and greatest in air, whereas for vitamin C this was reversed in some trials. The seawater recommendation is a compromise to minimise losses of both vitamins. Air-thawing, particularly at room temperature, accelerates oxidative processes and thus irreversible breakdown of antioxidants within fish tissue.
- Block feeding at sea is recommended over land-based thawing as rapid thawing (1-2 hours vs 1-2 days) may reduce vitamin losses
- In addition, fish should be kept on ice or refrigerated when transferred from the fishing vessel's refrigerated seawater tanks to the factory, and storage time limited.
- Good correlations between TBARS and remaining vitamin E suggest a practical utility of this measure as an indicator of antioxidant status, with 1-1.5mg/kg TBARS equating to 50% remaining vitamin E in each baitfish species. In view of this result it is recommended that TBARS be included in as an analytical requirement for baitfish quality analysis in Port Lincoln. Analysis of TBARS is also methodologically simpler and less time-consuming than for K factor.

Objective 3 was addressed in consultation with the ASBTIA. It was decided that the most effective means of industry extension would be the production of a laminated summary of the key results for distribution to sardine fishers, processors and tuna farm operators. This was carried out. Key results were also included in industry newsletters (Tuna Briefs; http://www.sardi.sa.gov.au/pages/aquafin/southern_bluefin_tuna_publications.htm:sectID=967&tempID=11#Newsletters) and discussed with industry at SBT Aquaculture Subprogram-Aquafin CRC meetings at Port Lincoln. Results were also presented at the Aquafin CRC conference in the Barossa Valley, May 2007.