Southern Bluefin Tuna: Its propagation in Australia

A strategic plan for the propagation of Southern Bluefin Tuna (*Thunnus maccoyii*)

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The financial and scientific information given in this Strategy is the best available to the Consultant at the time. The recommendations and advice contained herein and derived from this information are, to the best of his knowledge, accurate and realistic. Nevertheless the recommendations and advice are of a general nature and have not taken into account the investment objectives, financial situation, or particular needs of individuals. Those wishing to pursue this Strategy should assess personally or with the

assistance of a financial advisor whether the recommendations are appropriate in their circumstances.

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EXECUTIVE SUMMARY

FRDC 1999/376
Southern Bluefin Tuna Aquaculture Subprogram – development of a strategic plan for the propagation of Southern Bluefin Tuna (SBT)

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OBJECTIVES: To develop a strategic plan for the propagation and enhancement of Southern Bluefin Tuna (SBT) in Australia.

Southern Bluefin Tuna (SBT) is a fast growing fish species which commands high market prices. A substantial reduction in the wild stock size made necessary a considerable reduction in catches in 1988 and 1989 from the major fishing nations (Japan, Australia, and New Zealand). These historically low quotas now restrict their availability for capture, either for direct supply to the market in Japan, or for fattening in farms. Several agencies and farming companies have been discussing approaches whereby the value and volume of Australia's supply of SBT might be increased. They have been investigating improved aquacultural methods to provide a supply of fingerling fish for ongrowing and reseeding.

This present Business and R&D Strategy was commissioned by the Commmonwealth Department of Agriculture, Fisheries and Forestry – Australia (AFFA) and the Fisheries Research and Development Corporation (FRDC) on the suggestion of industry and government in May 2000 to determine the best way to develop a project to domesticate SBT in Australia for the production of fingerlings for farming and stock enhancement. The Strategy extends investigations on the feasibility of the aquaculture of SBT in Australia which was commissioned by AFFA in 1998 (see Lee 1998).

Financial feasibility

The feasibility of stock enhancement of wild populations of SBT, and farming from fingerlings is subject to both scientific and economic constraints. It was fairly clear that the technical difficulties associated with both could be resolved, albeit with difficulty. What was not known was if the biological characteristics of the species might make reseeding or farming economically impossible. To examine this premise, a simulation of the growth and natural mortality of wild animals was performed to estimate the number of fingerlings that would need to be released into the wild to replace the value of the presently caught Australian quota. A second simulation estimated the weight and feed costs of 3.5 and 4 years old fish raised from farmed fingerlings, over a range of feed conversion ratios. These simulations confirmed that farming SBT from fingerlings is not only financially feasible, but is also likely to be profitable, even with the present conversion ratios and prices. The size of the high value tuna market currently exceeds 30,000 tonnes and is growing both in Japan and elsewhere.

Conclusions:

- Stock enhancement and farming SBT from hatchery fingerlings is technically feasible and likely to be economically successful.

- There needs to be an evaluation of the best technical strategy for releasing hatchery fingerlings for stock enhancement, and a technical evaluation of the ways to rear fingerlings to market on commercial farms.

The Business Proposal

During discussions with stakeholders it soon became apparent that although there were many specific items on which different views were expressed, there were a number of issues that were shared. There was an industry concern that if the strategy was implemented, the current industry should benefit from the investment and also, that any investment in the strategy should not jeopardise the present farmers. Other major concerns related to the lack of a clearly articulated government policy on reseeding and farming, and fears that the intellectual property being generated cannot be safeguarded from use by others. Substantial finance for the research and development activities will be needed. Contributions will have to be harmonised and managed as an entity. Moreover, there will need to be a seamless passage from the initial research and development activities to the commercial production of fingerlings.

The diversity of stakeholders and potential funders for this project, and the control of issues such as ownership of intellectual property (IP) and financial benefits, need an entity specifically for the purpose of managing the research and its commercial extension. There is no existing organisation which satisfies all these needs.

While a major role is needed to manage the R & D program, there will also be a need for the construction and running of commercial broodstock holding facilities and a hatchery. It would be inefficient to unnecessarily duplicate research expertise and facilities available elsewhere, so "In house" research should be restricted to that which cannot be contracted elsewhere. The commercial extension of research results would be greatly facilitated by having the same organisation managing the R&D and constructing and running a commercial hatchery.

The operations of the management entity could be supported by capital investment from private industry investors and venture capital, with options for additional government support. This would need to be augmented by an ongoing contribution which could be levy-based, or alternatively funded, until commercial success. If required, a new share issue could be contemplated after eggs were successfully produced. Once the commercial production of fingerlings came on stream, an income flow would start to be generated from their sale.

The entity responsible for implementing the business and research and development plans for the propagation of SBT should own such capital or intellectual property that is generated, subject to such commercial agreements that may be negotiated.

Conclusions:

- There is no existing R&D management entity which will satisfy the needs of all the stakeholders and an alternative needs to be developed.
- The intellectual property (IP) arising from a successful R&D plan to produce fingerlings from captive broodstock includes both public and private benefit IP, and should attract both private and public investment.
- An independent incorporated private business entity should be established to manage the Research and Development Plan, to establish a commercial SBT hatchery, and to own the real and intellectual property derived from its activities.

Research and Development Plan

In preparing a Business strategy for the propagation of SBT in captivity to provide fingerlings in Australia for stock enhancement and farming it was necessary to identify a R&D plan which would be at the heart of the business activity until the primary research phase was completed.

The Plan outlined in this report is not an exhaustive blueprint for every step in the process. It does not give an extensive literature review of each objective. Some relevant literature is included, more is provided in Appendix I- Scientific Review of Published and Available Unpublished Work on the Propagation of Tuna and Similar Species.

The present plan outlines ways of:

- Capturing, holding and bringing to maturity and spawning adult SBT,
- Treating and transporting eggs,
- Determining the food and environmental needs of larvae and
- Determining the best feed and environmental conditions for growing post metamorphosis larvae to fingerlings.

The consultant's brief did not include aspects of the business relating to the ongrowing from fingerling to market. Nor did it include the research and activities needed for a successful stock enhancement program. Significant research aspects which have been omitted from this strategy, and which will need to be investigated before either stock enhancement or farming from fingerlings can be successful include;

- Genetics research
- Strategy for reseeding
- Methods of rearing of fish on farms from fingerlings to 3 year old fish
- Research into disease prevention

Some aspects of these are currently under investigation in Australia.

Primary broodstock development

Conclusions:

- Broodstock be acquired by catching 6-7 year-old, 40 kg fish from the Great Australian Bight and transported to a broodstock holding facility. Consideration be made to the use of existing captured fish for this purpose.
- Broodstock need to be acclimatised to land-based tanks for the induction of maturation and spawning.

- An appropriate methodology, type and concentration of anaesthetic needs to be identified to sedate fish during handling operations
- A suitable pelleted diet must be developed for the maturation of SBT which produces gonad growth and maturation and healthy eggs.
- Broodstock should be exposed to combinations of photoperiod and temperature to initiate vitellogenisis. Final maturation and spawning should also be investigated by the use of exogenous hormones.

Egg Collection, Sterilisation & Transport

Conclusions:

- The most robust developmental stage for egg transport and the best handling methodology and loading density for transport of eggs need to be identified.
- A methodology should be developed for the sterilisation of eggs with minimum toxicological effect on the subsequent larvae.

Egg Hatch & Larval Development To Metamorphosis

Conclusions:

- Comparative experiments on a range of feeds and environmental variables should be contracted out to research institutions. Eggs or yolk stage larvae, should be supplied for this purpose from the broodstock facilities.
- A small research hatchery should be built as part of the broodstock holding facility to confirm and commercialise the work done under contract. This to be expanded later for the production of commercial quantities of fingerlings.

Post-metamorphosis juvenile development to fingerlings

Conclusions:

- Comparative experiments on a range of feeds and environmental variables to rear post-larval fish to fingerling stage should be contracted out to research institutions after completion of the larval development work.
- The commercial hatchery should be used to confirm and commercialise the contracted work.
- A method needs to be developed for transporting fingerling SBT to sea farms for growout and for release into the sea for stock enhancement.

OUTCOMES ACHIEVED

This project has shown that stock enhancement and farming SBT from hatchery fingerlings is technically feasible and likely to be economically successful. In the short term this project will encourage a broad range of investors to consider investing in research and development of SBT propagation and stock enhancement. At time of publication the project had received a positive response from the SBT aquaculture industry, researchers and governments. Actions arising from presentation and discussion of the project outcomes during 2001 will indicate how useful the project truly proves to be.

In the longer term, and assuming investment is available for a propagation program, the research undertaken as part of this project will be available for use by investors and researchers to assist in developing and undertaking a long-term research program.

If this report can encourage investment in research in SBT propagation and ultimately allow the captive breeding of SBT then it will lead to an SBT aquaculture industry that is self-sufficient and whose growth is unrestrained by wild catch quotas; will provide Australian fisheries managers with a new capacity to manage the sustainability of wild-stocks through stock enhancement programs; expand existing and successful collaboration on fisheries research and development with countries in the Asia-pacific region.

PART 2: INTRODUCTION TO THE STRATEGY

Southern Bluefin Tuna (SBT) is a valuable resource with high market prices and fast growth. Catches are generally restricted by historically low quotas. A number of agencies and operators are discussing approaches whereby the value and volume of Australia's supply of SBT might be increased. They have been investigating improved aquacultural methods to provide a supply of juvenile fish for ongrowing and reseeding. A study on the feasibility of the aquaculture of the SBT in Australia was commissioned by AFFA in 1998 (Lee, 1998). This present Business and R&D Strategy was commissioned by AFFA and FRDC upon the suggestion of industry and government to further pursue these investigations, and to determine the best way to progress a project to domesticate SBT for the production of fingerlings for farming and stock enhancement. Although some preliminary evaluations of the likely financial and biological success of stock enhancement and farm growout from fingerlings has been included, the present Strategy is designed only for the propagation of SBT in captivity to the fingerling stage. Successful implementation of this Strategy for the propagation of SBT will need the commitment by governments and industry of facilities, people and finance for many years.

The propagation of SBT is a new venture and is likely to produce a number of difficulties of a practical and scientific nature, so relevant published and available unpublished information has been examined and reviewed. This review includes information about SBT and other species of tuna and where relevant, information from studies on other species of marine fish propagated in captivity. Observations have been drawn from a review of abstracted literature, primary scientific publications, and interviews with appropriate available specialists in the field, including scientists and those active in fish husbandry in Australia. The review is attached as Appendix I. A reference list of the publications cited throughout this entire strategy, including the appendices, is provided in Appendix III.

This strategy was developed by a process of investigation and consultation. Stakeholders were questioned on a face-to face basis regarding their views about the SBT propagation project. The questions were grouped into two areas. The first investigated the views of the stakeholder about the best way to run the business part of the project. The second asked views on the most appropriate research to achieve the propagation of the SBT. Questions were targeted to stakeholders on the basis of their occupational category. The results of the consultation process are attached as Appendix II.

During discussions with stakeholders it soon became apparent that although there were many specific items on which different views were expressed, there were a number of issues which were shared. The first was an industry concern that if the strategy were implemented, the current industry should benefit from the investment and also, that any investment in the strategy should not jeopardise the present farmers. Other major concerns related to the lack of a clearly articulated government policy on reseeding and farming, and fears that the intellectual property being generated cannot be safeguarded from use by others. One pervasive issue which will need resolution, is that state government

representatives were unwilling to commit financial support to the program unless most of the research and development activity was to take place in the state which they represented.

This Business and R&D Strategy attempts to identify a process for the commercial production of fingerling SBT. The current state of knowledge of the biology of SBT, other tunas, and relevant domesticated marine fish species is examined, and an R&D plan is proposed which should address all the relevant bottlenecks currently preventing the propagation of SBT is captivity in Australia. A strategy is suggested which takes account of the different outcomes required for the project to be able to receive public good funding as well as private funding for the R&D. A business structure is proposed which should enable all interested stakeholders to participate. The core of this part of the strategy is the successful incorporation of a business entity to encompass the views and ideals of the stakeholders, and to obtain finance and drive the activities proposed, the objectives identified, and the outcomes expected.

PART 3: THE BUSINESS PROPOSAL

Background

SBT can live to 40 years old, when they reach a length of about 200 cm and weigh about 200 kg. They are usually found in southern oceans between 30° and 45° S). The wild stock spawns only between 7 and 20 °S in the Indian Ocean south of Java. Postlarval juveniles migrate southwards during their first 6 months, adjacent to the coast of Western Australia to the coastal waters of southern and eastern Australia, where they are found during the Australian summer (December-April) and are harvested by Australian surface fisheries as 3-5 year old fish. Few fish older than five are found in coastal waters. They spend their winters in deeper oceanic waters where older fish are also caught by the Japanese long line fishery. The species has been considered depleted for over 20 years, and limits to the total allowable catch were first introduced in 1983. The quotas were reduced under an informal Trilateral Framework between Japan, Australia and New Zealand. In 1993 these countries formed the Commission for the Conservation of SBT (CCSBT) to facilitate global management of the species. Since then, catches from countries other than those party to the CCSBT have increased their unregulated take of the species and it is estimated that Indonesian, Korean and Taiwanese boats now take between them at least one third of the global catch.

The catch limits initiated in 1989 were intended to allow the spawning stock to regain the abundance levels that it had in 1980 by 2020. However there is disagreement between the Australian, New Zealand, and Japanese scientists as to the extent that this may or may not be happening. The Australian scientists involved with the assessments are of the opinion that the current global catches of SBT are unlikely to be sustainable, and in 2000 Australia requested that an Arbitral Tribunal be established in accordance with Annex VII to UNCLOS (United Nations Convention on the Law of the Sea) over the dispute between it and Japan about catches of this species.

Following the dramatic cuts in quota available to the Australian industry in 1988 and 1989, an experimental tuna farm was set up by the Japanese Overseas Fishery Cooperation Foundation (OFCF) in collaboration with the South Australian Government and the Tuna Boat Owners of Australia Association (TBOAA) in 1991 to try to fatten fish caught in the Great Australian Bight. Global management controls on SBT are now restricted to the imposition of a global total allowable catch which is distributed between the participating countries as a national catch quota. Within Australia this is subdivided and distributed as individual transferable quotas to individuals and companies who have bought the rights to own it. This quota is defined as tonnes of tuna, not numbers of individual fish, so if fish were to be caught at a small size and grown bigger on farms, then the quota could in effect be made larger. In recent years, all operators have switched from poleing the quota allocation, to catching schools in purse seines and transferring these to static cages at Port Lincoln for ongrowing in farms. There are now 16 fishing groups that utilise 20 farming sites off Port Lincoln, South Australia for this purpose. Fish are caught in mid January to February and fattened for 4-6 months.

While the restrictions on wild catch of SBT were intended to allow the stock to rebuild naturally, there is a considerable feeling among Australian scientists and regulators this is not happening, and some feel that the stock may even be continuing to get smaller. As a consequence, an alternative approach to rebuilding the stock by reseeding from hatchery derived juveniles has been proposed. Because of the quota restrictions on the number of fish that can be caught and fattened, the cost of leasing wild quota for catching and farming has reached as high as $A20 a kilo, and the market price for farmed fish as high as $A40 a kilo. At these prices some farmers are also considering the practicability of increasing the volume of product by producing fingerlings from domesticated adults to growout for the market. By doing so the present constraints on the numbers of fish that can be farmed would be lifted, and the industry's growth would only be constrained by the same market and environmental forces that apply to other species of farmed fish.

Issues to be Considered

The production of fingerling SBT can be technically achieved in Australia. However to do so, and also produce a financially viable hatchery it must be recognised that while there are strengths and opportunities associated with doing it in Australia, there are also very real threats and weaknesses to be overcome.

Strengths:

- Australia has a number of world class research scientists capable of performing fish aquaculture research with a high level of expertise.
- Committed support for SBT research from FRDC.
- There are numerous commercially based fish hatcheries in Australia with staff familiar with the bulk production of fish fingerlings, albeit in freshwater.
- All states have research hatcheries with facilities capable of performing much of the research and development needed.
- Australian SBT farmers, along with those in Spain and Croatia, are among the only people in the world with extensive knowledge and experience in growing tuna for the commercial market.
- As a result of the commercial farming of this species, there is a well trained work force that are used to handling large tuna.
- Australian aquaculture scientists have a high degree of expertise in the essential areas of reproductive endocrinology, nutrition research and larval rearing.
- Research and development is likely to be taken up quickly because farming is presently in the hands of a small (16 companies), well organised, financially resourced, farming sector who own most of the wild quota.

Weaknessses:

- Most of Australia's aquaculture research scientists have little expertise or experience in rearing marine pelagic fish species.
- Because the research and development is not producing a patentable product, it is difficult to perceive how the intellectual property from the R&D could be retained.

- If Australia develops a successful reseeding program, this could benefit the fishing industry of other countries who may just increase their catches to take up the extra numbers of fish without contributing to the cost of reseeding.
- Because the fingerlings are to be used for both farming and stock enhancement, there will need to be two subprojects because different physical and genetic attributes will be needed for each.
- Production of fingerlings will not produce a saleable product unless research is done to find out the best way to grow these to a marketable size.
- Without a well developed release strategy, releasing fingerlings into the wild could be merely feeding other predators.
- Despite the species being globally distributed and the project being national in intent, State governments will only contribute funding if the research is done in their State and if the results produce an industry in their State.
- When increased numbers of SBT are available from the hatchery production, the value of the quota would most likely fall, causing an instability in the quota market.
- Australia does not have a large aquaculture facility in one location, research expertise and facilities are dispersed thinly across the continent.
- There are no land-based facilities currently holding SBT broodstock.
- There is an almost complete lack of knowledge about the ecology of SBT between the larval metamorphosis stage and 100 days old.
- There have been no investigations of the responses of the markets to an increased supply of farmed SBT but economic theory suggests that the price would fall.
- There is only one market for SBT at current prices, which makes the product very exposed to weakness in that single market (eg currency fluctuations).
- The basic lack of knowledge on tuna health, and larval and juvenile feeds.

Opportunities:

- The profitability of the present farming activity suggests that there is room for expansion in this sector before the market is satiated.
- If the concerns of the Australian scientists and managers about the size of the wild stock are proved correct, then there is likely to be instability in the allowable quota available for catching for farming. Hatchery production of fingerlings will enable a reliable supply of product to be farmed each year.
- If a successful stock enhancement program is achieved, this may enable the wild fishery to be stabilised, and reduce the threat to the wild population and reduce international tensions.
- The rapid growth and high value of the finished product makes commercial profitability highly likely.
- The difficulty of obtaining fish for broodstock development from elsewhere than Australia's southern EEZ , together with their development in land based facilities may enable Australia to get a comparative advantage on fingerling production.
- The profitability of the present farming industry suggests that the current or new producers would probably be prepared to expand production even at a lower price and profit margin.

Threats:

- If the current Japanese work on cultivation of northern bluefin tuna produces a commercial product, then this is likely to compete with SBT product from Australia.
- If the proposed European research and development program for domestication of the northern bluefin tuna is funded and is commercially successful, this is likely to compete with SBT product from Australia.
- The proposed research project for work in Australia on yellowfin tuna and other fish species could dilute the availability of funds and time of skilled research workers for SBT research.
- There is a lack of suitably zoned water in inshore waters, even at current tonnages, this is likely to inhibit expansion.
- Although several sources have expressed an interest in the project, it is currently seen as a high risk investment, and there are no clear sources of identified funds at present.
- If overseas interests are brought in, these may appropriate the results for development overseas rather than in Australia.
- Perceived inadequate environmental management by fish farmers in the past has produced a community backlash to the presence of fish farms in coastal waters.
- Other countries possibly suitable for SBT propagation/growout (eg, South Africa, Chile) have both lower labour costs and greater feed availability.
- If core problems in the propagation of SBT are solved, then this would encourage the use of the technology elsewhere to propagate Northern Bluefin Tuna which spawns earlier and grows faster than SBT.

Any business plan that is developed must have a capacity to make best use of the strengths and opportunities identified, and to cope with the weaknesses and threats.

Commercial Feasibility

Stock Enhancement

There is some scepticism that reseeding of marine populations of wild fish produces any tangible benefits either to the wild populations, or to the economics of fisheries, and a belief that it is somehow an abrogation of responsible fisheries management. However an increasing number of studies suggest that populations of wild fish stock may be augmented by a carefully planned program of releases of cultivated juveniles for stock enhancement. This may be implemented responsibly, provided several significant issues are taken into account during the process. These include:

- developing a management plan that identifies stock rebuilding goals and measures of success;
- defining and using genetic and health management objectives;
- defining an optimum strategy to release fish and assess stocking effects, and;

- identifying economic and policy guidelines in forming an adaptive fisheries management plan for the restocked species.

The survival of hatchery-bred releases is usually related to the season released and size of released fish. Greatest recovery usually occurs when they are released at the same time as peak recruitment of similar sized wild juveniles. For SBT this is likely to be into the Leeuwin current off Western Australia between January and April.

Providing the natural mortality rate for restocked fish is similar to wild bred animals, the release numbers needed to achieve specific targets at age can be estimated from published instantaneous mortality rates at age in the literature. Extrapolation of these figures needs to be done with caution however, as the survival of released marine fish is very dependent upon the size and the time and location of release. By varying release protocols Leber and Lee (1997) increased the proportion of released striped mullet (*Mugil cephalus*) in the wild population after 10 months from 3% to over 50%.

Although age-based natural mortality rates for SBT from 1 year old and older have been estimated experimentally (Polacheck, Hearn, Millar and Stanley, 1998) the natural mortality from post metamorphosis to one year old is unknown and open to conjecture. SBT larvae have been estimated to have a natural mortality rate of 0.66 a day, and fish from post-metamorphosis to one year old will have a mortality rate somewhere between this, and the rate of 0.35 yr^{-1} estimated by Polacheck, Hearn, Millar and Stanley (1998) for one year old fish. It is most likely that the mortality rate drops dramatically with metamorphosis at about 20 days post-hatch and rapidly declines further as they grow up to 30 cm long during the next 80 days or so. An arbitrary estimate can be made from fitting a second order polynomial regression of natural mortality values against age. This produces a statistically significant fit to the values ($p < .0001$) and the M for 0+ fish estimated from its value at the intercept (t=0) is 0.389.

Information on comparative natural mortality rates for hatchery-bred releases of tuna versus wild bred fish are absent. However Kristiansen, Ottera and Svasand (2000) have shown that the survival of releases of hatchery bred cod (*Gadus morhua*) over their first three months of release, can be raised from less than 2% up to 70%, by releasing them at a larger size (a mortality coefficient of 1.2 yr^{-1}). It is likely that similar improvements could be realised by an appropriate study on release strategy for SBT. Adult cod have a natural mortality coefficient of about 0.2, similar to that of 6 year old SBT so a second estimate of the natural mortality of released SBT in their first year could similar to this figure, 1.2 yr^{-1}.

Notwithstanding all the above, it is likely that the natural mortality rates of released fish may be an order of magnitude greater that these estimates, so a third estimate of M is made here by multiplying the "best" estimate from extrapolation of published figures by 10. This gives a value of M at 0+ =3.89 yr^{-1}.

Applying these 3 rates to the restocked fingerlings and under the assumptions given above, it is estimated that somewhere between 800,000 and 25,600,000 fish would need to be released as fingerlings if the fish currently taken by Australian farmers are to be replaced by stock enhancement (Box 1) The differences in

numbers are due entirely to the assumptions made about natural mortality rates in the first year of release.

Box 1. Stock Enhancement: Numbers of Fingerlings needed to replace the Australian Quota

Global TAC	11,750 tonnes	Australian Quota	5,265 tonnes
Weight at capture for farming	22 kg	Age at capture for farming	3 .5 years old
Number of 22 kg fish potentially caught for farming			239,318

Age of SBT	Three scenarios of estimated natural mortality rates			% of fish remaining alive after release			Numbers needed at each age to replace quota		
	M[1]	M[2]	M[3]	M[1]	M[2]	M[3]	M[1]	M[2]	M[3]
Age 0+	0.389	1.200	3.890				772,624	1,738,527	25,611,409
Age 1+	0.350	0.350	0.350	67.8%	30.1%	2.5%	523,634	523,634	523,634
Age 2+	0.308	0.308	0.308	47.8%	21.2%	1.4%	368,999	368,999	368,999
Age 3+	0.250	0.250	0.250	35.1%	15.6%	1.1%	271,183	271,183	271,183
Age 3.5	0.250	0.250	0.250	30.1%	13.8%	0.9%	239,318	239,318	239,318

[1]The age based instantaneous natural and fishing mortality coefficients for SBT juveniles from 1+ are given in Polacheck , Hearn, Millar and Stanley, 1998. A second order polynomial regression of natural mortality values against age, produces a statistically significant fit to the values ($p < .0001$) and the value of M for 0+ fish was estimated here from its value at the intercept (t=0). This estimate assumes the natural mortality for hatchery fingerlings is the same as for wild-bred individuals. [2]This estimate replaces the natural mortality coefficient to that of hatchery released cod in their first year given by Kristiansen, Ottera and Svasand, 2000. Adult cod have a natural mortality coefficient of about 0.2, similar to that of 6 year old SBT. [3]This estimate increases the natural mortality rate of 0+ released fish estimated under scenario [1] by a factor of 10.

If marketed, and under the assumption that the quota lease value is $A15 a kilo, the theoretical lease value of these 22 kg fish is about $A79 million, (the lease value per kilo multiplied by their combined weight of 5,265 tonnes). However if the propagated

tonnage was additional to the current wild quota, there would in all likelihood be a fall in quota value of about one half to $A8 a kilo. This would place a value of $42.1 million on the lease value of the quota.

So to break even, the restocking program will need to produce and release enough fingerlings to produce about 239,000 extra 3.5 year old fish for less than about $A42 million. Under the most optimistic scenario above (M at 0+ = 0.389) the production and release costs per fingerling would have to be less than $54. Under the worst scenario (M at 0+ = 3.89) the cost per fingerling would have to be less than $1.64. In reality, the natural mortality rate of these fish in their first year is likely to be higher than that of wild reared individuals. It is unlikely however, that if properly researched release strategies are used, and fish held until they were 10-15 cm long before release, this would be higher than that identified for wild releases of hatchery reared cod (adult cod have a similar natural mortality rate to that of 6 years old SBT). These initial figures are promising.

To determine the level to which stock enhancement increases the size of the wild population, the released animals will have to be recognisable, for instance by DNA fingerprinting, their genetic constitution must be compared with, and kept the same as that of the wild population, and a continuing program will be needed to measure the extent to which the hatchery animals are increasing the size of each year class in the population. All these activities should be costed into the price of the fingerlings.

Increased farm production

The current marketing activity produces a high value sashimi quality product that is supplied either fresh or deep frozen for the Japanese market. If the current production is doubled, this is not likely to make a major impact on worldwide consumption of fresh and frozen sashimi, as this is about 400,000 tonnes, of which 300,000 tonnes is used in Japan. However, the about 30,000 tonnes premium sashimi market occurs mostly in Japan and SBT from both farmed and wild caught sources represents about 14,000 tonnes of this. The currently farmed product from Australia makes up about 50% of this market. Substantial increases of product into this market are likely to produce significant falls in the price of product.

The cost of leasing quota is between $A10-$A20 per kg, and wild fish are caught at about 22 kg, grown on for 4-6 months, then harvested for the Japanese market at about 32 kg at prices of up to $A40 a kilo. At this capture size, the present quota enables about 240,000 fish to be caught for ongrowing and marketed at 32 kg producing about 8,000 tonnes of product a year.

A simulation of the growth and cost of feed for 10,000 SBT from fingerling to 3.5 years and 4 years old is presented in Box 2. This simulation uses cage mortality rates of 12%, and 20% per year, and uses equations derived from literature values of wild growth rates and weight/length relationships. The simulation suggests that at a cage mortality of 12% there should be about 6,400 fish remaining at 3.5 years and 6,000 at 4 years old, each with a weight of 23.6 kg and 27.3kg, respectively. At a cage mortality of 20% there should be about 4,592 and 4,056 remaining of similar mean weights. Irrespective of the size of the farm mortality rate (12% or 20%) or the age of the fish (3.5 yr, 4 yr), the feed cost per kilo of live

fish remaining was similar, and differed in a large way only between food conversion rates. So to equal a presumed future cost of quota of about $A8 per kilo, the feed conversion ratios (FCR) of fish will need to be between 1:10 and 1:15. Current ratios based on baitfish are around 1:16. Formulated feeds for other species have food conversion ratios much better than this. If a 1:15 ratio is achieved, then overall feeding cost at current rates to achieve a 23.6 kg fish will be about $A18 a kg.

These simulations suggest that farming SBT from fingerlings is not only financially feasible, but is also likely to be profitable even with the present food conversion ratios and prices.

The major source of farm feed is frozen baitfish, principally pilchards (Thorpe, Van Landeghem, Hogan, and Holland, 1997). About 45-55,000 tonnes of baitfish are used each year to produce a weight gain of about 3,000 tonnes of SBT from an initial wild caught quota of about 5,000 tonnes. The food conversion ratio (FCR) for baitfish is not good, and there is a potential problem with the nutritional quality of the baitfish and environmental degradation due to waste products. A considerable proportion of the baitfish comes from overseas. In 1997, 50% was sourced this way and in 2001 around 85% is likely to be imported (B. Jeffriess *pers com*). The possibility of introduction of disease with their importation is an issue which will not go away. Recent wet feed FCRs have achieved better than 1:15, and attempts to produce a soft pellet formulated diet are now achieving a level of success, with improved moist pellets achieving a FCR of around 1:10.

Box 2. *Increased farm production of SBT- some costs*

Current Prices & Costs

Weight of Australian Quota	5,265 tonnes yr^{-1}	Nominal lease value of Quota	\$A15.00 kg^{-1}
Weight of Marketed Fish	8,000 tonnes yr^{-1}	Nominal market Value of Product	\$A40.00 kg^{-1}
Volume of feed used	50,000 tonnes yr^{-1}		

Simulation of Costs of feed and value of fish from fingerlings to farm size

Initial number of fingerlings	10,000	Nominal Cost of Feed	\$A1.2 kg^{-1}
Assumed mortality rate in cages	12% and 20% yr^{-1}	[1]Nominal Cost of fingerlings	\$A2.7 each
Estimated growth rate of fish from fingerlings to 3.5 years old	[2]Length (cm)= 11.55 + 0.113xdays- 0.00003088xdays^2		
Estimated weight/length relationship in fish from fingerlings to 3.5 years old	[3]Weight(kg)=0.0000313087xlength(cm)$^{2.9058}$		

	As fingerlings	As 3.5 years old fish	As 4 years old fish
Mean length of fish	11.55 cm	105.4 cm	110.7 cm
Mean weight of fish	0.038 kg	23.6 kg	27.3 kg
Number of fish remaining at 12% Farm Mortality	10,000	6,404	5,998
Total Weight of fish at 12% Farm Mortality	383 kg	151,198 kg	163,534 kg
Number of fish remaining at 20% Farm Mortality	10,000	4,592	4,096
Total Weight of fish at 20% Farm Mortality	383 kg	108,425 kg	111,681 kg

Feed Conversion Ratio	1:2	1:5	1:10	1:15	1:18
Lifetime feed costs for 3.5 years old fish 12% farm mortality					
Total Weight of Feed	301,629 kg	754,073 kg	1,508,147 kg	2,262,220 kg	2,714,664 kg
Cost of Feed	\$A361,995	\$A904,888	\$A1,809,776	\$A2,714,664	\$3,257,597
Feed costs kg^{-1} of fish	\$A2.57	\$A6.16	\$A12.15	\$A18.13	\$A21.72
Lifetime feed costs for 3.5 years old fish 20% farm mortality					
Total Weight of Feed	216,084 kg	540,211 kg	1,080,422 kg	1,620,634 kg	1,944,760 kg
Cost of Feed	\$259,301	\$648,253	\$1,296,507	\$1,944,760	\$2,333,713
Feed costs kg^{-1} of fish	\$2.39	\$5.98	\$11.96	\$17.94	\$21.52
Lifetime feed costs for 4 years old fish 12% farm mortality					
Total Weight of Feed	326,302 kg	815,755 kg	1,631,510 kg	2,447,266 kg	2,936,719 kg
Cost of Feed	\$A 391,562	\$A978,906	\$A1,957,812	\$A2,936,719	\$A3,524,062
Feed costs kg^{-1} of fish	\$A2.39	\$A5.99	\$A11.97	\$A17.96	\$A21.55
Lifetime feed costs for 4 years old fish 20% farm mortality					
Total Weight of Feed	222,596 kg	556,490 kg	1,112,980 kg	1,669,470 kg	2,003,364 kg
Cost of Feed	\$A267,115	\$A667,788	\$A1,335,576	\$A2,003,364	\$A2,404,037
Feed costs kg^{-1} of fish	\$A2.39	\$A5.98	\$A11.96	\$A17.94	\$A21.53

Total value of fish	Reported Food Conversion Ratios
Present SBT wet fish diet	1:18
[4]Experimental SBT Diet	1:15
[5]Freshwater trout	1:1.6
[6]Cichlids	1:0.6-1.2

[1]The target cost of fingerlings is estimated from the financial projections given in this strategy. [2]This growth rate equation for fish between fingerling and 4 years old was derived by fitting a second order polynomial ($p<.004$) to data of length at age for fish at 1-4 years old in Gunn, Davis, Farley, Clear, and Haskard, 1996. Age 0 (fingerling) was arbitrarily defined as 10 cm long in the derivation of this curve. [3]This weight/length relationship is that of Robins, 1963 which was derived for fish between 40 and 110 cm long. [4] Buchanan, 2000 [5] O'Sullivan, 1991. [6]Martinez-Palacios and Ross, 1996

While existing farms are all in the vicinity of Port Lincoln, South Australia, the propagation of SBT and enlargement of farming will become a reality only if additional water suitable for this activity is identified and zoned. This need not be in close proximity to existing farms, and suitable water could be identified as far away as the south-west coast of Western Australia, or even Northern Tasmania, provided the water temperatures were adequate.

Risks

The present SBT farmers have now successfully fattened SBT for many years and are aware of the difficulties with doing so. However most of the other activities needed to grow fingerlings are new ventures and as such must be considered speculative.

Although the implementation of this Strategy must initially be considered as a high risk venture, the degree of risk and uncertainty has been reduced by suggesting research to reduce the risk of failure. Many of the risks are common to aquaculture ventures worldwide, but some are specific to the propagation of SBT in captivity.

Potential risks and their minimisation include:

Risk	Risk Minimisation
1. Inadequate capital investment	Split capital investment into a) initial capital raising, b) ongoing levy based or similar, c) second capital raising after egg production.
2. Disagreements between partners	Incorporated company board with independent chairman , expertise-based directors, and grouped shareholder representational directors.
3. Delays in starting the project	Activities follow sequentially, no commitment to activity before identification and acquisition of finance. Agreement to R&D Plan before any R&D activity.
4. R&D Plan is inadequately managed	Financial provision made for a specialist high calibre R&D manager as CEO of the company.
5. Inadequate numbers of quality research scientists available to do the work	Contract research means that the best scientists can be targeted for the work. Financial provision has been made for importing expertise from overseas if not available in Australia.
6. Inadequate site and facilities	Site and facilities be predicated on the best location, not existing facilities.
7. Incapacity to hold adult SBT in land-based tanks	Best size holding tanks, must be based on known behaviour of SBT in cages, the biomass of fish per cubic meter successful in other species of tuna, and current

	practice with related marine pelagic fish species (yellowtail kingfish).
8. Failure to induce maturity and spawning	Acclimatisation of SBT to land-based tanks will allow for the use of all the currently successful methods of inducing maturity and spawning used in other marine fish species.
9. Inadequate quality eggs produced	A staged R&D Plan will mean that if inadequate quality eggs are produced, then the other parts of the R&D plan are left in abeyance until success. The use of 4 land-based tanks rather than sea-based nets will enable experiments to be carried out on diet and environmental stimuli to produce quality eggs.
10. Disease-induced mortalities	Diseases of juvenile and adult SBT are currently under investigation at the Aquaculture CRC and are part of the activities proposed for the Finfish Aquaculture CRC. A research objective on sterilisation of eggs will seek to minimise the possibility of disease in hatcheries.
11. Incapacity to transport eggs or larvae to remote laboratories	A research objective has been designed to address this topic.
12. Inadequate number of experimental tanks available to R&D contactors	Contract research will allow several research laboratories to apply their facilities to the work simultaneously.
13. Inadequate supplies of larval feeds	It is unlikely that supplies will run out in the R&D stage. Research into larval, post metamorphosis and maturational diets will enable the R&D entity to hold the IP needed for producing these diets.
14. Incapacity to grow larvae to metamorphosis	Research objectives related to nutrition and environment will address this issue.
15. Inadequate post-metamorphosis diet	Research objectives related to nutrition and environment will address this issue.
16. Incapacity to grow to fingerlings	Research objectives related to nutrition and environment will address this issue.
17. Incapacity to produce sufficient numbers of fingerlings	Commercial nursery expansion after the R&D stages will allow accurate projection of needs after the research has been done, and a capacity for building appropriate facilities.
18. Market failure reduces the price for SBT	Simulations in this Strategy suggest that there will have to be a significant market

failure to make the production of fingerlings uneconomic.

Options for managing the R&D

Substantial finance for the research and development activities will be needed. Potential sources of some of this have been identified, however the form of contribution varies with the source. These contributions will have to be harmonised and managed as an entity. Potential sources of initial finance identified include:

- State Government funds for capital works
- Research staff as "in kind" funds
- Industry levies currently passed on to FRDC
- Leverage on FRDC from industry levies
- Leverage on a CRC from FRDC funds from industry levies
- Industry facilities as "in kind" funds
- Direct funding through individual farming companies
- Foreign and Commonwealth government funding through the CCSBT process.
- Commonwealth Government stand alone finance from CCSBT
- Leverage on industry contributions to a CRC
- Individual foreign companies or groups
- Venture Capital

The diversity of these sources of income could produce a management nightmare for the implementation of a research and development plan. Formal agreements must be negotiated between each of the potential funding sources in regard to the uses to which the funds are to be put and ownership of the outcomes. Moreover, there will need to be a seamless passage from the initial research and development activities to the commercial production of fingerlings.

Staff from a number of government research agencies have expressed an interest in becoming involved in the research of the project. In the past, to keep ahead of competitors, some agencies have tried to "own" research areas, and have often been difficult to negotiate with in regard to ownership of the intellectual property generated. Almost all government research agencies are also strongly influenced by political policies, which at the State level, can mean that financial contributions may only be spent in that state, or that the principal research and development expenditure should be done in that State.

Informal collaboration between industry and government scientists are often hailed as productive, however many attempts at this type of collaboration have usually been restricted to the provision of "in kind" industrial facilities which are provided when they are not needed for commercial activities. This approach is unwise as facilities can be withdrawn with a minimum notice if the commercial activities warrant it. The commercial partners may also become disillusioned with the collaboration when the activities of the scientists are seen as irrelevant or not communicated well.

The R&D plan could be implemented by requesting FRDC or the new finfish aquaculture CRC, to subsume management control of the project. It is possible for

FRDC under its act to manage the research and development and even the commercialisation of results under its subprogram structure. However its role to date has been primarily that of a research funder and coordinator involved with organising the oversight and synthesis of research. To become involved with commercialisation and the running of a commercial broodstock and hatchery facility would require a major policy change from current practice.

The finfish aquaculture CRC will be responsible for managing research, and will have a commercial structure which could facilitate its management of this project. However SBT will be only a part of its activities and it is unlikely that commercial production of fingerlings would fit within its mandate. Moreover, because its board may well be constituted on a representational basis across the entire fin fish aquaculture and research sectors, the emphasis on the propagation of SBT would be only one of a number of priorities for the CRC.

TBOAA has experience of managing SBT research, and itself could possibly manage the project. It is intimately involved with current ongoing SBT research, and is well aware of industry priorities, e.g. propagation competing with a high cost pellet formulation project and a medium cost health and environmental projects. However in view of the possibility of financial contributions from third parties, and governments, it is hard to envisage the association with sufficient capacity for their interests to be heard and accommodated without large changes in its constitution.

One company (The Stehr Group) has voiced an intention of pursuing the captive propagation of SBT. The company is currently engaged in discussions with the CRC for Finfish Aquaculture about a collaborative agreement incorporating an experimental hatchery at Arno Bay, South Australia. The company has retained fish collected from the Great Australian Bight in the 19989/99 and 1999/00 seasons, with the intention of rearing these for broodstock. The company has also made a number of business proposals to both the ATBOA and the CRC.

A Limited Liability Proprietary Company

Because of the potentially wide range of participation, it is difficult to conceive of any management structure other than that of an independent private business entity for implementing the business and research and development plans for the propagation of SBT, and to own the real and intellectual property generated. The proposed entity would be similar to that of SALTAS, however in this case it is not proposed that the government holds a majority shareholding, but ownership of shares be based upon the respective equity in the company. Because of the diversity of participants, and differences in their capacity to contribute, it is only by setting up an incorporated business entity that suitable financial and business jurisprudence and continuity can be ensured. It will own such capital or intellectual property that is generated, subject to such commercial agreements that may be negotiated, and will be responsible for the implementation of the business and research and development plans.

Objectives and Structure

The objectives of the company are to:

1. Acquire capital and operating finance to establish and manage an agreed research and development program for the propagation of the SBT
2. Establish and manage a hatchery for commercial propagation of SBT for fingerling production.

Participation in the company should be by the ownership of shares. These should be allotted to shareholders on the basis of their direct contribution of financial resources to the company, and not include "in kind" contributions. Shares may be held by individuals, companies, or nominees of associations, or government agencies. The company should be administered by a Board of Management with an independent Chairman selected by the Board itself. The company should be governed by a board of not more than 10 directors, including the managing director. Apart from the Managing Director and the Chairman, the board of directors should be elected by a general meeting of the shareholders. Directors should be selected by the shareholders on the basis of their understanding of a number of issues. These should include a knowledge of the duties and responsibilities of directors in the Corporations Law environment, their ability to read and understand financial statements, a working knowledge of the production and market for fin-fish fingerlings, a knowledge and understanding of the aquaculture research and development environment, a knowledge of the international aspects of the fishery and markets for SBT. To ensure that the Board of Management is not dominated by the 'big players' it may be advisable to divide the shareholders into, say 3 groups, each with the right to nominate directors to represent them. One such a division could be 2 directors for large industry / venture capital, 3 directors for small industry, and 3 directors for public investors such as FRDC, AFFA or the states. The exact basis for representation would have to be negotiated during the setting up of the company.

Equity

During the process of consultation, a number of options to finance the project were canvassed. Representatives from at least two state governments (WA and SA) suggested that if the project was based in their state, it was likely that it would attract substantial amounts of state government finance. To attract public funds in this way the project should have a substantial degree of public good as well as private benefit attached to it. In practice, the public and private benefit pertaining to a project such as this are inextricably linked. The enhancement of wild stocks of SBT produces a public good aspect that flows from the commonwealth government's responsibility to maintain fisheries resources which are shared between the fishing industry, recreational fishers and the community at large. Moreover, in investing public funds for this purpose, governments are supporting international conventions and agreements on the sustainable use of fish species which straddle jurisdictions, or are highly migratory. In supporting R&D to develop generic technologies for the aquaculture of SBT, government funds are facilitating the development of industry in rural Australia, the provision of jobs and economic development, and the use of sustainable aquaculture practice.

Most of the current tuna farmers suggested that a quota-based levy would be their preferred method of financing the project. It was also made clear that if levies were collected for this purpose, then FRDC would be in a position to provide matching funds. A number of company representatives suggested that they would prefer to make an investment directly into the project, and thereby gain more control over its activities. There was a strong view that the benefits of the R&D should not be restricted to a few initial investors and there should be an opportunity for late comers to participate. While overseas contributions were not excluded, there was a desire to keep control of the project and its results in Australia.

It is therefore proposed that the initial equity in the company consist of up front capital investment by private industry investors with options for additional government support. This to be augmented by an ongoing contribution which could be levy-based, or alternatively funded, for at least 6 years. In the fourth year of the project it is anticipated that an income flow will start to be generated from the sale of fingerlings. If required, a new share issue may be contemplated after eggs have been successfully produced. It is anticipated that the company will become financially self-sufficient from fingerling sales after commercial production comes on stream.

The provision of "in kind" support to the activities of the company should not entitle the provider to participation in the ownership of the company, but may be the subject of separate arrangements between the company and the third party.

Ownership & Management of IP

It is intended that the intellectual property (IP) generated by the company during the process of R&D shall be owned by the company, and used for the welfare of its shareholders. However, while IP will no doubt be generated, it is open to question whether much of it is capable of protection, raising doubts as to whether it can even be considered property. Two important products from the R&D process will be the generation of domesticated livestock, and "know how". Whilst the latter can be transferred by the process of staff "poaching", the domesticated livestock will be held on site and will be the exclusive property of the company. The benefits of this IP can be distributed to shareholders by way of entitlements to purchase fingerling production, or from the proceeds of fingerling sales to third parties. Possession of shares can give the owner an entitlement to purchase a specific share in the production of SBT fingerlings from the hatchery after its establishment.

So while some of the IP produced by the R&D activities of the company will be private in nature and therefore capable of protection and commercialisation, other IP will be difficult to protect and of public benefit. Indeed there would be little purpose for government involvement in the project if there were to be no public benefit. Examples of public benefit where IP would be difficult to protect and commercialise include:

- Broodstock capture
- Broodstock transport and sedation protocols
- Broodstock holding facilities
- Broodstock management, including health monitoring and management

- Larval fish tank design
- Post-metamorphosis fish management protocols, including design of optimum holding facility design
- Larval fish transport protocols.

Examples of IP which may be protected and of commercial value include:

- Broodstock maturational diet
- Egg management protocols for fertilisation and health maintenance
- Larval fish management protocols
- larval and post-metamorphosis feed formulation

These two categories of IP would need to be identified and agreed before any R&D activity started. In this way public and private investors would be clear about the products they were investing in, and the likely benefits from their investment.

The protection of IP need not be an insurmountable problem to the successful propagation of SBT. Similar difficulties were present at the start of salmon farming in Norway, Scotland, and Canada and yet it is clearly apparent that despite the lack of clearly definable ownership of IP, the experience gained by being 'first off the rank' enabled those farming companies to ensure large market shares and efficiencies which remain today, 40 years after the first R&D started.

At a later stage the process of hatchery production of SBT could be licensed or sold to other companies. This could include the sale or licence to use, the company's broodstock or egg production.

Management of Research & Development

The Company will initially play a major role in the R & D program. As these activities are completed, its activities will change to become a commercial provider of fingerling SBT for the market. The two activities will require different roles and skills. The management of R & D will encompass work that is done by external providers, and also work done "in house". In commissioning R & D, the company will need to follow an effective process to ensure that money is not wasted. The process should include a) the production of a register of R&D providers from which to invite tenders, b) the specification of outcomes required from each tender, including the confidence levels acceptable from the results, c) the review and management of the contracted research, including an evaluation of the completion of milestones, and the timeliness and cost-effectiveness of the research. The company need not employ its own staff for this purpose, it may choose to contract out the process to an expert independent panel, however, it should employ and use at least one officer of its own with a detailed knowledge of R & D. Research contracts should be audited after completion, and evaluated for effectiveness against efficiency criteria

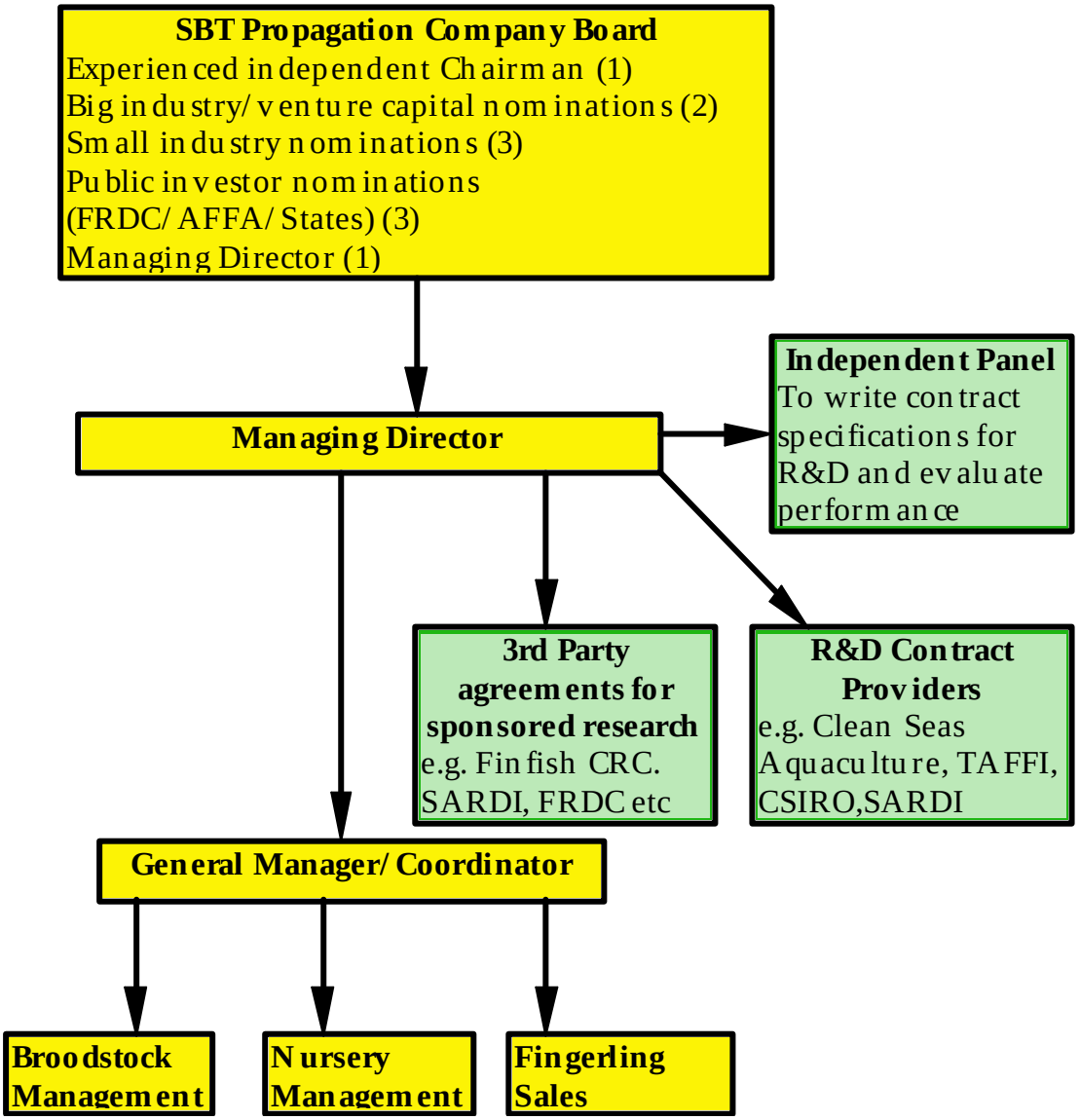
"In house" research and development should be restricted to that which cannot be contracted out. It will consist mostly of work done on the acclimatisation of broodstock, their maturation and spawning. The work need not be done by employees of the company. Staff used for this purpose can be seconded staff or collaborators. The company may find it useful to consider itself as a provider of research facilities, and commission the R & D broodstock work by letting research

contracts. Research on specific topics could either be contracted across several R&D providers simultaneously to maximise capital facilities and increase replication in experiments, or specific parts of the research could be contracted to individual specialist R&D providers. Although the first of these approaches has been taken in this strategy, both have merit, and a final choice would be best decided at the time of letting the contracts.

As the R&D matures, the company should develop its broodstock holding facility, and run full scale commercial trials to raise eggs, larvae, and fingerlings. When the trials are successful, the company will need to expand its production to meet demand, and move into a phase of fingerling production. At this stage, the staff activities and structure of the nursery facility will need to change, moving from R & D into full scale commercial production.

It is not envisaged that the company will employ many staff during the R & D phase. There is a need for a CEO and administrative assistant to oversee the running of the company and initiate its contracted work. The core activities of the broodstock facility and nursery should employ about 8-9 staff. A general manager/coordinator, 2 for broodstock and one each for nursery, algal feeds, rotifers, larval rearing and *Artemia*, and maintenance. The requirement for staff in addition to this will very much depend upon the work load, and amount of R & D performed "in House", and the degree to which the process of letting research tenders is contracted out.

A corporate organisational chart for the SBT Propagation Company might look similar to :



PART 4 THE RESEARCH AND DEVELOPMENT PLAN

Introduction

In preparing a Business strategy for the propagation of SBT in captivity to provide fingerlings in Australia for stock enhancement and farming it was necessary to identify a R&D plan which would be at the heart of the business activity until the primary research phase was completed.

In developing the plan, the views of research workers, industry and managers involved with finfish aquaculture and SBT were canvassed, and the literature extensively reviewed. There will always be more than one view on the best way to achieve some of these objectives, and not everyone will agree with all the ways presented below. However this present Plan is a reasoned summary of the consultant's views of the best options on how to propagate SBT to fingerlings in captivity

There has been a suggestion that because of the costs and difficulties with obtaining SBT, then it would be useful to start work on yellowfin tuna as a surrogate species for SBT. Yellowfin tuna has already been grown in captivity overseas, and the details of that work could relatively easily be obtained from those involved. Yellowfin tuna is a tropical species with very different life history characteristics from SBT. While there is a case for farming yellowfin tuna in Australia, and a need to find the best way of doing so, this should be argued on its own merits. Spending time and money on more yellowfin tuna research because of perceived difficulties with SBT will only indirectly assist the propagation of SBT, and could easily divert resources from SBT to yellowfin research.

The Plan is not an exhaustive blueprint for every step in the process. It does not give an extensive literature review of each objective. Some relevant literature is included, more is provided in Appendix I- Scientific Review of Published and Available Unpublished Work on the Propagation of Tuna and Similar Species. Nor does the plan try to give a detailed breakdown of the costs of each activity. Rather it gives the consultant's view on the quickest and most cost-effective way of proceeding, and the indicative costs of doing so.

If the plan is to proceed further there will be a need for specialist research workers with in depth knowledge of the problems and logistics involved with each objective to build upon the overview given in this document. Most of this would best be done as part of the design and letting of research contracts by the expert panel consulting to the MD.

The consultant's brief did not include aspects of the business relating to the ongrowing from fingerling to market. Nor did it include the research and activities needed for a successful stock enhancement program. The present plan outlines ways of:

- Capturing, holding and bringing to maturity and spawning adult SBT,
- Treating and transporting eggs,
- Determining the food and environmental needs of larvae and

- Determining the best feed and environmental conditions for growing post metamorphosis larvae to fingerlings.

Significant research aspects which have been omitted from this strategy, and which will need to be investigated before either stock enhancement or farming from fingerlings can be successful include;

- Genetics research
- Strategy for reseeding
- Methods of rearing of fish on farms from fingerlings to 3 year old fish
- Research into disease prevention

Some aspects of these are currently under investigation in Australia.

Costs associated with the R&D Plan are the best available to the consultant at the time. Some of these could be reduced by sharing equipment e.g. with the TBOAA experimental farm or the CRC. However the present plan assumes that none of these savings are currently available. Some of the costs may escalate, for instance the cost of land for the broodstock facility will depend on where is situated, and hatchery costs will need to be more closely examined, depending upon the numbers of larvae needed. All these variations to the cost structure should be investigated at the time of implementation of the Plan.

1. **Primary Broodstock Development**

Propagation of marine fish species under aquaculture can only proceed after domestication of initially wild-caught animals. These will usually be mature fish, collected during maturation, and these are then induced to spawn in captivity by the manipulation of one or several of photoperiod, temperature and hormones.

Large and highly active pelagic species such as the SBT present formidable challenges to achieve even the capture and successful holding of mature individuals. To this must be added the difficulty of monitoring their breeding condition prior to maturation, spawning and the production of viable eggs.

Objective 1.1 ***Identification and Capture of Wild Fish***

Background:

Maturing SBT are found in the southern fishing grounds (40-50°S) off the southeast and south of Australia, but spawning does not take place in these waters. These individuals all have small gonads and it is believed that they move to an area in the southern Indian Ocean based at about 20-30°S, 90-110°E. where maturation continues, thereafter moving northwards to spawn between the Sunda Islands and Australia about 7-20°S, 102-124°E. (Davis, Lyne, and Jenkins, 1991). The spent fish then move south with the Leeuwin current, at least some of them reappearing in the Tasman Sea from November onwards.

Mature spawning fish are caught with long lines by the Indonesian fishery (Davis, Bahar, and Farley, 1995). They spawn in tropical water, possibly within 1 metre of the surface (T. Davis *pers com*), however they are usually caught between 50 and 220m depth and even down to 350m. Water temperatures in this region in January are around 27-29°C on the surface, 25°C at 50°m, 13-17°C at 200m and 10°C at 350m (Davis and Clementson, 1989). Mature but unripe adults are also caught by the long line fishery off New Zealand, south of Tasmania, and in the Southern Indian Ocean (Farley and Davis, 1998). Fish caught in these locations are up to 150 kg weight (T. Santic *pers com*) and in these grounds they are fished between 40-160m depth in water temperatures of 10-12°C. Data retrieved from archival tags suggests that they are capable of, and frequently do, move vertically through all these water depths during the course of their daily swimming activity (J. Gunn *pers com*).

Adults could be collected from long lines either from the spawning grounds south of the Sunda Islands, or from the southern feeding grounds south west of Tasmania and held in the currently used towing cages until sufficient numbers are caught. However the catch rates from the spawning grounds is only about 1 individual per 10,000 hooks, (T. Davis *pers com*). This fishery is principally for yellowfin and bigeye tuna, so targeted fishing for SBT may produce a better catch rate. The catch statistics from Japanese operations targeting SBT suggest that in the areas currently fished (which does not include the spawning grounds)

although the catch rates of 8-20 years old fish is better than the Indonesian catch, it is still below 2 fish per 1,000 hooks set (Polacheck and Bailey, 1998).

Indonesian longlines are between 27 and 50 km in length, setting between 700 and 2,000 hooks, depending on the vessel and method used (Davis, Bahar and Farley, 1995). Japanese longliners generally set between 2-3,500 hooks in a single set up to 80 km long and when longlines are used it is normal to make one set per day when fishing. This means that on average below about 4 individual SBT are likely to be caught per set in either location. To obtain 400 mature adults from long lines would require about 100 days of fishing even under the most optimistic scenario (ie. all fish caught are alive and mature).

Because of the length of the long lines, it would not be feasible to hold and bring the fish to a moored holding cage. This would have to be towed beside the catching vessel while it was retrieving the long line. Under current technology the maximum speed the holding net could be towed is about 1 knot, which is far below the speed that the line boat would need to go to retrieve the long line. Collection of spawning fish from the northern spawning grounds is also impracticable because of the high temperatures of the surface waters there. Captured adults are unlikely to have the capacity to live in 27°C water for any length of time, for apart from spawning time, the species is usually found in temperate waters. Whilst on the spawning grounds the species is caught in the cooler deeper waters from 50-220 m depth where it is likely to be spending most of its time.

Apart from all the above, it is likely that the trauma of capture and transportation would produce gonadial atresia in these fish so they could not be used as broodstock until the next spawning season in one year's time.

Schools of juvenile SBT, occur in surface waters of the Great Australian Bight where they are caught by encirclement and held in modified purse seine nets by the present SBT farmers. Thereafter they are towed considerable distances after which they are transferred to large static open water sea cages at Port Lincoln for ongrowing for 4-6 months (Lee, 1998).

Seven year old fish which had been previously caught this way as two years old from the Great Australian Bight and grown in net cages, showed signs of the onset of maturity when autopsied after they died in a storm event. Sections of the ovaries of some of the females (about 147 cm long & greater than 80 kg in weight) showed perivitellogenic oocytes (P.C. Young *pers obs*), and ovarian weights had reached over 0.3 kg in 8 out of 17 females autopsied (Table 1).

Table 1 Length, total weight and gonad weight of 7 year old SBT in net net cages. (Data provided by K.M. Rough, TBOAA)				
Autopsy Date	Sex	Total Weight (kg)	Length (m)	Gonad weight kg)
23/4/96	Female	68.30	1.410	0.420
26/4/96	Female	77.30	1.570	0.375
22/4/96	Female	82.10	1.520	0.356
23/4/96	Female	73.35	1.430	0.332
23/4/00	Female	80.15	1.470	0.327
25/4/96	Female	66.40	1.430	0.322
26/4/96	Female	66.00	1.520	0.319
22/4/96	Female	87.00	1.580	0.308
25/4/96	Female	75.30	1.450	0.280
26/4/96	Female	76.00	1.600	0.270
22/4/96	female	70.25	1.460	0.262
23/4/96	Female	72.15	1.520	0.262
25/4/96	Female	67.00	1.450	0.255
23/4/96	Female	79.35	1.490	0.237
26/4/96	Female	67.60	1.490	0.209
26/4/96	Female	66.80	1.540	0.180
26/4/96	Female	52.30	1.380	0.138
23/4/96	Male	90.70	1.530	0.467
24/4/96	Male	81.92	1.600	0.295
23/4/96	Male	91.10	1.500	0.211
23/4/96	Male	95.00	1.570	0.208
24/4/96	Male	68.49	1.500	0.194
26/4/96	Male	93.40	1.630	0.177
22/4/96	Male	51.40	1.330	0.175
26/4/96	Male	87.10	1.620	0.159
26/4/96	Male	82.10	1.620	0.153
26/4/96	Male	74.30	1.560	0.150

The testes of males showed crypts of spermatogonia, and some development of spermatocytes was present in at least one male (*P C Young pers obs*). Male gonads were smaller than those of the females, reaching over 0.3 kg in only 1 of 12 males. These fish died in April at a time when the water temperature (18°C) was declining. These fish were of a size and gonad development that, with the right environmental conditions and diet, some might have been expected to have become ripe in the next spawning season when they were eight years old.

While most fish that are caught and held by this method are around 15-20 kg, the odd fish is caught at 60-70 kg. When they occur, bigger fish (about 40 kg) are usually deeper, with the smaller (15-20 kg) fish swimming above them (J. Nelligan *pers com*). These larger 40 kg fish would be 6-7 years old. They could be caught by targeting them during the process of capture, or by selecting larger individuals from fish captured by the fleet.

Despite the difficulties and cost associated with capture and holding fish from the Great Australian Bight, this is likely to be the most feasible way to obtain

broodstock. The difficulties of separating and handling large 40 kg fish from the smaller fish caught should not be underestimated as there is no current methodology in use to do so. Methods for handling and transporting mature fish will need to be developed as part of the research process.

Proposed Activities:

It is proposed that 6-7 year-old broodstock be caught as 40 kg fish from the Great Australian Bight. Fish will be caught by purse seining and transporting them to the broodstock holding facility by towing in the usual commercial way. Schools of larger fish may be targeted, or big fish could be selected from catches across the fleet. The fish must be collected and established in the broodstock holding facilities with minimal handling or stress. Handling cradles and sedatives must be developed to minimise trauma and physical damage (see 1.2 below).

Outcomes Required:

Capture and establishment in net cages of 400, 6-7 year old SBT.

Costs

Objective 1.1	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Identification and Capture of Wild Fish						
Capital Items						
2 Pontoons, nets, & mooring lines	$300,000					
200, then 100, 40 kg wild SBT fish from quota @ $15 kg-1	$120,000	$120,000	$60,000	$60,000	$60,000	$60,000
Net tender and feeding barge	$100,000					
2 Vehicles	$60,000					
Operating						
Costs of capture and transport of fish from wild to holding net cages	$25,000	$25,000	$12,500	$12,500	$12,500	$12,500
Feed costs per kg of average weight 40 kg fish at 7% body weight day-1	$245,280	$245,280	$245,280	$245,280	$245,280	$245,280
Running costs of vehicles and barge	$25,000	$25,000	$25,000	$25,000	$25,000	$25,000
Staff						
Managing Director	$120,000	$120,000	$120,000	$120,000	$120,000	$120,000
General Mngr / Coordinator	$22,500	$90,000	$90,000	$90,000	$90,000	$90,000
Maintenance and general assistance worker	$35,000	$35,000	$35,000	$35,000	$35,000	$35,000
2 Broodstock handlers	$90,000	$90,000	$90,000	$90,000	$90,000	$90,000
Administrative assistant	$45,000	$45,000	$45,000	$45,000	$45,000	$45,000
Total	$1,187,780	$795,280	$722,780	$722,780	$722,780	$722,780

Objective 1.2 ***The Handling and Sedation of Captured Fish***

Background

Juvenile northern bluefin tuna have been caught on troll lines, using barbless hooks and successfully transferred to net cages. Yellowfin tuna have also been caught at between 2-7 kg and transferred successfully into circular concrete tanks, (Lee, 1998). However relatively large 40 kg SBT present major problems in minimising physical and stress based trauma during handling for transportation between cages and onto shore facility.

Anaesthetics are primarily used to minimise stress during handling operations such as these and if used on fish not intended for human consumption, a range of anaesthetics may be used with varying effects. However anaesthetics can in themselves be very potent stressors, and the very different natures of chemicals in use as fish anaesthetics, as well as the differing responses of fish species to a particular anaesthetic emphasise the need to assess the effective dose for particular water quality, fish size and species, and the desired level of anaesthesia (Iwama, McGeer and Pawluk, 1988).

Solutions of 2.66 g sodium bicarbonate/L or 60 mg clove oil/L have been found to be optimal as anaesthetics for use in fresh water on walleyes salmon *Stizostedion vitreum* at 10°C. At these concentrations, complete immobilisation occurred in about 7 and 4 min respectively, and recovery occurred in about 5 and 10 min respectively, for sodium bicarbonate and clove oil. These concentrations were also found to be suitable for use on small-mouth bass, *Micropterus dolomieu*, northern pike, *Esox lucius*, and lake sturgeon, *Acipenser fulvescens*. Clove oil was recommended over sodium bicarbonate for surgical procedures, however, recovery took substantially longer when time under clove oil anaesthesia exceeded 5 min (Peake, 1998).

There is concern that because of the "ram jet" method of gill ventilation of tunas, the use of anaesthetics may not be possible with SBT due to asphyxia associated with anaesthesia. However low levels of MS-222 (0.1g per litre) have been used successfully to aesthetise skipjack tuna, *Katsuwonus pelamis* (Arthur, West, Brill, Schulte and Hochachka, 1992). Moreover, in a series of experiments designed to reduce pre-harvest stress, both carbon dioxide and Aqua-S™ (containing the active constituent of clove oil) were effective in anaesthetising SBT. Individual fish were observed to recover after anaesthesia, however, because the intention of the experiments was to reduce pre-harvest stress, the best concentrations for recovery were not determined (B. Goodrick, *pers com*).

Cradles have been used to hold juvenile SBT for tagging purposes, and in conjunction with anaesthetics, are used to handle broodstock of yellowtail kingfish, *Seriola grandis* (A. Tindale *pers com*). A portable hinged aluminium restraint cradle has been described for handling wild caught salmonids up to 130

cm fork length. Fish that were caught by hook and line were transferred from the hook to a 2.5 cm stretch mesh soft knotless nylon dip net, then rolled into the cradle which was suspended in the water This method was used successfully to process over hooked 400 salmon with only a 7% mortality (Larson, 1995).

Large fish are now commonly held in Oceanaria such as in Boston, Monterey and Tokyo, but the methodology used for handling them is not readily available in the literature. These sources of expertise should be investigated at an early stage in the research. If possible existing expertise should be brought to the project from where it is in use, rather than repeating work which has already been done elsewhere.

Proposed Activities:

A series of experiments will be conducted to investigate the use of anaesthetics on captive SBT. These experiments will use animals currently held at the experimental farm facilities. The aim of the research will be to determine a method of restraining the fish to enable the administration of anaesthesia, transport of the fish, the determination of the appropriate type and concentration of anaesthetic, and identification of a methodology to prevent post-anaesthetic trauma.

Outcomes Required:

1. A demonstrated capacity to anaesthetise and revive SBT under captive conditions

2. A demonstrated capacity to hold, transport and revive anaesthetised fish between net cages and from net cages to land-based tanks.

Costs

Objective 1.2 *The Handling and Sedation of Captured Fish*	Year 1
Operating	
Purchase of fish from farms for experiments	$10,000
Purchase of anaesthetics	$5,000
Development of fish handling and holding equipment and procedures	$10,000
Miscellaneous costs of equipment	$20,000
Travel	$10,000
Staff	
Fish anaesthetics and handling expert 6 months at $80,000 pa	$40,000
Total	$95,000

Objective 1.3 ***Development of Broodstock Holding Facilities***

Background:

Although maturation and spawning has been reported from sea cages with northern bluefin tuna, the establishment and holding of broodstock will require the development of land-based holding facilities. Although reasonably large yellowtail kingfish are currently held in tanks in at least two hatcheries in Australia, SBT have not yet been acclimatised to land-based facilities. Broodstock of similarly sized tunas have been held in Japan, and large fish are regularly held in oceanaria in Australia. There is no unsurmountable reason why broodstock of SBT cannot be acclimatised to land-based tanks.

The location of the broodstock facility is heavily contingent upon the availability of suitable land adjacent to the sea, and the provision of adequate supplies of clean temparate oceanic sea water of a suitable temperature. SBT is not a tropical tuna, spending most of its time in waters of between 5-20°C (Collette and Nauen, 1983). Although the water temperatures in the tanks can be either cooled or heated, and will need to be for some of the experiments, it is a more cost effective process to heat water than to cool it. Broodstock will also need to be held at sea in ocean-based cages as well as on land, so the land based- facility must be located in the temperate regions of Australia where to water temperatures are conducive for holding the fish.

Land based tanks are normally used in the commercial production of marine fish for aquaculture. They are essential for the use of photoperiod and temperature to control maturational stages. They will also greatly facilitate:

- The development of pelleted diets
- Hormonal induction of maturation and spawning
- Egg collection
- Controlling the genetic identity of eggs and larvae using individually identifiable fish

Sufficient generic heterogeneity should be retained if about 200 individuals were held for broodstock purposes, however these should be preferentially taken from a diversity of wild schools. Although between 300 and 1000 fish may be needed to perform statistically rigorous experiments at least 400 fish should be held during the first year to allow for accidental mortality, repeat experimentation and any need to sacrifice individuals during experiments. These do not all need to be retained in land-based tanks, however once individuals are acclimatised to tanks they should be retained there.

The size of holding tanks is a critical issue. Yellowfin tuna have spawned with a stocking density of 44 adults of an average weight of 32.6 kg in a concrete tank 17m diameter by 6 m deep (1,362m^3) a stocking density of about 1.1 kg fish m^3 (Lee, 1998). This is between half to a quarter of the current stocking ratio of between 2.5- 4 kg fish m^3 used by Australian SBT farmers.

While the minimum number of SBT that can be held in a tank without interfering with their normal behaviour is unknown, this is unlikely to be much less than 25 fish. On the assumption that 25 broodstock fish per tank are likely to grow to about 100 kg, and at a stocking ratio of 1.1 kg fish m^3, this will require tank size of at least 2272 m^3. This could be contained in a tank 6 m deep and 22 m in circumference. Fish have been seen to have difficulty in turning within an experimental net cage of a diameter of 11 m, and it would be unwise to consider

holding broodstock in a tank with such a small diameter. Tanks of 30m diameter and 10 m depth would be a size comparable with the present netting cages used to hold SBT for fattening. The cost of tanks is closely associated with their depth and the engineering strength needed. It is proposed to construct these in ground out of reinforced concrete lined internally with an appropriate inert lining. These have been priced on the basis that they will project 1 metre above ground and have an internal diameter of 30 metres at the top. After a two metre vertical drop, each will slope to form a truncated cone of 20 metres bottom diameter at 10 metres total depth.

The number of tanks needed is a factor of cost and statistical rigour. The more tanks that are built, the greater is the number of experimental treatments that can be used and the greater the accuracy and quicker the research can proceed. However this must be tempered by the costs associated with constructing tanks and feeding the broodstock. Four tanks are considered to be the minimum needed. This will allow for example, 4 treatments with one variable such as photoperiod, or two treatments of two variables such as photoperiod and temperature to include interactions.

Induction of maturation and spawning needs the capacity to vary illumination and water temperature. Fish, especially pelagic species, have a tendency to leap out of the water when startled. They are especially sensitive to abrupt switching off or on of lights. Consequently the tanks will have to have suitable restraints to prevent fish from jumping out of them and a capacity for control of illumination by not only slow dimming, but also timing switches to vary and temperature for environmental control of maturation. Although a commercial hatchery may be able to run with only two broodstock tanks, the experimental facility will require at least 4 tanks, each with separate environmental controls. The tanks will need gantry access and a capacity for isolating fish for sedation and lifting cradles for handling broodstock.

There will be a need for feeders, skimmer boxes and egg traps, water destratifiers, biological filters and aerators and built-in vacuum hose cleaners for the sides and bottom.

Proposed Activities:

A suitable site, both at land and at sea will have to be identified for acquisition and building of the broodstock holding facility. While proximity to existing industrial infrastructure and activity would facilitate activities, this is not essential. However the land site should be in southern Australia adjacent to the sea, and with clean, temperate, oceanic quality water. The sea site should be close to the land site, but will consist only of net cages for holding developing broodstock. These net cages and their moorings and a site lease will need to be acquired, and a suitable boat provided to maintain them. Three phase power and road and sea access are essential. The site will require a jetty for access to fish held in sea cages, and transport of these to the land-based holding tanks.

A small hatchery facility with suitable space for expansion to commercial size should be planned in conjunction with this activity (see Objective 3.1).

Outcomes Required:

1. A broodstock facility which includes at least 4 broodstock holding tanks with associated filtration, cleaning, egg collection equipment and adequate fresh and recirculating oceanic quality sea water supply.

2. A broodstock tank building with a capacity for independent light and temperature control to each tank.

3. A small preparatory laboratory associated with the facility for preparation of hormones, sedatives etc used in the process of handling and maturing broodstock.

Costs

Objective 1.3 *Development of Broodstock Holding Facilities plus* Objective 3.1 *Development of a Hatchery Facility*	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Capital						
Site Acquisition	$80,000					
Establishment of power, water and roads	$150,000					
Office, laboratory, accommodation and Amenities	$170,000					
Settlement ponds for waste	$50,000					
Backup power generator	$50,000					
4 @ 30m tapered to 20m circular 10 m deep in-ground concrete broodstock tanks	$1,000,000					
Insulated buildings for broodstock tanks 4 @ $400,000	$1,600,000					
Engineering associated with Broodstock tanks	$100,000					
Hatchery tanks	$85,000					
Hatchery plumbing and electricals	$125,000					
Algal / live feeds equipment	$5,000					
Temperature control	$30,000					
Blowers and aerators	$10,000					
Water intake, pumps etc	$150,000					
Office & Lab general equipment	$100,000					
Operating						
Repairs & Maintenance buildings, equipment and site	$10,000	$20,000	$25,000	$25,000	$25,000	$25,000
Electricity gas & water usage, rates etc	$10,000	$20,000	$20,000	$20,000	$20,000	$20,000
Office supplies etc	$5,000	$5,000	$5,000	$5,000	$5,000	$5,000
Staff						
Algal maintenance	$22,500	$45,000	$45,000	$45,000	$45,000	$45,000
Rotifer maintenance	$22,500	$45,000	$45,000	$45,000	$45,000	$45,000
Nursery half time	$11,250	$22,500	$22,500	$22,500	$22,500	$22,500
Larval Rearing & Artemia	$22,500	$45,000	$45,000	$45,000	$45,000	$45,000
Coordination	$22,500	$90,000	$90,000	$90,000	$90,000	$90,000
Total	$3,831,250	$292,500	$297,500	$297,500	$297,500	$297,500

Objective 1.4 ***Development of a Maturation Diet***

Background:

The influence of broodstock diet on egg condition and survival is now well documented for many species of marine fish, and it is unlikely that tuna species will differ to any marked extent. The importance of adequate diet for maturation and egg production in captive marine fish species is shown in differences in the fatty acid profiles from eggs of wild striped bass, *Morone saxatilis*. compared with those from domesticated broodstock. Wild eggs were significantly higher in total lipid, n-3 highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) than from domesticated individuals fed a commercial diet. The mean ratio of n-3/n-6 fatty acids from wild fish was almost an order of magnitude higher (Harrell, and Woods, 1995).

Egg quality, hatching rate, larval growth and survival, swim bladder inflation and metamorphosis variously been described as positively influenced by the addition of cephalopod or crustacean components to the diet of broodstock in Red Sea bream, *Chrysophrys major*, gilthead seabream, *Sparus aurata* and yellowtail, *Seriola quinqueradiata* (Watanabe, Itoh, Murakami, Tsukashima, Kitajima, and Fujita, 1984, Tandler, Harel, Koven and Kolkovski, 1995, Verakunpiriya, Watanabe, Mushiake, Kawano, Kobayashi, Hasegawa, Viswanath, Satoh, Watanabe, Visuthi and Kiron, 1997).

Juvenile (1-4 yrs) SBT, like other species of tuna, feed on fish, crustaceans and molluscs. They, and younger sub-adults feed on surface schools of sardines, jack mackerel, and krill (B. Stanley *pers com*). Off eastern Australia the diet is extremely variable and while depending on prey availability, consists mostly of jack mackerel, pilchard and juvenile squid, *N. gouldi* (Young, Lamb, Le, Bradford, and Whitelaw, 1997).

Mature SBT have quite a different diet. Off South Africa they feed in deep water from May to September on fish (64%), prawns (30%), squid (4%) and tunicates (2%). They feed mainly in the early morning, then again in the evening (Talbot and Penrith, 1963). In waters off southern Australia a diverse mix of cephalopod species make up the largest component in the diet of offshore adults. When in subantarctic waters they eat relatively more squid than when in the East Australian Current. In the latter, fish and crustacea are more important (Young, Lamb, Le, Bradford and Whitelaw, 1997). Adult fish in the south Indian Ocean (40-45°S 80-105°E) feed on myctophids, rays bream, *Brama brama* and other species opportunistically. In winter there is a significant squid component in their diet. Fish are caught on squid bait when the sea surface temperature is between 8-13°C. When above 17°C pilchards are more effective, presumably relating to the availability of their feed. The peak of fishing activity in this area is on tuna caught in association with schools of krill in surface waters, where they feed close to the surface, most fishing ending in April/May. A second peak of catches on squid finishes around October and this is believed to be the trigger for a run of animals going north to spawn.

While the principal diet of wild-caught SBT consists of various species of cephalopods, crustaceans and fishes, the species eaten and ratio of each

taxonomic group in the diet varies between species, age of individual, location and time of year. The present diet fed to farmed SBT is almost exclusively frozen clupeids or mackerel, and while producing a marketable commodity in the short term, it is unlikely to be satisfactory for developing healthy broodstock and viable eggs and larvae.

Commercially fabricated diets allow greater control over the composition of biochemical components and reduce the risks of disease introduction. However, satisfying the dietary lipid requirements of marine broodstock using artificial diets has proved difficult, particularly with respect to their HUFA composition. Manipulation of dietary arachidonic acid (20:4 n - 6;), EPA (20:5 n - 3) and DHA (22:6 n - 3) in dry pelleted diets has been shown to improve levels and ratios in European sea bass, *Dicentrarchus labrax* , which were then transferred to the resulting eggs with improvements in early survival and hatching success repeated over successive spawning seasons. (Bruce, Oyen, Bell, Asturiano, Farndale, Carrillo, Zanuy, Ramos and Bromage, 1999).

Existing diets used for fattening juvenile SBT have been used in conjunction with a Nippai formula (which is fed to spawning northern bluefin tuna in Japan) to produce the gonadial development in seven year old fish held at Port Lincoln.

Proposed Activities:

Broodstock must be weaned off the fresh fish diet as soon possible after they are introduced into the broodstock holding facility. Soft pellet formulas must be trialed on the basis of the reported percentages of fish, crustaceans and squid in the diet of adult fish during their maturational process. Levels of dietary components of the natural diet of adults should be measured, and replicated by the use of crustacean, fish and squid meal, together with appropriate additives. Fish on these diets should be compared with controls and a methodology identified for measuring the levels of HUFA in the blood of fish, degree of gonadial development, and levels of HUFAs in eggs and larvae when spawning occurs.

Outcomes Required:

1. Identification of the nutritional profile of eggs from wild SBT.

2. A suitable pelleted diet for the maturation of SBT which produces growth and gonad maturation, and a similar nutritional profile in fish and subsequently produced eggs to that of the eggs from wild fish.

Costs

Objective 1.4 *Development of a Maturation Diet*	Year 2	Year 3
Operating		
Cost of feed for 25 fish by 4 formulations in experimental trials (100 experimental fish)	$102,000	$102,000
Travel	$10,000	$10,000
Staff		
Fish Nutritionist half time	$45,000	$45,000

Food chemist half time	$45,000	$45,000
Total	$202,000	$202,000

Objective 1.5 ***Broodstock maturation and spawning***

Background:

Two spawning times were detected from counting daily otolith growth increments in SBT from off west and south west Australia between January 1 and February 5, 1990-92. A smaller September-October spawning, and a larger January-February spawning (Itoh and Tsuji, 1996).

Although successful production of eggs and larvae from captive tuna has been reported for northern bluefin, black skipjack, and yellowfin tuna, there is very little information currently available in the literature relating to their reproduction in captivity. Adult northern bluefin tuna held in cages in Japan, and black skipjack and yellowfin tuna held in concrete tanks in Panama have spawned naturally without hormonal or photoperiod manipulation, apparently in response to increasing water temperatures. Spawning in northern bluefin tuna usually occurs from 23-28°C, usually between 1730 and 1800 hr at the surface or just below it. Black skipjack tuna spawned at 26-28.7°C and 29-31 ppt salinity. Yellowfin tuna spawned when the daytime average water temperature reached higher than 24°C and spawned repeatedly at 28.1-29.5°C Each spawning occurred around sunset and was preceded by courtship behaviour (Lee, 1998).

Primary gonad maturation in other species of fish can frequently be induced by variation in the periodicity of illumination and water temperature. Spawning of rainbow trout, *Oncorhynchus mykiss* broodstock was advanced by 4 months in groups of fish exposed to 14 weeks of long days (18-h light: 6-h darkness), followed by short days (6L:18D) (Davies and Bromage, 1991). When the photoperiod of broodstock was manipulated and enriched live feed used, it was possible to produce halibut, *Hippoglossus hippoglossus* fry at any time of the year (Holmefjord, Gulbrandsen, Lein, Refstie, Leger, Harboe, Huse, Sorgeloos, Bolla, Olsen, Reitan, Vadstein, Oie and Danielsberg, 1993). Year-round production of juvenile halibut was subsequently achieved from a broodstock of Atlantic halibut maintained on a 6-month delayed photoperiod, (Naess, Harboe, Mangor-Jensen, Naas and Norberg, 1996).

The effects of four combinations of light regime and temperature on the maturation of the striped mullet, *Mugil cephalus*. concluded that short days and low temperature had the most stimulatory effect while long days and high temperature had the most inhibitory effect on oocyte growth. The short photoperiod stimulated the onset of the cortical vesicle stage at both temperatures, while the lower temperature stimulated the onset of vitellogenesis under both photoperiods. The higher temperature caused atresia of vitellogenic oocytes under both photoperiods. Complete regression to primary growth-stage oocytes required both the higher temperature and the longer photoperiod (Kelley, Tamaru, Lee, Moriwake and Miyamoto, 1991)

Most research on the control of reproduction in fishes has focused on female physiology because ovarian development and maturation are easily disturbed by environmental stresses. Chemical methods of manipulating reproductive activity are widely used in fish aquaculture, particularly in the induction of oocyte maturation, and ovulation. Final gonadal growth and spawning usually can be achieved by treatment with gonadotropin-releasing hormone analogues (GnRHa)

either by injection, implant, or feeding, and in most species, have to be applied in combination with dopamine antagonists to enhance responsiveness to GnRHa (Patino, 1997).

As in other fishes, two different molecular forms of gonadotropins, have been isolated from the pituitary glands of bigeye tuna. Both stimulated estradiol-17-beta and testosterone production in tuna ovarian follicles in vitro. As with salmonids, each gonadotropin consisted of alpha- and beta-subunits. Unlike salmonids, the alpha subunits of both had identical amino acid sequences (Okada, Kawazoe, Kimura, Sasamoto, Aida and Kawauchi, 1994). Immunocytochemical identification of GTH I and GTH II cells in the pituitary of the northern bluefin tuna suggested that, as with other fish, GTH I and GTH II are synthesized in separate cells in its pituitary. Both GTH I beta- and GTH II beta-immunoreactive cells were observed in immature northern bluefin tuna, although there were greater numbers of the latter. It has been suggested that despite the chemical similarity of these two gonadotropins to those of salmonids, the pattern of their production and secretion in northern bluefin tuna may be different, and GTH II may also play an important role in the early phases of gonad development. (Kagawa, Kawazoe, Tanaka and Okuzawa, 1998). However this is unlikely, as GTH II was described as playing a major role in final maturation of another, non-salmonid fish, the Japanese eel (Nagae, Todo, Gen, Kato, Young, Adachi and Yamauchi, 1996). The diurnal variation of plasma GTH II of female red seabream also increased at the migratory nucleus-stage maturation of oocytes, to a peak at oocyte maturation at 08.00 h, followed by a rapid decrease at 18.00 h, when spawning occurred (Tanaka, Kagawa, Okuzawa and Hirose, 1991).

Proposed Activities:

In conjunction with exposure to a maturational diet, broodstock should be exposed to a varying photoperiod and temperature to mimic that which occurs in the wild. It is likely that the combination of diet, photoperiod and temperature will initiate the start of vitellogenisis, which should be monitored by the use of non-invasive techniques (see 1.6 below). Although it may be possible to initiate final maturation and spawning by environmental means, the use of gonadotropin-releasing hormone analogues and dopamine antagonists either as implants or with food (Patino, 1997) is more likely to be successful in the first instance. Attempts to control this process the use of elevated temperatures should also be investigated after investigation of the use of the hormone analogues is completed.

Outcomes Required:

1. Development of mature gonads in captive male and female SBT held in land-based tanks

2. Successful spawning of male and females fish in land-based tanks

3. Control of maturation and spawning by environmental manipulation to produce spawners throughout the year.

Costs

Objective 1.5 *Broodstock maturation and spawning (in conjunction with 1.4)*	Year 2	Year 3
Operating		
Purchase of gonadotropins, incorporation in diets etc	$15,000	$15,000
Travel	$6,000	$6,000
Staff		
Post Doctoral fellow	$60,000	$60,000
Total	$81,000	$81,000

Objective 1.6 ***Monitoring Gonad development***

Background:

Induction of maturity, and spawning requires ongoing monitoring of the sex and stage of maturity of broodstock. While visual differentiation between male and female SBT is possible in 7- 8 year old fish this is not possible for younger animals (K. Rough *pers com*).

Low resolution ultra sound images have been used to determine both the sex and maturity of pacific herring *Clupea harengus* and Atlantic salmon, *Salmo salar* by the use of ultra sonic scanners (Bonar, Thomas, Pauley and Martin, 1989, Mattson, 1991). However in these cases the fish were either anaesthetised or killed and held in small tanks prior to scanning.

A less invasive way of determining the sex and reproductive activity of adult females fish has been shown to be the use of an enzyme linked immunoabsorbent assay for vitellogenin in skin mucus swabs. This was demonstrated to reflect the gonad condition in the greater amberjack, *Seriola dumerili* (Takemura, Taeuchi, Teruya, Oka, and Kanematsu, 1999) and identified the sex of yellowfin tuna, *Thunnus albacares* (Takemura and Oka, 1998) and striped bass, *Morone saxatilis* (Kishida, Anderson, and Specker, 1992).

Proposed Activities:

The extent to which this method is appropriate for SBT should be investigated by the use of existing vitellogenin probes. Probes for gonadotropic hormones should also be developed if possible, to facilitate assay of the levels of the two gonadotropins in maturing fish as an indicator of the level of maturity.

Outcomes Required:

1. Production of a non-invasive methodology to identify male from female SBT
2. Production of a non-invasive measure of gonadial development during maturity and spawning in female SBT

Costs

Objective 1.6 *Monitoring Gonad development (in conjunction with 1.4)*	Year 2	Year 3
Operating		
Development of existing probes for skin mucopus swabs	$10,000	$10,000
Travel	$6,000	$6,000
Staff		
Post Doctoral fellow	$60,000	$60,000
Total	$76,000	$76,000

2. Egg Collection, Sterilisation & Transport

Capture for domestication of wild SBT could possibly be avoided if freshly hatched larvae could be obtained by stripping at sea and airfreighting to experimental facilities. The eggs and larvae obtained from wild caught fish are likely to be of better quality than those from fish held in tanks. However there are many difficulties with obtaining these. Problems include:

- If the fish are caught as part of the ongoing CSIRO research on commercial vessels, the number of fish caught per day is likely to be much less than one per 5 days fishing (10,000 hooks).
- The proportion of fish that are caught when running ripe is unknown.
- The capacity for stripping SBT is untested and the size and muscular body wall of adult SBT may well produce difficulties with manual expression of the eggs. Although harvesting gonads and maturing them *in vitro* is one possible way of getting around this problem, it has never been tried in this species, and is unlikely to be successful without considerable investigation.
- Stripped eggs would have to be fertilised so they, or stripped sperm would have to be held on board the vessel until suitable gametes were available for fertilization.
- Fertilised eggs would need to be flown to a hatchery within 24 hours (before hatching), or held on the commercial vessel and hatched prior to dispatch. This would require a capacity to have a helicopter standing by, a capacity to transport eggs or larvae to the helicopter, and a laboratory on standby to receive the fertilised eggs. If cryopreservation of gametes was possible then this would not be needed. However while cryopreservation of sperm is a possibility, fish eggs have not been successfully cryopreserved to date.

If eggs were successfully collected from adults on the spawning grounds, although they could potentially produce fingerlings within a year, the source of eggs and larvae would continually be at risk to the vagaries of the environment. If fingerlings are to be used for reseeding or farming, they should come from a known and controlled genetic source that is pathogen free, and reared in such a way that they can be genetically identified for the reseeding project.

It has also been suggested that it might be possible to catch larvae over the spawning grounds by the use of "plankton trawls and other means", because of the reported high densities there (200 / m^2, Lee, 1998). However, the actual density reported was a maximum of 205 / 100m^2 (Davis, Jenkins and Young, 1990a). The highest densities reported during these studies were about 22m^{-3} in surface tows (Davis, Jenkins and Young, 1990b). Apart from problems with obtaining sufficient numbers of larvae, SBT larvae cannot be differentiated from bigeye tuna larvae below 3.5 mm (Davis, Lyne and Jenkins, 1991) or from other tuna larvae and juveniles bigger than 15 mm (Nishikawa and Rimmer, 1987).

Apart from these technical difficulties, any political sensitivities would also have to be overcome, as the best chance of obtaining either wild larvae or ripe adults

would be from fish caught in the Indonesian fishery, and permits to operate in Indonesian waters would need to be obtained.

For the above reasons, collection of eggs from wild fish spawning on the spawning grounds is not considered to be the best appropriate option, and it is recommended that eggs be obtained from captive broodstock fish.

Objective 2.1 ***Egg Handling and Transport***

Background:

The implementation of this R&D strategy is contingent on transporting eggs or yolk stage larvae to contract R&D providers at locations remote to the broodstock facility. The pivotal nature of this transportation activity brings a much greater need for this research into egg handling and transport than might otherwise have been the case. Unless a standard handling and transportation system is developed, then the results of experiments from different R&D providers may appear different, but actually be due to differences in handling procedures during transport.

Eggs collected from captive broodstock are subjected to a variety of physical and biological influences. If they are to be transported to other hatcheries for ongrowing it is critical to have finished transporting the eggs either before they hatched (less than one day), or immediately after hatching, as the waste products associated with hatching easily contaminate the transport water and newly hatched larvae. If not transported as eggs, the early yolk stage before first feeding would probably be the best time (day 2-3). Northern bluefin tuna larvae have been successfully transported by air as 2 day yolk sac larvae (Kaki, Tanaka, Takahashi, Oka and Ishibashi, 1996).

Fertilised eggs from spontaneous spawning of northern bluefin tuna have been collected from net cages by manual removal by hand nets. However it is more usual with cultivated fish to hold broodstock in spawning tanks. Eggs are usually collected after spawning by an overflow skimmer and holding tank until they are moved for further development and hatching to nursery tanks or to other hatcheries. During the process they may vary in their sensitivity to damage at different times of embryological development. This degree of sensitivity and stage of sensitivity depends upon the species. Eggs of milkfish, *Chanos chanos* are most sensitive to physical shock at the beginning and end of larval development (Hilomen-Garcia, 1998), while eggs of grouper, *Epinephelus coioides* and coho salmon (Caberoy and Quinitio, 1998, Jensen, and Alderdice, 1983) are much less sensitive to handling stress at the later stages of development.

Proposed Activities:

Series of experiments on eggs at all stages of development from freshly laid to immediately pre-hatch to determine the best egg age for transport, most appropriate loading density (eggs/litre) and degree of agitation of the holding water.

Outcomes Required:

1. Identification of the most robust developmental stage for egg transport

2. Identification of the best handling methodology and loading density for transport of eggs

Costs

Objective 2.1 *Egg Handling and Transport*	Year 2
Operating	
Experimental tanks and equipment for experiments	$10,000
Travel	$6,000
Staff	
Post Doctoral Fellow half time	$30,000
Total	$46,000

Objective 2.2 ***Egg Sterilisation***

Background:

During the process of spawning, collection, embryogenesis and hatching eggs are collected under intensive conditions, and are also susceptible to a number of fungal, viral and bacterial diseases which may at times be derived from the parental fish. Most Australian states now have requirements that if fish stocks are to be transported from place to place they must have a disease-free status.

Several procedures have been examined to sterilise eggs to prevent transmission of disease. Eggs of chinook salmon *Oncorhynchus tshawytscha* were disinfected with buffered free-iodine for 10 minutes, then treated daily with formalin until the eyed stage of development in an attempt to eliminate mortality due to infection with the fungus *Saprolegnia parasitica*. This produced a significantly lower mortality from hatch to swim-up compared with daily hand picking of dead eggs and fry (Barnes, Cordes and Sayler, 1997). Iodophores are generally considered to be effective as antimicrobial agents, however their effectiveness is reduced by their toxic effects on the fish themselves. The effectiveness of Povidon-iodine varied on the time of its application to eggs, and concentrations for its use in spotted halibut, *Verasper variegatus* and Red Sea bream (Hirazawa, Hara, Mitsuboshi, Okazaki, and Hata, 1999). Bufodine, when tested on Atlantic halibut significantly affected the eggs, the survival and the development by both the developmental stage at which the eggs were treated and the concentration of disinfectant used (Bergh and Jelmert, 1996). Surface disinfection of the eggs of the Atlantic halibut with ozonised seawater was shown to be effective in inactivating a notovirus (VER) when eggs were exposed to this pathogen. However delayed hatching or non-hatching occurred in some eggs of all groups that were treated (Grotmol and Totland, 2000). The toxicological effect on larvae from use of the disinfectants benzalkonium chloride, formalin, hydrogen peroxide, thimerosal (merthiolate), polyvinylpyrrolidone iodine and sodium hyperchlorite on eggs of the red drum, *Sciaenops ocellatus* were tested independently by Douillet and Holt (1994). They concluded that the early tail-free stage of development was the most resistant to all germicides but that successful disinfection of eggs was achieved after a 5-min exposure to hydrogen peroxide at 3% concentration. However, the other two species tested (yellowtail snapper, *Ocyurus chrysurus*, and spotted seatrout, *Cynoscion nebulosus* were more sensitive to hydrogen peroxide.

Proposed Activities:

Experiments to test the effectiveness of a range of disinfectants in sterilising eggs, and their toxicity at varying stages of embryogenisis.

Outcomes Required:

1. Identification of a methodology for the sterilisation of eggs with minimum toxicological effect on the subsequent larvae.

Costs

Objective 2.1 *Egg Sterilisation*	Year 2
Operating	
Purchase of sterilising equipment and reagents	$15,000
Travel	$6,000
Staff	
Post Doctoral Fellow half time	$30,000
Total	$51,000

3. Egg Hatch & Larval Development To Metamorphosis

Most "in house" research and development activity will consist of work on the acclimatisation of broodstock, their maturation and spawning, and the development of methodology for transporting eggs or yolked larvae.

Feeding of marine fish larvae in captivity is, in most cases, limited to two types of live prey, rotifers and *Artemia.* This reduction in the range of food available for the cultured larvae may lead to nutritional imbalances or deficiencies. The use of green water is generally felt to enhance the passage of the enrichment medium through to the larval fish. However, for a commercial industry, the dependence on live food is a complication, especially the difficulties associated with a reliable *Artemia* supply. Considerable research will need to be done on establishing the best food for larval SBT. Provided an adequate process of transporting eggs or early yolk larvae is identified, this need not be done at the hatchery, but contracted out to research agencies for comparative experiments. After the successful establishment of a diet, the hatchery would be used to confirm its suitability for commercial production.

The infrastructure for a small basic hatchery should be developed during the establishment of broodstock holding facility. This hatchery will confirm results from contract research done at other locations, and run commercial trials to build on the results from the contract research. After successful completion of the trials, the hatchery facilities will need to expand to produce sufficient fingerlings to meet demand. This expansion need not be at the same site as the broodstock facility, particularly if the fingerlings were to be used at a remote locality.

Objective 3.1 ***Development of a Hatchery Facility***

Background:

The larval stages of SBT inhabit the surface mixed layer of the ocean in December and January in the spawning grounds south of Java. Temperatures at this time in this layer vary between 27° and 28.6°C and salinities between 34.6-35.0 ppt. In this location variability in salinity is likely to be low, however during cyclonic events temperatures may well vary due to upwelling of cooler waters. Far more larvae occur near the surface (0-2 m) during the day than at night and the highest natural densities were found to be greater than 22 larvae per cubic metre. (Davis, Jenkins and Young, 1990b). The larval stage of SBT appears to last about 20 days in the wild (Jenkins and Davis, 1990).

Behavioural studies on captive larval fish suggest that most need a minimal threshold light intensity to be able to develop normally and grow, probably related to the aptitude to localise, catch and ingest prey. Light is also indispensable for body pigmentation, an important phenomenon involved in early development and growth. Too intense light can be stressful or even lethal due to the increased swimming activity associated with phototaxic larvae. Generally, long day length and availability of food improves larval rearing quality (Boeuf and Le Bail, 1999).

Newly hatched larvae of northern bluefin tuna have been successfully reared from 2-day yolk sac larvae in a plastic tank 5x1x1m deep at a density of 6 larvae per litre . The water was maintained at 25°C, and the photoperiod was natural and between 100 and 1000 lux (Kaji, Tanaka, Takahashi, Oka and Ishibashi, 1996).

Pelagic species such as SBT feed on copepods in the wild (Young & Davis, 1990). However there is not yet a suitable copepod feed for commercial use. If rotifers and *Artemia* are going to be used, they should be enriched and it will be worth trying both microparticles and algae for this purpose.

Air bladder inflation is also important, however in other species the use of surface skimmers has solved most of the problems associated with this.

Proposed Activities:

Suitable tanks for rearing larval need to be designed and built. These will need to be of circular design and equipped with aeration and filtration facilities and have a capacity for inducing a circular water flow. Appropriate surface skimmers should be fitted. The tanks should have a fully variable level of recirculating capacity. Boilers and chillers together with suitable heat exchangers will need to be designed so the water temperature at which the larvae is held can be controlled. Illumination will require similar control. Suitable facilities will be developed for growing algae, rotifers and *Artemia.* A capacity for harvesting algae and enriching rotifers and *Artemia* will also need to be developed. The number of tanks will initially be low, and used for testing commercial application of the results of research contracts on larval rearing. Algal, rotifer and *Artemia* rearing tanks will have to be installed in proportion to the number and size of the larval tanks installed at each phase of the plan.

Outcomes Required:

1. The water system capable of running elevated and lowered temperatures simultaneously.

2. A building holding a minimum of 6 circular larval rearing tanks with a capacity for controlled temperature and light, surface skimmers, waste disposal and variable levels of recirculation

3. Algae, rotifer and *Artemia* growing facilities.

4. A capacity for algal harvesting and rotifer and *Artemia* enrichment.

Costs

See Objective 1.3

Objective 3.2 ***Identification of a suitable Larval Diet***

Background:

The diet of wild larval SBT consists mainly of copepod nauplii, cyclopoid copepods, *Corycaeus* spp, calanoid copepods, *Clausocalanus* spp and cladocerans, *Evadne* spp (Uotani, Matsuzaki, Makino, Noda, Inamura and Horikawa, 1981). Of the larvae between 2 and 14 days old caught, 52.7 % that were caught during daylight (0600-1800) had food in their stomachs. Incidence of stomachs with food

increased with fish size. Feeding only occurred in daylight, peaking after sunrise and in mid afternoon after a decline about 1100. Stomachs are empty by 2200. Fish larvae are a principal diet of post-flexon larvae. Overall mouth width is correlated with prey width. Fish smaller than 4.75 mm (less than 5 days old) fed mostly on copepod nauplii, prey width is 0.08-0.16 mm, mean 0.087 for fish less than 5 mm SL. The prey is gradually replaced by cyclopoids as they grow bigger (prey width 0.06-0.30 mm, mean 0.171 and then calanoids (prey width 0.08-0.32 mm, mean 0.20. Fish larvae greater than 7 mm SL feed on fish larvae, including SBT larvae (Young and Davis, 1990).

In captivity, newly hatched larvae of northern bluefin tuna were fed rotifers enriched by "Super Rotifer II" from day 3, *Artemia* nauplii and coral trout eggs and larvae were supplied from day 12, *Artemia* larvae and an unspecified artificial diet was added several days before metamorphosis at 30 days. Survival was very low, 0.19% at 35 days post hatching (Kaji, Tanaka, Takahashi, Oka and Ishibashi, 1996). Higher growth rates have been reported, larvae were described as reaching 40 mm TL after feeding rotifers from day 2 to 20, *Artemia* from day 13 to 28, live fish larvae from day 16 to 29, anchovy from day 20 to day 30 and sand lance from day 25 to day 30 (Kumai, 1998).

Yellowfin tuna larvae that were reared from hatching to beyond metamorphosis were fed on rotifers from day 4, on fish larvae and *Artemia* nauplii from Day 16, and then on frozen fish and minced fish meat. Survival rates were not given (Kaji, Tanaka, Oka, Takeuchi, Ohsumi, Teruya and Hirokawa, 1999).

A large amount of research has been devoted to the study of the dietary requirements of marine fish, especially of their essential fatty acid needs. Studies on the biochemical composition of developing eggs and larvae, as well as the comparison of the patterns of loss and conservation during starvation, shows the importance of n-3 HUFAs and arachidonic acid for larvae of marine fish (Izquierdo, 1996). Assays from eggs of northern bluefin tuna demonstrated that at the early cleavage stage, eggs were composed almost entirely of protein, free fatty acids, triglycerides and phospholipids. Because of the declining levels of triglycerides with embryogenesis, this is considered a main endogenous energy fuel (Takii, Miyashita, Seoka, Tanaka, Kubo and Kumai, 1997).

Microbound and micro-encapsulated diets have been designed to supplement live feed in the culture of fish larvae, to substitute for live food during the early stages of rearing.

There is a recognition that it is best to wean larvae from live feed on to a pelleted formula, but no strong views on how to do it. Microbound and micro-encapsulated diets have been designed to supplement live feed in the culture of fish larvae, to substitute for live food during the early stages of rearing The growth rate and survival of larval gilthead bream fed a microencapsulated diet following 4 days feeding on rotifers were similar to that of larvae fed on rotifers. (Yufera, Pascual and Fernandez-Diaz, 1999). The addition of *Artemia* to microdiet-fed seabass larvae positively affected rates of assimilation and growth, regardless of the age of the fish (Kolkovski, Tandler and Izquierdo, 1997)

Ingestion rates were also markedly improved when inert microdiets were co-fed to larval seabream *Sparus aurata* together with live *Artemia* nauplii. This was

considered to be due to one or both of visual and chemical stimuli of the live diet to ingestion, or the direct influence of the biochemical composition of nauplii on larval digestion and assimilation (Kolkovski, Koven and Tandler, 1997). Survival of larvae of this species fed only microcapsules ranged from 11%, when the capsules were added from first feeding, to over 50% when pre-fed rotifers. The addition of live prey (5% of the total food supplied on dry weight basis) improved the survival (42%) when the microcapsules were supplied from the start of feeding. Feeding incidence on microcapsules was similar to that obtained with rotifers (Fernandez-Diaz and Yufera, 1997).

Proposed Activities:

Treated eggs or if not possible, yolk stage larvae, should be supplied to several research institutions to examine the effects of a variety of diets on larval development. Water temperatures and salinities will be standardised and held at those characteristic of the spawning grounds for wild larvae. Each institution should compare an experimental diet with a standard enriched rotifer / *Artemia* green water diet. The composition of this diet has yet to be established.

Experimental treatments can include a considerable permutation of diets, but should initially include at least combinations of rotifers and *Artemia* with high and low EPA / DHA ratios, inert microdiets and fish larvae. If a satisfactory diet is not achieved using one of these combinations, or if a suitable method of mass production of copepods is established before this, similar experiments should be contracted substituting copepods for *Artemia.* It is estimated that if three contracting institutions tested four different diets three times a year for two years this would allow for the testing of 36 diets each year. This would require a minimum of five larval tanks in each institution (4 diets plus control).

The hatchery should not be used for these first experiments. Once constructed, and before results are forthcoming, existing techniques with other species should be tested to familiarise staff with the methods of larval rearing and to bring the hatchery into a smooth operating condition. After results have been produced by the contract research then the hatchery should seek to implement these under commercial conditions.

Outcomes Required:

1. A cost effective larval diet which produces satisfactory growth, low mortality, and high percentage of satisfactory metamorphosis.

Costs

Objective 3.2 *Identification of a suitable Larval Diet*	Year 3	Year 4
Letting of 3 Research Contracts each of 4 months for larval diet research		
Operating		
On costs for of use of hatchery facilities and support staff	$70,000	$70,000
Costs of Diets	$15,000	$15,000
Staff		
Research scientist 3 x 4 months	$70,000	$70,000
Research assistant 3x 4 months	$37,000	$37,000

Total	$192,000	$192,000

Objective 3.3 ***Environmental Conditions for Larval Development***

Background:

Studies on growth and survival of marine fish under differing environmental conditions of light and temperature are scarce. After 35 days, growth and survival of haddock (*Melanogrammus aeglefinus*) larvae were not significantly different between photoperiod treatments of 24L : 0D and 15L : 9D. Overall larval survival was poor, but greater in white versus black tanks after 41 days (2% versus 1%, respectively). Growth of larvae was impaired in black tanks at low light intensity. Transmission and reflection of light was low in black tanks at low incident light, and there was very little upwelling light. The resultant poor prey to background contrast was thought to result in larvae being unable to consume sufficient food to sustain a level of growth comparable to that in other treatments, (Downing and Litvak, 2000).

Barramundi larvae 2-10 days old grew progressively faster under conditions of 8, 16 and 24 h light per day; survival rates did not differ between the treatments. Larvae 8-20 days old also grew significantly faster in 16 and 24 h light than in 8 h light; survival rates did not differ between the treatments. Juveniles, initially 11-12 mm total length, showed no significant difference in growth or survival rates in either 12, 18, or 24 h light (Barlow, Pearce, Rodgers and Clayton, 1995).

Larval SBT are found in the surface mixed layer of tropical waters (to about 30 m depth) where they undergo vertical migrations to shallower waters to feed during daylight hours. Two spawning times have been detected from counting daily otolith growth increments in fish from off west and south west Australia, a smaller September-October spawning, and a larger January-February spawning (Itoh and Tsuji, 1996). The surface water temperatures in this region at this time are around 27-28°C.

Proposed Activities:

The light intensity and photoperiod of surface waters (0-2m) of the spawning grounds should be reproduced in the larval rearing tanks. Water currents should be induced by surface recirculation of the water, and the surface kept clean by surface skimmers. Water temperatures should be kept at 27-28°C until metamorphosis, thereafter the fish should be transferred to nursery tanks. Once a successful diet for rearing to post-metamorphosis is achieved, experiments can be done to vary the photoperiodicity and temperature to maximise growth and survival.

Outcomes Required:

1. Successful growth and metamorphosis of larval SBT

Costs

Objective 3.3 *Environmental Conditions for Larval Development*	Year 3	Year 4
Letting 3 of 3 research contracts each of 4 months for larval environment research		

Operating		
On costs for of use of hatchery facilities and support staff	$70,000	$70,000
Costs of Diets	$15,000	$15,000
Staff		
Research scientist 3 x 4 months	$70,000	$70,000
Research assistant 3x 4 months	$37,000	$37,000
Total	$191,000	$191,000

4. Post-Metamorphosis Juvenile Development To Fingerlings

Nothing is known of the distribution, growth or feeding of SBT between metamorphosis and about 80 days old. Juvenile fish about 100 days old and (ca 30 cm long) have been found between early December and late January off Western Australia in the surface waters of the Leeuwin current (Itoh and Tsuji, 1996). The water temperature in this region (off Shark Bay) peaks at around 24-25°C from January to May, thereafter dropping to a low of 20-21°C in September before increasing again in summer. All fish are believed to be out of tropical waters by the time they are 100 days old (J. Gunn *pers com*). Juveniles (2-4 years old) are found in 13°C water and dive to 8-9°C water to feed. Studies of the otolith structure of juvenile fish suggest that there are three major parts to the deposition of the increments to the otolith. The first is from about day 9 to 16, the second from about the 13th day to the 40-50th day, and the third part from there on (Itoh and Tsuji, 1996). The otoliths of demersal fish show a characteristic mark when the larva metamorphoses and settles on the bottom. It is possible that these changes in the otoliths of young SBT are recording changes in the environmental conditions with age. Thus the first part from day 9 to 16 may represent the larval growth stage and the 13th day to the 40-50 day the elevated growth in late larvae and post metamorphosed juveniles suggested by Jenkins and Davis (1990). The part thereafter may record the movement of the juvenile fish out of tropical waters and into the Leeuwin current.

Considerable variation in growth rates of juvenile SBT from year to year was first postulated by Serventy (1956) who believed that growth took place entirely in the summer months between October and May. A similar conclusion was reached by Thorogood, (1987) who suggested that growth was rapid from November to June, thereafter decreasing significantly. Hearn (1986) also showed that growth was much faster in summer / autumn than in winter / spring.

Holding conditions for post-metamorphosed fish have yet to be determined, as has their diet and the best age for transport to net cages for grow-out. Comparison of their growth rates with northern bluefin tuna suggests that 4 cm fingerlings might be achieved within one month post-hatch.

Objective 4.1 ***Development of a post-metamorphosis diet***

Background:

Post-metamorphosis northern bluefin tuna have been reared from day 30 to day 120 on minced fish (sand lance, *Ammodytes personatus* or sardine, *Sardinops melanostictus*) (Miyashita, Sawada, Hattori, Nakatsukasa, Okada, Murata and Kumai, ms), and yellowfin tuna on chopped or whole fish fry and chopped thread herring (Lee, 1998).

Despite these reports of the use of fish as food in the development of tuna fry, a switch from a live or fish diet to formulated feed is a critical stage in the culture of any fish species and essential for the developement of commercial quantities of fingerlings. The switch may take place relatively quickly. Juvenile winter

flounder were weaned from live, cultured *Artemia* onto a dry feed originally formulated for turbot *Scophthalmus maximus* and cod *Gadus morhua* after one week. Increased specific growth rates and feed efficiency ratios in weaned juveniles indicated that prepared diet was a better food source than *Artemia* (Lee and Litvak, 1996).

After metamorphosis, the juvenile cod offered a commercial pelleted diet in addition to the natural zooplankton available showed from 3 weeks after metamorphosis, there was a gradual increase of the commercial diet in gut contents, and by day 55 most of the diet consisted of this feed. Analysis of fish, zooplankton and the commercial diet indicated that the fatty acid composition of cod neutral lipids was significantly influenced by the polar lipid classes of the zooplankton during the first 3 weeks after metamorphosis. Thereafter there was an increase in the fish triacylglycerols typical for the neutral lipid classes of both the live feed and the artificial diet (Olsen, Henderson and Pedersen, 1991).

A suitable feeding programme for juvenile barramundi included rotifers (*Brachionus plicatilis*) and *Artemia* sp. nauplii for larvae, followed by dry starter and production feeds for juveniles. When 15- to 71-g barramundi were fed on a series of 6 dry diets, the feed containing the most fish meal (60%) and fat (16.9%) produced the best feed conversion (0.89); however, a diet containing 20% fish meal and 13.4% fat gave practically the same results (Tucker, MacKinnon, Russell, O'Brien and Cazzola, 1988).

There is little information available on the best diets for post-metamorphosis tunas. Juvenile yellowfin tuna smaller than 39 cm FL are planctivores, gradually increasing their consumption of fishes with increasing size (Maldeniya, 1996). The diet of small SBT includes organisms such as small pelagic amphipods and euphausians (sic), and a range of fish fry, including mackerel, pilchard, anchovies, leatherjackets, bellows fish, scorpaenids, billfish, morwong, barracouta, king barracouta, scad, jack mackerel, and a range of other fish families (Serventy, 1956).

Proposed Activities:

A series of experiments to replace live larval diets with inert formulated diets for post-metamorphosed fish (weaning). These experiments should investigate the use of mixtures of live crustaceans and fish fry, and formulated "weaning" diets. It may be possible to introduce weaning diets directly with the enriched *Artemia* diet. These experiments are a logical extension of the contracted experiments on feeding larvae to metamorphosis.

As the fish grow there is likely to be a need for grading these by size to avoid cannibalism and maximise growth. This should be investigated.

Outcomes Required:

A suitable diet for growing post-metamorphosed fish to healthy fingerling stage (5-10 gm)

Costs

Objective 4.1 *Development of a Post-Metamorphosis diet*	Year 3	Year 4
Letting of 3 Research Contracts each of 4 months for post-		

metamorphosis diet research		
Operating		
On costs for of use of hatchery facilities and support staff	$70,000	$70,000
Costs of Diets	$15,000	$15,000
Staff		
Research scientist 3 x 4 months	$70,000	$70,000
Research assistant 3x 4 months	$37,000	$37,000
Total	$192,000	$192,000

Objective 4.2 ***Development of post-metamorphosis holding facilities***

Background:

Severe problems have been reported in the survival of post-metamorphosis juvenile northern bluefin tuna due to death from collisions with the tank walls (Miyashita, Sawada, Hattori, Nakatsukasa, Okada, Murata and Kumai, ms). These fish were reared in indoor 90m^3 octagonal tanks 1.5m deep and in a sea cage 12 x 12 x 3 m deep. Yellowfin tuna juveniles have been reported as being raised in circular tanks of either 2.4 or 4.6m diameter of 4,800 or 11,000l capacity. (equating to ca 1m and .66m deep) however collision fatalities have not been reported as being a problem in this species (Lee, 1998).

Proposed Activities:

It is not known if collision mortality will be a problem with SBT. This is likely to be minimised by the use of appropriate illumination, lack of disturbance, and the use of either circular tanks with circular current flow, or the use of raceways. A series of experiments should be initiated to investigate the most effective way to hold post-metamorphosis fish.

Outcomes Required:

A tank system sufficient to reduce environmentally induced mortality to an acceptable minimum

Costs

Objective 4.2 *Development of post-metamorphosis holding facilities*	Year 3
Operating	
Engineering of works for tank and raceway setups at hatchery	$50,000
Staff	
Research scientist	$70,000
Total	$120,000

Objective 4.3 ***Design and development of suitable transport technology for fingerlings***

Background

While the location of the commercial hatchery could be in a number of suitable locations, the time needed for transporting fingerlings to farms or to Western Australia for stock enhancement could vary from a couple of hours to up to 2-3 days. The handling associated with loading and transport has been demonstrated to cause major stress on fingerlings.

Transported Atlantic salmon smolt had plasma cortisol concentrations increased up to 15 times from resting values, with a peak 1 h after transport. A severe discrepancy in both fresh- and seawater osmoregulatory ability was observed 24 and 48 h after transport. Low recapture rates and survival of the hatchery-reared salmon smolts were, in part, caused by the handling and transport of the smolts prior to the release.(Iversen, Finstad, Nilssen, and Kjell, 1998).

Dip-netting 1 and 2 year old sea-ranched smolts of Atlantic salmon (*Salmo salar* L.). just prior to release reduced the survival of 1- but not of 2-year-old-smolts. Adding an additional transport stress lasting 4 h gave similar results. Handling and chlorobutanol anaesthesia immediately before release reduced the survival of both smolt groups.(Hansen and Jonsson, 1988)

The main objective of this study is to maximise the recovery from loading and transport stress of hatchery-reared SBT fingerlings.

Proposed Activities:

To examine the most recent methods of transporting fingerling fish under aquaculture, and in conjunction with objective 1.2 determine a methodology for sedation and transport.

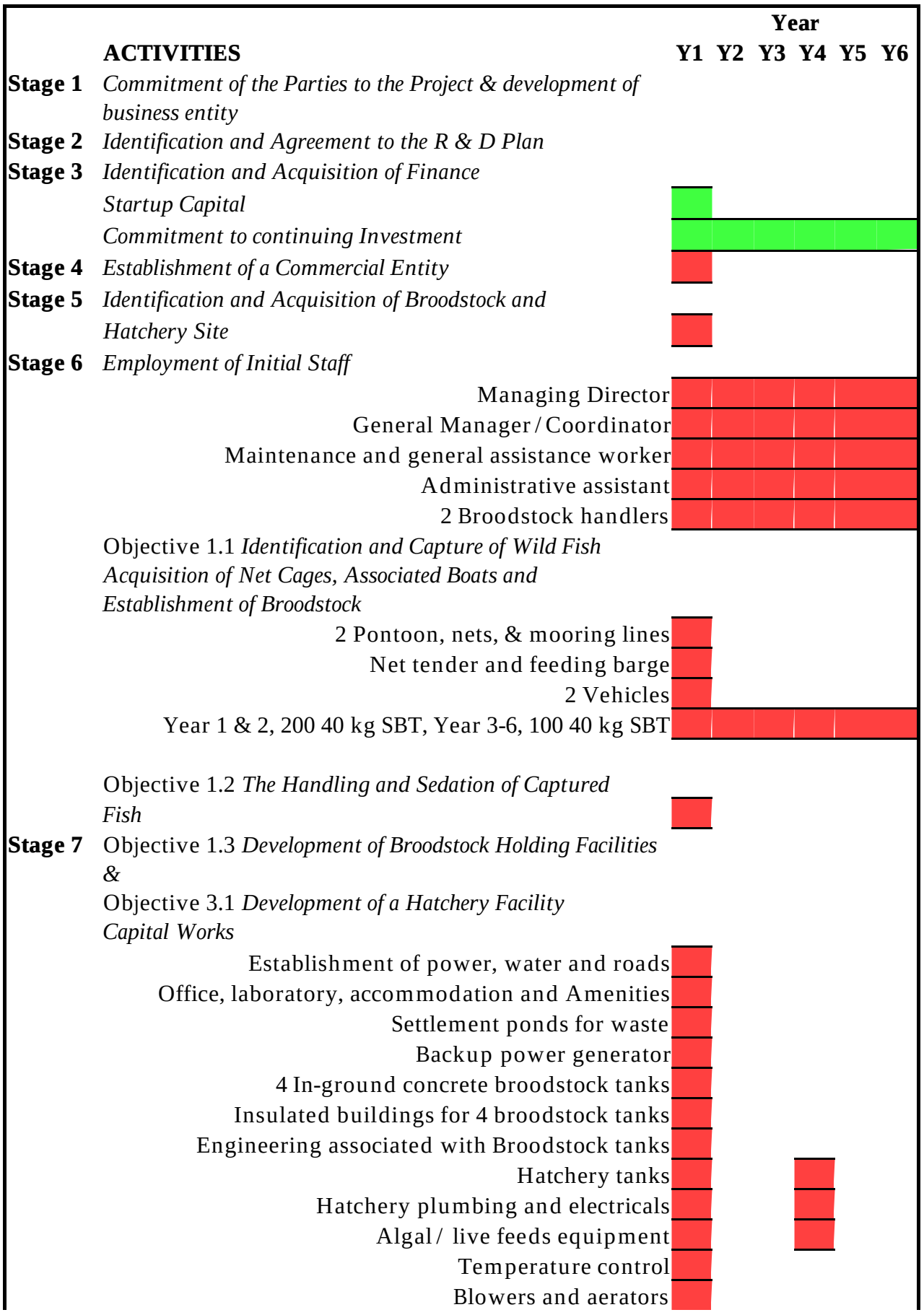
Outcomes Required:

A method for transporting fingerling SBT a) to sea farms for growout b) for release into the sea for stock enhancement.

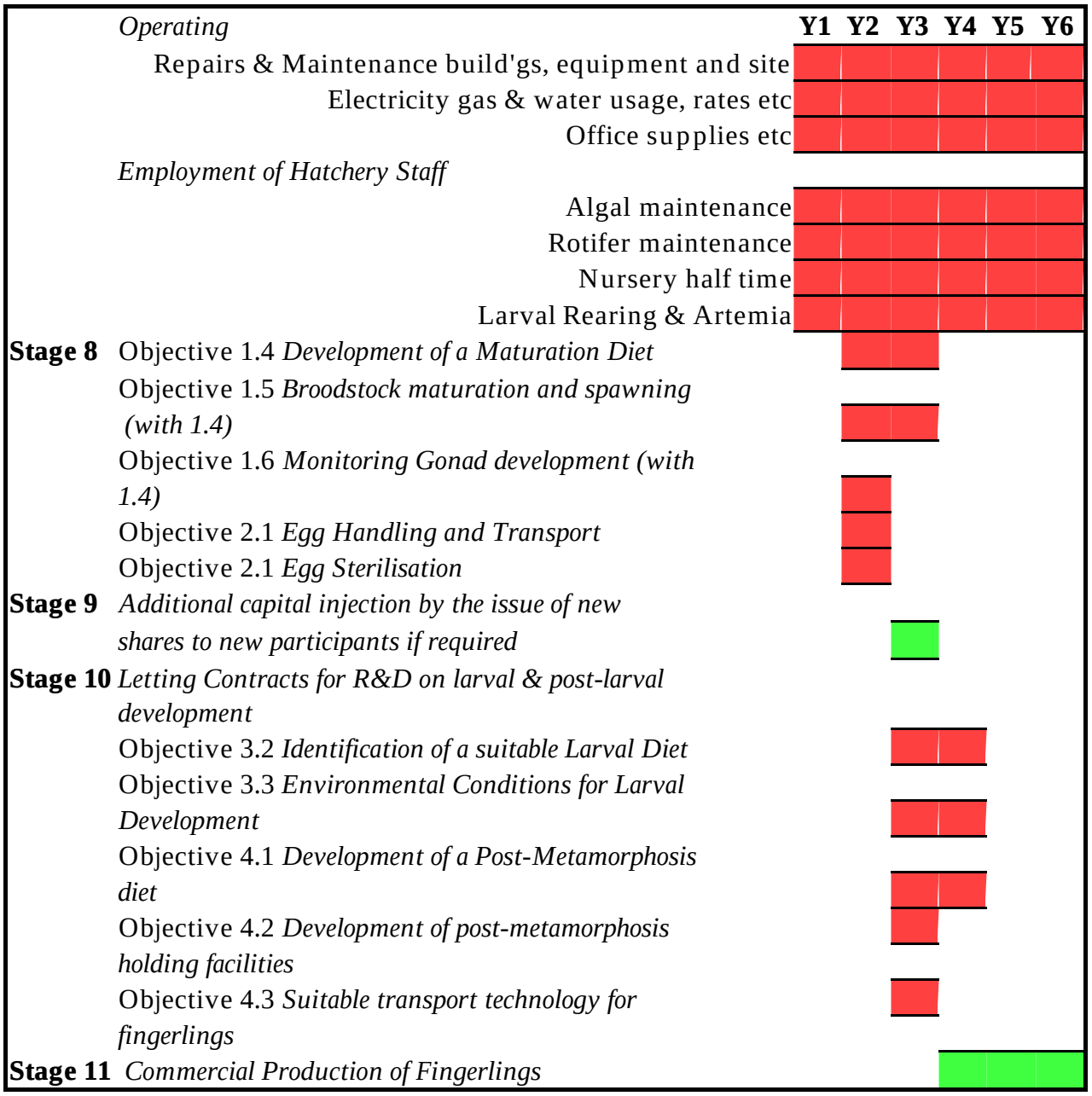
Costs

Objective 4.3 *Design and development of suitable transport technology for fingerlings*	Year 3
Operating	
Purchase of anaesthetics	$5,000
Equipment & Engineering	$10,000
Staff	
Research Scientist	$70,000
Total	$85,000

PART 5: SEQUENCE OF ACTIVITIES



Water intake, pumps etc	
Office & Lab general equipment	


Y1 Y2 Y3 Y4 Y5 Y6
Operating
Repairs & Maintenance build'gs, equipment and site
Electricity gas & water usage, rates etc
Office supplies etc
Employment of Hatchery Staff
Algal maintenance
Rotifer maintenance
Nursery half time
Larval Rearing & Artemia
Stage 8
Objective 1.4 Development of a Maturation Diet
Objective 1.5 Broodstock maturation and spawning (with 1.4)
Objective 1.6 Monitoring Gonad development (with 1.4)
Objective 2.1 Egg Handling and Transport
Objective 2.1 Egg Sterilisation
Stage 9
Additional capital injection by the issue of new shares to new participants if required
Stage 10
Letting Contracts for R&D on larval & post-larval development
Objective 3.2 Identification of a suitable Larval Diet
Objective 3.3 Environmental Conditions for Larval Development
Objective 4.1 Development of a Post-Metamorphosis diet
Objective 4.2 Development of post-metamorphosis holding facilities
Objective 4.3 Suitable transport technology for fingerlings
Stage 11
Commercial Production of Fingerlings

PART 6: FINANCIAL PROJECTIONS

		Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Stage 1- Commitment of the Parties to the Project							
Stage 2- Identification and Agreement to the R & D Plan							
Stage 3- Identification and Acquisition of Finance							
Stage 4-Establishment of a Commercial Entity							
Stage 5-Identification and Acquisition of Broodstock and Hatchery Site							
Capital Items	Site Acquisition	$80,000					
Stage 6- Acquisition of Net Cages, Associated Boats and Establishment of Broodstock							
Objective 1.1 *Identification and Capture of Wild Fish*							
Capital Items	Item						
	2 Pontoon, nets, & mooring lines	$300,000					
	200, then 100, 40 kg wild SBT fish from quota @ $15 kg^{-1}	$120,000	$120,000	$60,000	$60,000	$60,000	$60,000
	Net tender and feeding barge	$100,000					
	2 Vehicles	$60,000					
Operating	Costs of capture and transport of fish from wild to holding net cages	$25,000	$25,000	$12,500	$12,500	$12,500	$12,500
	Feed costs per kg of average weight 40 kg fish at 7% body weight day-1	$245,280	$245,280	$245,280	$245,280	$245,280	$245,280
	Running costs of vehicles and barge	$25,000	$25,000	$25,000	$25,000	$25,000	$25,000
Staff	Managing Director	$120,000	$120,000	$120,000	$120,000	$120,000	$120,000
	General Manager / Coordinator	$22,500	$90,000	$90,000	$90,000	$90,000	$90,000
	Maintenance and general assistance worker	$35,000	$35,000	$35,000	$35,000	$35,000	$35,000
	2 broodstock handlers	$90,000	$90,000	$90,000	$90,000	$90,000	$90,000
	Administrative Assistant	$45,000	$45,000	$45,000	$45,000	$45,000	$45,000

Objective 1.2 *The Handling and selection of Captured Fish*							
Operating	Purchase of fish from farms for experiments	$10,000					
	Purchase of anaesthetics	$5,000					
	Development of fish handling and holding equipment and procedures	$10,000					
	Miscellaneous costs of equipment	$20,000					
	Travel costs for anaesthetics scientist	$10,000					
Staff	Fish anaesthetics and handling expert 6 months at $80,000 pa	$40,000					
Stage 7- Capital works for Broodstock tank building, hatchery and nursery and employment of core staff							
Objective 1.3 *Development of Broodstock Holding Facilities*							
Objective 3.1 *Development of a Hatchery Facility*							
Capital Items	Site Acquisition	$80,000					
	Establishment of power, water and roads	$150,000					
	Office, laboratory, accommodation and Amenities	$170,000					
	Settlement ponds for waste	$50,000					
	Backup power generator	$50,000					
	4 @ 30m tapered to 20m circular 10 m deep in-ground concrete broodstock tanks	$1,000,000					
	Insulated buildings for broodstock tanks 4 @ $400,000	$1,600,000					
	Engineering associated with Broodstock tanks	$100,000					
	Hatchery tanks	$85,000			85,000		
	Hatchery plumbing and electricals	$125,000			125,000		
	Algal / live feeds equipment	$5,000			5,000		
	Temperature control	$30,000					
	Blowers and aerators	$10,000					
	Water intake, pumps etc	$150,000					
	Office & Lab general equipment	$100,000					
Operating	Repairs & Maintenance buildings, equipment and site	$10,000	$20,000	$25,000	$25,000	$25,000	$25,000
	Electricity gas & water usage, rates etc	$10,000	$20,000	$20,000	$20,000	$20,000	$20,000

Office supplies etc	$5,000	$5,000	$5,000	$5,000	$5,000	$5,000

Staff	Algal maintenance	$22,500	$45,000	$45,000	$45,000	$45,000	$45,000
	Rotifer maintenance	$22,500	$45,000	$45,000	$45,000	$45,000	$45,000
	Nursery half time	$11,250	$22,500	$22,500	$22,500	$22,500	$22,500
	Larval Rearing & Artemia	$22,500	$45,000	$45,000	$45,000	$45,000	$45,000
	Coordination	$22,000	$90,000	$90,000	$90,000	$90,000	$90,000
Stage 8- Establishment of Broodstock in land tanks and production of eggs							
Objective 1.4 *Development of a Maturation Diet*							
Operating	Cost of feed for 25 fish by 4 formulations in experimental trials (100 experimental fish)		$102,000	$102,000			
	Travel		$10,000	$10,000			
Staff	Fish Nutritionist half time		$45,000	$45,000			
	Food chemist half time		$45,000	$45,000			
Objective 1.5 *Broodstock maturation and spawning (in conjunction with 1.4)*							
Operating	Purchase of gonadotropins, incorporation in diets etc		$15,000	$15,000			
	Travel		$6,000	$6,000			
Staff	Post Doctoral fellow		$60,000	$60,000			
Objective 1.6 *Monitoring Gonad development (in conjunction with 1.4)*							
Operating	Development of existing probes for skin mucous swabs		$10,000				
	Travel		$6,000				
Staff	Post Doctoral fellow		$60,000				
Objective 2.1 *Egg Handling and Transport*							
Operating	Experimental tanks and equipment for experiments		$10,000				
	Travel		$6,000				
Staff	Post Doctoral Fellow half time		$30,000				
Objective 2.2 *Egg Sterilisation*							
Operating	Purchase of sterilising equipment and reagents		$15,000				
	Travel		$6,000				
Staff	Post Doctoral Fellow half time		$30,000				

Stage 9- Seeking a capital injection by the issue of new shares to new participants

Stage 10- Letting of contracts for R & D work on larval and post-larval development

Objective 3.2 *Identification of a suitable Larval Diet*			
	Letting of 3 Research Contracts each of 4 months for larval diet research		
Operating	On costs for of use of hatchery facilities & support staff	$70,000	$70,000
	Costs of Diets	$15,000	$15,000
Staff	Research scientist 3 x 4 months	$70,000	$70,000
	Research assistant 3x 4 months	$37,000	$37,000
Objective 3.3 *Environmental Conditions for Larval Development*			
	Letting 3 of 3 research contracts each of 4 months for larval environment research		
Operating	On costs for of use of hatchery facilities and support staff	$70,000	$70,000
	Costs of Diets	$15,000	$15,000
Staff	Research scientist 3 x 4 months	$70,000	$70,000
	Research assistant 3x 4 months	$37,000	$37,000
Objective 4.2 *Development of post-metamorphosis holding facilities*			
Operating	Engineering of works for tank and raceway setups at hatchery	$50,000	
Staff	Research scientist	$70,000	
Objective 4.1 *Development of a Post-Metamorphosis diet*			
	Letting of 3 Research Contracts each of 4 months for post-metamorphosis diet research		
Operating	On costs for of use of hatchery facilities	$70,000	$70,000
	Costs of Diets	$15,000	$15,000
Staff	Research scientist 3 x 4 months	$70,000	$70,000
	Research assistant 3x 4 months	$37,000	$37,000
Objective 4.3 *Design and development of suitable transport technology for fingerlings*			
Operating	Purchase of anaesthetics	$5,000	
	Equipment & Engineering	$10,000	
Staff	Research Scientist	$70,000	

Stage 11- Commercial Production of Fingerlings						
Total Annual Expenditure	$5,194,030	$1,543,780	$2,084,280	$1,596,280	$1,020,280	$1,020,280

EQUITY						
Stage 3 Startup Capital						
Mix of individual Company investments and government grants	$4,500,000					
Stage 3 Continuing R & D Funding						
Levies on quota	$526,500	$526,500	$526,500	$526,500	$526,500	
Matching funds from FRDC etc	$526,500	$526,500	$526,500	$526,500	$526,500	
Other Government contributions	$606,500	$606,500	$606,500	$606,500	$606,500	$606,500
INCOME						
Stage 10- Commercial Production of Fingerlings						
100,000 Fingerlings produced at $A2.7 each from one run a year				$270,000		
Stage 11- Commercial Production of Fingerlings						
300,000 Fingerlings produced at $2.7 each from one run a year					$810,000	
Stage 12- Commercial Production of Fingerlings						
700,000 Fingerlings produced at $2.7 each from two runs a year						$1,890,000
Total annual income	$6,159,500	$1,659,500	$1,659,500	$1,929,500	$2,496,500	$2,496,500
Issued Capital	$6,159,500	$7,819,000	$9,478,500	$11,138,000	$12,797,500	$13,404,00
#$A1 Shares	6,159,500	7,819,000	9,478,500	11,138,000	12,797,500	13,404,000
Fingerlings Produced				100,000	300,000	700,000
CASH FLOW	$965,470	$115,720	($424,780)	$333,220	$1,449,220	$1,476,220

Appendix I

Scientific Review of Published and Available Unpublished Work on the Propagation of Tuna and Similar Species

Contents

INTRODUCTION TO SCIENTIFIC REVIEW

Australia has a good recent history of producing and ongrowing fish in aquacultural systems. However SBT is a new venture and is likely to produce a number of difficulties of a practical and scientific nature. Eckert, Kalish, Majkowski and Petherbridge (1987) collated the titles of English or English abstracted scientific and technical reports and articles published before 1985 on the biology, fisheries and management of SBT. However, they did not review the content of the publications, listing them by author, and the subject of each publication.

This present review summarises the results of published and available unpublished information relevant to the propagation of tuna. It is based on published work identified and abstracted in the Biological Abstracts, AGRICOLA, Commonwealth Agricultural Bureaux, and Current Contents electronic databases from 1970, and also on references derived directly from the primary literature. It includes information about SBT and other species of tuna and where relevant, information gleaned from studies on other species of marine fish propagated in captivity. Observations have been drawn from an examination of abstracted literature, primary scientific publications, and interviews with appropriate available specialists in the field, both scientists and those active in fish husbandry in Australia. Several conference proceedings and articles in the Japanese literature which have been cited in articles related to tuna aquaculture were unavailable during the period of this consultancy. These have not been referenced.

The review is presented in three parts, 1 Broodstock, 2) Eggs & Larvae, and 3) Issues of Reseeding. The first investigates the current state of knowledge relating to the husbandry, growth, diet, reproduction and mortality of broodstock. The second to the collection and treatment of eggs and the growth, development, diet, and environmental relationships of larvae. The third part deals with the issues surrounding the augmentation of wild fish stocks, and includes an examination of the survival and economic success of releases of some marine fish species, problems of genetic mixing between released fish and wild stocks, and a description of the genetic relationships between tuna species and within the SBT species itself.

1. *BROODSTOCK*

1.1 *Broodstock Husbandry*

Propagation of wild marine fish species under aquaculture can only proceed after domestication of initially wild-caught animals. Large and highly active pelagic species such as the SBT present formidable challenges to achieve even the capture and successful holding of mature individuals. To this must be added the difficulty of monitoring their breeding condition prior to maturation, spawning and the production of viable eggs.

1.1.1 *Catching and handling*

Capturing and handling large wild fish present major problems in minimising physical and stress based trauma. Juvenile SBT, *Thunnus maccoyii*, occur in surface waters of the Great Australian Bight where they are encircled and held in modified purse seine nets. Thereafter they are towed considerable distances before being transferred to large open water sea cages at Port Lincoln for ongrowing (Lee, 1998). While most fish that are caught and held by this method are around 20 kg, the odd fish is caught at 60-70 kg. When they occur, bigger fish (about 40 kg) are usually deeper, with the smaller (15-20 kg) fish swimming above them (Nelligan, J. *pers com*). Mature but unripe adults are also caught by the long line fishery off New Zealand, south west of Tasmania, and in the Southern Indian Ocean (Farley and Davis, 1998). Fish caught in these locations are up to 150 kg weight (Santic *pers com).* Mature spawning fish are caught with long lines by the Indonesian fishery in the Eastern Indian Ocean between the Sunda Islands and Australia (Davis, Bahar, Farley, 1995).

Juvenile northern bluefin tuna have also been caught on troll lines, using barbless hooks and successfully transferred to net cages. Yellowfin tuna have also been caught at between 2-7 kg and transferred successfully into circular concrete tanks, between 1.5 and 6 m deep and up to 37, 000 L in volume (Lee, 1998).

Cradles have also been used to hold juvenile SBT for tagging purposes, and in conjunction with anaesthetics, are used to handle broodstock of yellowtail kingfish, *Seriola grandis* (Tindale *pers com*). A portable hinged aluminium restraint cradle has been described for handling wild caught salmonids up to 130 cm fork length. Fish that were caught by hook and line were transferred from the hook to a 2.5 cm stretch mesh soft knotless nylon dip net, then rolled into the cradle which was suspended in the water This method was used successfully to process over hooked 400 salmon with only a 7% mortality (Larson, 1995).

1.1.2 *Sedation*

Domesticated fish are usually handled during the normal processes of moving them from tank to tank, assessing the stage of maturity, treatment for disease or the induction of ovulation and spawning. Anaesthetics are primarily used to minimise stress during these handling operations and if used on fish not for human consumption, a range of anaesthetics may be used with varying effects. However anaesthetics can in themselves be very potent stressors, and the very different natures of chemicals in use as fish anaesthetics, as well as the differing responses of

fish species to a particular anaesthetic emphasise the need to assess the effective dose for particular water quality, fish size and species, and the desired level of anaesthesia (Iwama, McGeer and Pawluk, 1989). Thus a comparison of the effects of four anaesthetics on the heart rate, dorsal and ventral aortic blood pressure, and electrocardiograms of rainbow trout, *Oncorhynchus mykiss* showed that exposure to the local anaesthetics tricaine methanesulfonate (MS-222) and benzocaine hydrochloride (BZH) produced minimal cardiovascular alterations compared with the general anaesthetic 2-phenoxyethanol or the hypnotic agent etomidate (Fredericks, Gingerich and Fater, 1993).

Solutions of 2.66 g sodium bicarbonate/L or 60 mg clove oil/L have been found to be optimal as anaesthetics for use in fresh water on walleyes salmon *Stizostedion vitreum* at 10°C. At these concentrations, complete immobilisation occurred in about 7 and 4 min respectively, and recovery occurred in about 5 and 10 min respectively, for sodium bicarbonate and clove oil. These concentrations were also found to be suitable for use on small-mouth bass, *Micropterus dolomieu*, northern pike, *Esox lucius*, and lake sturgeon, *Acipenser fulvescens*. Clove oil was recommended over sodium bicarbonate for surgical procedures, however, recovery took substantially longer when time under clove oil anaesthesia exceeded 5 min (Peake, 1998).

There is concern that because of the "ram jet" method of gill ventilation of tunas, the use of anaesthetics may not be possible with SBT due to asphyxia associated with anaesthesia. However, a series of experiments designed to reduce pre-harvest stress, demonstrated that both carbon dioxide and AQUIES (containing the active constituent of clove oil) were effective in anaesthetising this species and individual fish were observed to recover after anaesthesia. However, because the intention of the experiments was to reduce pre-harvest stress, the best concentrations for recovery were not determined (Goodrich, B., *pers com*).

Low levels of MS-222 (0.1g per litre) have also been used successfully to aesthetise skipjack tuna, *Katsuwonus pelamis* (Arthur, West, Brill, Schulte and Hochachka, 1992).

1.1.3 *Gonad Monitoring*

Induction of maturity, and spawning requires ongoing monitoring of the sex and stage of maturity of broodstock. While visual differentiation between male and female SBT is possible in 7- 8 year old fish this is not possible for younger animals (Rough, K., *pers com*).

Low resolution ultra sound images have been used to determine both the sex and maturity of pacific herring *Clupea harengus* and Atlantic salmon, *Salmo salar* by the use of ultra sonic scanners (Bonar, Thomas, Pauley and Martin, 1989, Mattson, 1991). However in these cases the fish were either anaesthetised or killed and held in small tanks prior to scanning.

A less invasive way of determining the sex and reproductive activity of adult females fish has been shown to be the use of an enzyme linked immunoabsorbent assay for vitellogenin in skin mucus swabs. This was demonstrated to reflect the gonad condition in the greater amberjack, *Seriola dumerili* (Takemura, Takeuchi, Teruya, Oka, and Kanematsu, 1999) and identified the sex of yellowfin tuna, *Thunnus albacares* (Takemura and Oka, 1998) and striped bass, *Morone saxatilis*

(Kishida, Anderson, and Specker, 1992). The extent to which this method is appropriate for SBT has still to be determined.

1.2 *Broodstock Growth*

One of the most attractive features about the farming of SBT is their attractive growth rate. Yet until recently, there was considerable speculation about the size at which this species reached one or two years old, and there is still speculation on the size or age of first maturity. Much of this is due to the remarkable variation in growth from year to year, and the restricted nature of the data obtained from analyses of fish that are either caught or tagged from the Australian fishery, as this only targets a restricted age group. Recent studies of otolith increments have tended to confirm earlier results based on modal analyses, of the fast growth of one and two year olds, and discredit results based on modelling tag returns.

1.2.1 *Wild Populations of SBT*

Considerable variation in growth rates of SBT from year to year was first postulated by Serventy (1956) who believed that growth took place entirely in the summer months between October and May. A similar conclusion was reached by Thorogood, (1987) who suggested that growth was rapid from November to June, thereafter decreasing significantly. Hearn (1986) also showed that growth was much faster in summer/autumn than in winter/spring.

Two spawning times were detected from counting daily otolith growth increments in fish from off west and south west Australia between January 1 and February 5, 1990-92. A smaller September-October spawning, and a larger January-February spawning (Itoh and Tsuji, 1996).

Two or more groups of one year old fish were also identified from Western Australia by Leigh and Hearn (2000). In February/March 1986, one group was around 52 cm long and a second group was around 65 cm. Catch numbers in the lower modes were more scarce, especially after 1969, the start of the Western Australian fishery. The two groups appeared to merge as two-year-olds. Two size modes were also found in juvenile stages of the albacore, *Thunnus alalunga* in the southern Tyrrhenian sea, from individuals born at the beginning or end of the reproductive season (May-Sept)). As the fish grew, the difference between the two modes also reduced there (Cefali and Potoschi, 1982).

Serventy (1956) by analysis of modal lengths of fish catches, believed that the species attained a mean length of 41 cm after its first year's growing period and after the second year, a mean length of 61 cm. Two size groups of fish were also identified by the same method off Western Australia between 3/5- 4/7/62 by Hynd (1965) with modal sizes of approximately 60 cm and 70 cm.

The results of early studies on the growth of one- and two-year-old fish were recently corroborated by counting daily growth increments on otoliths. Itoh and Tsuji (1996) estimated the mean length of fish at age 1 and two as 50.8 cm and 78.6 cm respectively and confirmation that one-year-old SBT are 40-55 cm FL. was supplied by Gunn, Davis, Farley, Clear and Haskard (1996) who examined otoliths, vertebrae and scales as a basis for aging.

The mean size of fish at age calculated from modal analysis of length frequency data from commercial catches (Leigh and Hearn, 2000, Serventy, 1956) are given in table 1. Lengths given from Leigh and Hearn's mean for 1 year olds is the mean of multiple modes and the reference month for WA and SA is the first half of March, whereas in NSW it is the 1st half of December. Also included in table 1 are estimates for mean length at age from tagged fish released between 1962 and 1978 using 7 different models on the data (Hampton, 1991a), and three studies which estimated mean lengths at age from counts of annual otolith increments (Thorogood, 1987, Gunn, Davis, Farley, Clear and Haskard, 1996, Itoh and Tsuji, 1996).

The results given in table 1 show considerable variability in size at age. Part of this may be due to the intrinsic variability of growth in tuna species. However two recent investigations of the use of annual growth increments in the otoliths to determine age suggest that this method is likely to be the most accurate. The first study (a detailed examination of the potential of injection of strontium chloride in conjunction with a large scale tagging exercise) confirmed that for fish with between 1 and 6 increments in their otoliths, one increment is laid down annually. During the same study two previously tagged fish were recaptured. Eleven annual increments were present on the fish released as a 1 year old (45 cm) and that had been at liberty for 9 years 7 months. and thirteen increments were counted on the other, released as a 2 year old (82 cm) and that had been at liberty for 10.75 years (Clear, Gunn, and Rees, 2000). The second study measured the concentration of atomic bomb radiocarbon in fish otoliths and suggested that increments were laid down annually, and that individual fish were capable of living for over 30 years (Kalish, Johnston, Gunn and Clear, 1996).

Table 1 Estimates of length (cm FL) at age (years) of SBT

Age (Years)	1	2	3	4	5	6	7	8	9
Leigh and Hearn,									
2000.	58.3	74.8							
WA	59.6	78.4	94.1	107.8					
SA	71.8	89.2	102.0						
NSW									
Seventy, 1955	41	61	73	83					
Hampton, 1991									
Model 1	38.3	57.4	74.3	89.2	102.4	114.0	124.2	133.3	141.2
Model 2	33.1	54.5	72.2	86.9	99.0	109.0	117.3	124.2	129.9
Model 3	36.3	56.0	73.1	88.0	100.9	112.3	121.9	130.4	137.8
Model 4	36.6	56.0	73.1	88.0	100.9	112.1	121.8	130.3	137.6
Model 5	35.6	55.4	72.3	86.6	98.8	109.3	118.2	125.8	132.4
Model 6	36.6	56.2	73.2	88.1	101.0	112.2	122.0	130.5	138.0
Model 7	36.6	56.1	73.2	88.0	100.9	112.1	121.9	130.4	137.8
Thorogood, 1987	30.6	54.3	75.4	94.5	111.5	126.9	140.6	153.0	164.1
Gunn et al 1996	52.0	77.6	95.8	112.7	122.8	129.4	139.5	147.5	147.3
Itoh and Tsujii, 1996	50.8	78.6							

1.2.2 *Growth of Captive SBT*

Juvenile SBT are caught between 3 and five years old in purse seines from the Great Australian Bight at about 20 kg weight. They are towed in specially modified nets to Port Lincoln where they are transferred to cage farms and transferred to holding cages. They are fed a frozen pilchard diet while being on-grown in the cages and harvested after reaching a weight of about 30 kg after four to nine months (Lee, 1998). The only existing estimate of the long term growth rates of captive populations is from fish were held at Port Lincoln, SA over a number of years to investigate maturity and spawning. All these died in a mass mortality event at the site in April/May, 1966 (Munday and Hallegraeff, 1998). These fish were caught as two year olds and were seven years old at the time of death. They had been fed a diet of frozen sardines and mackerel and Nippai formula pellets and had achieved a mean size of 149 cm FL (68.3 kg) for 18 females and 154 cm (79.9 kg) for 12 males. (Rough *pers com*.).

1.3 *Broodstock Diet*

The influence of broodstock diet on egg condition and survival is now well documented for many species of marine fish, and it is unlikely that tuna species will differ to any marked extent. The principal diet of wild-caught tunas consists of various species of cephalopods, crustaceans and fishes. The species eaten and ratio of each taxonomic group in the diet varies between species, age of individual, location and time of year. The present diet fed to farmed SBT is almost exclusively frozen clupeids, and while producing a marketable commodity in the short term, it is unlikely to be satisfactory for developing healthy broodstock and viable eggs and larvae.

1.3.1 *Diet of Wild Tuna Populations*

The prey of skipjack tuna, *Katsuwonus pelamis* is mainly crustaceans or pelagic fish. Gonostomatids, *Maurolicus muelleri* and euphausids, *Euphasia similis* were described as its principal food off Brazil (Ankenbrandt, 1985) while off the northeast coast of Cuba, fishes were 87% of the diet (Guevara, Carrio, Wetango, and Amato, 1987).

Yellowfin tuna, *Thunnus albacares* have generally been described as feeding mainly on mesopelagic squid and fish in both the Pacific and Atlantic Oceans (Borodulina, 1981, Manooch and Mason, 1983). They were also described as feeding mainly on fish, crustaceans and cephalopods in the western Pacific Ocean (Kim, Moon, Kwon, Kim, and Jo, 1997). Off southern Brazil, squid and fish were more important in winter while hyperiid amphipods were the main food in spring (Vaske and Castello, 1998). Diurnal patterns in the stomach fullness and the frequency of occurrence of some prey in the diet of yellowfin tuna caught around FADs indicated that they do not feed at night but that they may prey on vertically migrating mesopelagic organisms at dusk and dawn (Buckley and Miller, 1994).

Crustaceans and cephalopods comprised 31% of the food volume found in the stomachs of blackfin tuna (*Thunnus atlanticus*). The rest consisted mostly of fishes (75%) (Manooch and Mason, 1983).

1.3.2 *Diet of Wild SBT*

Juvenile (1-4 yrs) SBT, like other species of tuna, feed on fish, crustaceans and molluscs. They are omniverous and random feeders, their stomach contents reflecting the relative components of the macroplankton and smaller nekton of the areas in which they are caught. Juveniles are surface schooling. They, and younger sub-adults feed on surface schools of sardines, jack mackerel, and krill (Stanley *pers com*). Off eastern Australia its diet is extremely variable and depends on prey availability. Serventy, (1956) described anchovies and pilchards as important in one year, while in the next the crustacean *Nyctiphanes australis* was common The largest fish habitually taken are jack mackerel *Scomber australicus* and pilchards *Emmelichthys nitidus.* Other fish included a range of small, mostly pelagic species, while the most common cephalopod is the squid *Notodarus gouldi* (Serventy, 1955). In another study forty-two years later crustaceans were described as contributing little to their diet which now consisted principally of jack mackerel, pilchard and juvenile squid, *N. gouldi* (Young, Lamb, Le, Bradford, and Whitelaw, 1997).

Diet items varied between locations, in South Australia it was less variable, consisting mainly of clupeoids, including pilchards, blue sprats, and anchovies. In Western Australia pilchards were overwhelmingly the preferred diet (Serventy, 1956).

Mature SBT occur off Southern Africa from May to September. While there, they feed in the inshore upwellings of the Benguela current, mostly on fish (64%), prawns (30%), squid (4%) and tunicates (2%). They feed mainly in the early morning, thereafter slackening until evening, then increasing. Probably no feeding occurs at night (Talbot and Penrith, 1963). Most feeding is in deep waters, preying on hake, *Merluccius capensis,* frost fish, *Lepidopus caudatus.* and the prawn, *Funchalia woodwardi.*

In waters off southern Australia a diverse mix of cephalopod species, but mostly *Lycoteuthis lorigera* and adult *N. gouldi* made up the largest component in the diet of offshore adults. The offshore larger (greater than 150 cm) tuna had macrozooplankton prey prevalent in their diet (eg *Phronima sedentaria*). When in subantarctic waters they ate relatively more squid than in the East Australian Current. In the latter, fish and crustacea were more important (Young, Lamb, Le, Bradford and Whitelaw, 1997).

Adult fish in the south Indian Ocean (40-45°S 80-105°E) feed on myctophids, rays bream and other species opportunistically. In winter there is a significant squid component in their diet. Fish are caught on squid bait when the sea surface temperature is between 8-13°C. When above 17°C pilchards are more effective, presumably relating to the availability of their feed. The peak of fishing activity in this area is on tuna caught in association with schools of krill in surface waters, where they feed close to the surface, most fishing ending in April/May. A second peak of catches on squid finishes around October and this is believed to be the trigger for a run of animals going north to spawn. In this area bigger fish are caught from deeper, colder water (Stanley, R., *pers com*). Big fish are also found south-west of Tasmania mostly in summer where the mixed zone is about 150-200 m deep and water temperatures are between 12-14°C. In this location a trigger for a northerly migration to off the coast of New South Wales appears to be a warm southerly movement of water in late April (Lyne, V., *pers com*).

1.3.3 *Maturation Diets for Marine Teleost Fish*

The importance of adequate diet for maturation and egg production in captive marine fish species was demonstrated in a study of the fatty acid profiles from eggs of wild striped bass, *Morone saxatilis*. These were significantly higher in total lipid, n-3 highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in wild fish than in domesticated individuals fed a commercial diet. The mean ratio of n-3 / n-6 fatty acids from wild fish was almost an order of magnitude higher (Harrell, and Woods, 1995). Dietary experiments have confirmed the key role of broodstock diet in several species of marine fish.

Red Sea Bream (Chrysophrys major): Egg quality, hatching rate, larval growth, survival and metamorphosis of Red Sea bream have all been described as positively influenced by addition of cephalopod or crustacean components to the diet of broodstock. An increase in total eggs produced and percentage of buoyant eggs also occurred after feeding frozen raw krill shortly before spawning. This was reduced when cuttlefish liver oil in the diet formulation was replaced with maize oil. The rate of hatching was not improved by addition of pigments such as beta-carotene, canthaxanthin or astaxanthin, but abnormality in the number and position of oil globules was reduced and the total number of normal larvae produced was higher than in the original broodstock (Watanabe, Itoh, Murakami, Tsukashima, Kitajima, and Fujita, 1984). More recently, the phospholipid and astaxanthin fractions of krill meal fed prior to spawning has also been shown to improve egg quality (Watanabe, Fujima, Lee, Fukisho, Satoh and Takeuchi, 1991).

Similar results were obtained fish given a diet containing cuttlefish meal rather than white fish as the protein source. Here the percentage of buoyant eggs, total hatch and normal larvae obtained with different dietary protein levels were markedly changed only with the cuttlefish meal (Watanabe, Itoh, Kitajima, and Fujita, 1984).

Defatted or intact cuttlefish meal, or frozen raw krill all increase the percentage of buoyant eggs and normal larvae of this species above that obtained from a white fish diet. Equally good results are obtained by replacement of cuttlefish liver oil in the control diet with 2.5% krill polar lipid or 2.5% krill nonpolar lipid. However defatted krill meal or the fat-soluble fraction of cuttlefish meal did not have a good effect on egg quality. The effective components in raw krill, aiding the reproduction of red seabream, has been suggested to be the polar and nonpolar lipid fractions. Vitamin E was also found to have the same efficiency in improving egg quality (Watanabe, Lee, Mizutani, Yamada, Satoh, Takeuchi, Yoshida, Kitada and Arakawa, 1991).

Gilthead seabream (Sparus aurata): Larval growth, survival and swim bladder inflation in the gilthead seabream are also improved by the inclusion of squid meal in the diet of broodstock. In this species both the protein and lipid fractions in squid meal have been shown to play an important role in improving the fecundity and egg quality. A dietary inclusion in broodstock of n-3 HUFA, when compared with soy oil, was associated with increased growth and more larvae with a functional swim bladder. The protein fraction of squid meal was also shown to have a significant effect on the egg quality and the amino acid composition of squid shown similar to that of the seabream egg. The presence of a balanced composition of essential amino acids expressed itself in vitellogenin synthesis and selective uptake (Tandler, Harel,

Koven and Kolkovski, 1995). The specific component of squid meal which enhances spawning quality appeared to be in the fat-insoluble fraction. Increased dietary levels of EPA in diets correlated with the number of fertilised eggs (Fernandez-Palacios, Izquierdo, Robaina, Valencia and Salhi, 1997).

After 3 weeks of feeding, significantly improved spawning quality in terms of fecundity, hatching and larval survival, was also observed in broodstock fed 1.6% n-3 HUFA. Unfertilised egg rates were significantly reduced by the increase in dietary 20:5n-3. The n-3 HUFA content in the eggs showed a positive correlation with that in the diets, mainly due to the change in 20:5n-3 content of the eggs (Fernandez-Palacios, Izquierdo, Robaina, Valencia, Salhi and Vergara, 1995). By contrast, egg viability was shown to significantly decrease within 10 days of feeding broodstock with a diet deficient in n-3 HUFA. The levels of n-3 HUFA in both polar and neutral fractions of egg lipid were also directly correlated with their levels in the broodstock diet. (Harel, Tandler, Kissil and Applebaum, 1994)

Yellowtail, (Seriola quinqueradiata) : The quality of eggs and larvae from broodstock of the yellowtail were also considered to benefit from the addition of krill meal to a commercial diet (Verakunpiriya, Watanabe, Mushiake, Kawano, Kobayashi, Hasegawa, Viswanath, Satoh, Watanabe, Visuthi and Kiron, 1997). The addition of astaxanthin for 5 months prior to spawning, also produced more eggs, of better quality, and greater numbers of larvae in this species than unsupplemented diets or diets with lower levels of astaxanthin (Verakunpiriya, Mushiake, Kawano, Watanabe and Visuthi, 1997). However when Atlantic salmon (*Salmo salar*) were fed a supplementary diet of astaxanthin there was no relationship between the astaxanthin concentration of the eggs and their fertilisation rates, survival from fertilisation to eyed stage or from eyed stage to hatching. Nor did free embryos that hatched from eggs with a high astaxanthin concentration perform better than those hatched from eggs with a low astaxanthin concentration (Christiansen and Torrissen, 1997).

Turbot (Scophthalmus maximus) : When turbot broodstock were fed diets of mackerel or trash fish varying in omega-3 highly unsaturated fatty acid (HUFA) and vitamins C and E, for 2-3 months prior to the reproductive season, the oil globule significantly increased in eggs of the vitamin-supplemented groups. These observations were not correlated with hatching or fertilisation rates. High HUFA levels in the broodstock diet also resulted in a significant increase of egg diameter, oil globule diameter and fertilisation rate. The control groups, with the lowest fertilisation rate, had the highest hatching rates, significantly higher than the HUFA / non-vitamin enriched groups. Enrichment of trash fish diets with ascorbate-2-polyphosphate resulted in a tripling of the vitamin C content The major fatty acids in turbot eggs and freshly hatched larva were 16:0, 18:1 omega-9, 20:5 omega-3 and 22:6 omega-3, but apart from the level of 20:4 omega-6 which was significantly higher in the control group, no obvious changes in their pattern could be detected for the different broodstock treatments. The hatching percentage was also positively correlated with levels of 20:4 omega-6. (Lavens, Lebegue, Jaunet, Brunel, Dhert, and Sorgeloos, 1999).

European sea bass (Dicentrarchus labrax): Commercially fabricated diets allow greater control over the composition of biochemical components and reduce the risks of disease introduction. However, satisfying the dietary lipid requirements of

marine broodstock using artificial diets has proved difficult, particularly with respect to their HUFA composition. The survival of the progeny of the European sea bass were greatly affected by the levels of a dietary protein and carbohydrate (Cerda, Carrillo, Zanuy, Ramos, and de la Higuera, 1994). Long-term dietary deficiencies in n-3 fatty acids were also shown to affect the patterns of plasma lipids in broodstock of this species and may induce early gonadal atresia, reducing the production of gonadal steroids, fecundity and subsequent egg survival (Cerda, Zanuy, Carrillo, Ramos and Serrano, 1995). By contrast, manipulation of dietary arachidonic acid (20:4 n - 6;), EPA (20:5 n - 3) and DHA (22:6 n - 3) in dry pelleted diets improved levels and ratios of each, which were then transferred to the resulting eggs with improvements in early survival and hatching success repeated over successive spawning seasons. (Bruce, Oyen, Bell, Asturiano, Farndale, Carrillo, Zanuy, Ramos and Bromage, 1999).

1.4. *Reproductive behaviour in Wild Populations*

Many tuna species undergo extensive migration, and are highly specific in the locations and time that they choose to spawn. If the propagation of SBT is to be achieved, then a way must be found to successfully induce gonadial maturation and reproduction in captivity. Clues to this may be found in the reproductive behaviours of tunas in the wild.

1.4.1 *Tunas in General*

Tunas are highly fecund, and those investigated are capable of multiple spawning. The yellowfin tuna reproduces in summer. In the Mexican Pacific it was reported as spawning from March to November with peaks in activity in April-May and October-November. (Gonzales and Ramirez, 1990). In the Coral Sea, spawning lasted from October to late February, starting in the north-west, and finishing in the central-west. It spawned in late evening and early morning. Final oocyte maturation occurred in less than 24 hours and postovulatory follicles could not be identified 24 hours after spawning. The average spawning frequency of females was once every 1.54 days, larger fish spawned more frequently (McPherson, 1991). In the Western Indian Ocean an analysis of otolith microstructure suggested that they spawn successfully throughout the year, but that most occurred between November and March (Stequert, Panfili and Dean, 1996).

In the Eastern Pacific Ocean an examination of this species caught by the purse seine fleet, suggested that spawning was widespread from 26°N to 14°S and from the coast to 140°W, the western edge of the fishery. Between 0° and 20°N spawning occurred throughout the year, with no pronounced seasonal patterns in intensity. North of 20°N spawning occurred mostly from July to November, when sea-surface temperatures exceeded 24°C. South of 0° spawning occurred between November and February, when the sea surface temperature exceeded about 24°C. Over 85% of the spawning occurred between 26-30°C and spawning occurred almost entirely at night between 2200 h and 0600 h and the average female spawned every 1.52 d. There was a positive nonlinear relationship between the fraction of mature females spawning per day and length (Schaefer, 1998).

Spawning bigeye tuna, *Thunnus obesus* were caught between January and March in the waters off Java, and off Hawaii from May to July (Nikaido, Miyabi and Ueyanagi,

1991). From histological examination it was considered that they spawn from around 1900 to 2400 in both areas. They considered that spawning occurred once daily.

In the Western Indian Ocean the age at first maturity for skipjack tuna, *Katsuwonus pelamis* is about 1.5-years old. Spawning occurs all year with more intense sexual activity during the two monsoon seasons, from November to March and from the beginning of June to the end of August. (Stequert and Ramcharrun, 1996).

The albacore, spawns between November to February off New Caledonia (Ramon and Bailey, 1996).

In three areas of the eastern Pacific Ocean, black skipjack tuna, *Euthynnus lineatus* spawned from August to October from the Revillagigedo Islands to Clipperton Atoll, from October to June off Central America, and from November to March in the Gulf of Panama. No evidence of spawning was observed off Colombia and Ecuador. The estimated average time between spawning a new batch of eggs was between 2.1 and 5.7 days and the average mature female spawned between 43 and 58 times a year in these areas (Schaefer, 1987).

The two major areas for spawning of Atlantic northern bluefin tuna (*Thynnus thynnus*) are approximately 4,000 miles apart, in the Gulf of Mexico and in the Florida Straits in April, May and June, and in the Mediterranean Sea during May, June and July (Baglin, 1982). An examination of the ovaries of fish taken from the Western Atlantic and off the North-east coast of North America showed little development of maturity in fish estimated from a length-age - weight table (Coan, 1976) to be between 1 and 7 years old, (Baglin, 1982). However, in the Mediterranean Sea 50% have been reported to mature at 3 years of age (Rodriguez-Roda, 1967). Individuals from populations on each side of the North Atlantic are believed not to mix (Nemerson, Berkeley and Safina, 2000). In the Pacific, a tagging study has shown that although this species is distributed across the entire north Pacific Ocean individuals freely mix and, it spawns only in the western Pacific between April and July (Bayliff, Ishizuka and Deriso, 1991).

1.4.2 *SBT*

An early study of tuna larvae collected in a series of cruises in the Indian Ocean found SBT larvae only present between October and March, and then only from a restricted area in the Eastern Indian Ocean between the Sunda Islands and Australia (Ueyanagi, 1969).

Lean SBT with brownish coloured flesh were also found in catches from this region (the "Oka" grounds) in October and March. The lean fish were all over 130 m in length, a size below which fish were considered to be immature. They were also found in higher altitudes from November to June. It was concluded that in August and September fat adults move northwards to the spawning grounds from an area based at about 40-50°S, 105°E. After spawning, the spent fish moved south with the Leeuwin current, at least some of them reappearing in the Tasman Sea from November onwards, dominating the southern fishing grounds (40-50°S) between February and March (Warashina and Hisada, 1970).

This scenario was supported by data collected on gonad condition. Ovarian weights were greatest in samples from the “Oka” grounds, most individuals with ovaries weighing between 1.6 and 3.2 kg . In the “Oki” grounds (20-30°S, 90-110°E) most

individuals had ovaries between 300 g and 1.8 kg, whilst in the southern grounds (40-50°S off the southeast of Australia and off New Zealand) individuals with an ovary weight heavier than 400 gm were rare. Most of the fish taken in the “Oki” or “Oka” grounds were either in a pre-spawning or spawning condition (Shingu, 1978). A subsequent study in similar regions, which included both smaller and larger fish, confirmed these results. All females collected from the "Oka" spawning grounds were mature, 69.2% spawning, 30.2% nonspawning, and 0.6% (3 individuals in October) postspawning. Fish from the southern feeding grounds were found with gonads smaller than 1 kg, whereas ovaries from the S E Indian Ocean fish weighed up to 2.8 kg. Females from the spawning grounds had larger ovaries of up to 7 kg in weight. (Farley and Davis, 1998).

The ovaries of fish in catches from the southern grounds off the southeast and south of Australia in August, March and January 1984-5 all contained animals that had reached primary yolk stage, largest individuals had ripe gonads in March and January, while atretic ovaries were detected in January. In August, even the biggest fish only reached a primary yolk stage (Thorogood, 1986). All ovaries examined by Farley and Davis (1998), from the same regions were either immature, post spawning, or nonspawning (mature). These last were characterised by the possession of ovaries with advanced yolked oocytes but no evidence of spawning activity (migratory nucleus or hydrated oocytes or post-ovulatory oocytes).

Although this species was also taken from waters adjacent to South Africa, juveniles were not found there, and adults were only found from May to September, occurring in the inshore upwellings of the Benguela current and further offshore in the South Atlantic surface waters. From January to March the vast bulk of the stocks disappear from the area. Adults have never been found to be ripe there (Talbot and Penrith, 1963).

SBT catches are caught by the Indonesian fishery from the "Oka" spawning grounds in all months except July. Catches show a marked seasonality in abundance, lowest from May to August, and highest in October and February (Davis, Bahar and Farley, 1995). There is also a trend for larger, heavier fish to be caught at the beginning of the season (August) and smaller fish later in the year, but the data presented are indicative rather than conclusive. Japanese CPUE data suggests that early in the fishery there were 2 peaks in fish abundance, the first between September and October, the second between February and March (Farley and Davis, 1998). These coincide well with the two modes in spawning determined by back calculation from counting daily rings in otoliths (a smaller September-October mode, and a larger January-February mode (Itoh and Tsujii, 1996).

Females are capable of multiple spawning doing so on average every 1.1 days (Farley and Davis, 1998). The size at which sexual maturity was attained was initially considered to be 130 cm in length as only fish of this size or greater were found on the spawning grounds (Shingu, 1978). Warashina and Hisada (1970) came to the same conclusion finding all leaner fish with brownish coloured flesh to be longer than 130 cm, and hence considered to be post spawners. Thorogood (1986) suggested that the smallest size at which fish were seen with ripe gonads in southern waters was about 118 cm. The smallest fish with mature gonads found on the spawning grounds by Farley and Davis (1998) was 147 cm FL. This may have been due to the

fact that fish examined by them were caught from the Indonesian fishery which generally sets its lines shallower than the Japanese boats and in this region more small and nonspawning fish are caught deeper than larger and spawning fish (Davis and Farley, 2001).

Gunn, Davis, Farley, Clear and Haskard (1996) aged 150 fish from the spawning grounds and 261 from the southern fishing grounds south of 30°S by counting annual otolith increments. They concluded that the mean age of first spawning of this species may be as high as 12-13 years old. However fish as young as 8 years old were also found on the spawning grounds.

1.5 *Induction of maturation and spawning*

Primary gonadial maturation in fishes can frequently be induced by variation in the periodicity of illumination and water temperature. However chemical methods of manipulating reproductive activity are widely used in fish aquaculture, particularly in the induction of oocyte maturation, and ovulation.

1.5.1 *Hormonal Stimuli*

Most research on the control of reproduction in fishes has focused on female physiology because ovarian development and maturation are easily disturbed by environmental stresses. Final gonadal growth and spawning usually can be achieved by treatment with gonadotropin-releasing hormone analogues (GnRHa) either by injection, implant, or feeding, and in some species, have to be applied in combination with dopamine antagonists to enhance responsiveness to GnRHa (Patino, 1997).

In salmonids gametogenisis is regulated by 2 gonadotropins (GTHs) that are chemically and biologically similar to FSH and LH of tetrapods. GTH I is present in the plasma of immature fish, and fish during the time of vitellogenisis and spermatogenisis, while GTH II levels increase at the time of ovulation and spermiation (Swanson, 1991). Two homologus gonadotropins have also been found in marine non- salmonid fish, e.g. Mediterranean yellowtail, *Seriola dumerilii* (Garcia, Koide, Diaz, and Kawauchi, 1997), Atlantic croaker *Micropogonias undulatus* (Copeland and Thomas, 1993), Japanese eel, *Anguilla japonica* (Nagae, Todo, Gen, Kato, Young, Adachi and Yamauchi, 1996), and red seabream, *Pagrus major*, (Tanaka, Kagawa, Okuzawa and Hirose, 1991)

Two different molecular forms of gonadotropins, designated tGTH I and tGTH II, have also been isolated from the pituitary glands of bigeye tuna. Both tGTH I and tGTH II stimulated estradiol-17-beta and testosterone production in tuna ovarian follicles in vitro, although responses to tGTH II were significantly greater than those to tGTH I. As with salmonids, each gonadotropin consisted of alpha- and beta-subunits. Unlike salmonids, the alpha subunits of tGTH I and tGTH II had identical amino acid sequences. The tGTH I-beta was structurally more similar to salmon GTH I-beta than to salmon GTH II-beta, whereas tGTH II-beta was also more similar to salmon GTH II-beta. (Okada, Kawazoe, Kimura, Sasamoto, Aida and Kawauchi, 1994).

Immunocytochemical identification of GTH I and GTH II cells in the pituitary of the northern bluefin tuna suggested that, as with other fish, GTH I and GTH II are

synthesized in separate cells in its pituitary. Both GTH I beta- and GTH II beta-immunoreactive cells were observed in immature northern bluefin tuna, although there were greater numbers of the latter.

It has been suggested that despite the chemical similarity of these two gonadotropins to those of salmonids, the pattern of their production and secretion in northern bluefin tuna may be different, and GTH II may also play an important role in the early phases of gonad development. (Kagawa, Kawazoe, Tanaka and Okuzawa, 1998). However, this is unlikely as GTH II was described as playing a major role in final maturation of another, non-salmonid fish, the Japanese eel (Nagae, Todo, Gen, Kato, Young, Adachi and Yamauchi, 1996). The diurnal variation of plasma GTH II of female red seabream also increased at the migratory nucleus-stage maturation of oocytes, to a peak at oocyte maturation at 08.00 h, followed by a rapid decrease at 18.00 h, when spawning occurred (Tanaka, Kagawa, Okuzawa and Hirose, 1991).

1.5.2 *Light and Temperature Stimuli*

Primary gonadial maturation in fishes can frequently be induced by variation in the periodicity of illumination and water temperature. Spawning of rainbow trout, *Oncorhynchus mykiss* broodstock was advanced by 4 months in groups of fish exposed to 14 weeks of long days (18-h light: 6-h darkness), followed by short days (6L:18D). The number of eggs spawned per kg body weight and survival to the eyed stage were not affected by day length or temperature but when compared with the natural photoperiod, long days reduced the egg diameter (Davies and Bromage, 1991).

When the photoperiod of broodstock was manipulated and enriched live feed used, it was possible to produce halibut, *Hippoglossus hippoglossus* fry at any time of the year (Holmefjord, Gulbrandsen, Lein, Refstie, Leger, Harboe, Huse, Sorgeloos, Bolla, Olsen, Reitan, Vadstein, Oie and Danielsberg, 1993). Year-round production of juvenile halibut was subsequently achieved from a broodstock of Atlantic halibut maintained on a 6-month delayed photoperiod, (Naess, Harboe, Mangor-Jensen, Naas and Norberg, 1996).

The effects of four combinations of light regime and temperature on the maturation of the striped mullet, *Mugil cephalus.* concluded that short days and low temperature had the most stimulatory effect while long days and high temperature had the most inhibitory effect on oocyte growth. The short photoperiod stimulated the onset of the cortical vesicle stage at both temperatures, while the lower temperature stimulated the onset of vitellogenesis under both photoperiods. The higher temperature caused atresia of vitellogenic oocytes under both photoperiods. Complete regression to primary growth-stage oocytes required both the higher temperature and the longer photoperiod(Kelley, Tamaru, Lee, Moriwake and Miyamoto, 1991)

Although successful production of eggs and larvae from captive tuna has been reported for northern bluefin, black skipjack, *Euthynnus lineatus* and yellowfin tuna, there is very little information currently available in the literature relating to their reproduction in captivity. However adult northern bluefin tuna held in cages in Japan, and black skipjack and yellowfin tuna held in concrete tanks in Panama have spawned naturally without hormonal or photoperiod manipulation, apparently in

response to increasing water temperatures. Spawning in northern bluefin tuna usually occurs from 23-28°C, usually between 1730 and 1800 hr at the surface or just below it. Black skipjack tuna spawned at 26-28.7°C and 29-31 ppt salinity. Yellowfin tuna spawned when the daytime average water temperature reached higher than 24°C and spawned repeatedly at 28.1-29.5°C Each spawning occurred around sunset and was preceded by courtship behaviour (Lee, 1998).

1.6 *Pathogens and Predators of Tuna*

Long lived fish such as SBT are generally assumed to have a relatively low mortality once they have reached adult size. However their extremely high fecundity suggests that as larvae they may have a huge mortality. This was confirmed by Davis, Lyne and Jenkins (1991) who estimated an instantaneous natural mortality coefficient of 0.66 per day (a daily survival rate of 52%). Adults, with orders of magnitude less likelihood of dying in the wild, nevertheless have a number of pathogens which under aquaculture conditions, may cause major problems.

1.6.1 *Wild Populations*

Natural mortality : In wild fish, the instantaneous natural mortality coefficient (M) is an estimate of the pooled mortality of a population from all sources. Hayashi, Honma and Shingu (1969) considered a value of 0.2 per year to be appropriate for the instantaneous natural mortality coefficient of SBT, based on comparisons with growth parameter estimates. (Hearn, Sandland and Hampton, 1987) described a method for estimating the instantaneous natural mortality rate of wild fish, and their analysis, of tagging returns from SBT experiments suggested that it may be in excess of 0.4 per year. Hampton (1991b) reviewed the literature on methods of estimating the natural mortality rate in wild fish, and used two models to estimate the instantaneous natural mortality rate from selected tagging experiments. One of his models also estimated the movement rates of fish between geographically separated fisheries. The first of these analyses derived estimates of M that varied from about 0.2 to 0.4 depending if a constant or decreasing tag shedding rate was assumed. However if estimations of movements rates of fish between fisheries was incorporated, and M assumed to be the same in each fishery, then an estimate for the Japanese fishery was reached that was similar to that of Hayashi, Honma and Shingu (1969) ie 0.2 per year. Polacheck, Hearn, Millar and Stanley (1998) updated estimates of instantaneous mortality rates to determine age-specific fishing and natural mortality rates. Their estimates of natural mortality rates declined with age from 0.35 $year^{-1}$ at age 1 to 0.15 $year^{-1}$ at age 8.

Pathogens: Tuna species are known to harbour a variety of parasites in the wild. Parasites found in albacore in the south-west Pacific included 1 apicomplexan, 3 nematode species, 4 cestode species, 1 acanthocephalan, 12 digenean species and 3 copepod species (Jones, 1991). Didymozoid trematodes are particularly prevalent in blood vessels of the tongue, gills and intestines of tuna species, (Nikolaeva and Dubina, 1985). A camallanid nematode has also been described from, northern bluefin tuna , yellowfin tuna , blackfin tuna (*Thunnus atlanticus*) and frigate tuna (*Auxis thazard*), (Moravec, Kohn, and Santos, 1999).

About 60% of yellowfin tuna greater than 3 kg caught near the Hawaiian Islands had plerocercoids of a larval cestode in the anterior dorsal aorta, (Brill, Bourke, Brock and Dailey, 1987)

Monogenean trematodes of the family Capsalidae have also been described from the nasal cavity of northern bluefin tuna (Wheeler and Beverley Burton 1987).

1.6.2 *Captive Populations*

Significant mortality, due to encephalitis from a scuticocilliate, was described in captive SBT held in South Australian waters for 3 to 8 months (Munday, O'Donoghue, Watts, Rough and Hawkesford, 1997). The disease was characterised by atypical swimming behaviour followed by rapid death. The parasites were assumed to initially colonise the olfactory rosettes and then ascend the olfactory nerves to invade the brain. An indirect fluorescent antibody test was also developed to identify the parasite for diagnostic and epidemiological purposes (Watts, Burke, and Munday, 1996). The cilliate involved (*Uronema nigricans*) is opportunistically parasitic with epizootics recorded from marine larval rearing systems and marine aquaria. It is susceptible to commonly used chaemotheraputics such as formalin, malachite green and hydrogen peroxide (Crosbie and Munday, 1999).

Mass mortality of captive SBT over two days was also described from South Australia (Munday and Hallegraeff, 1998). A diagnosis of the reason for the mortality was not given, but the authors postulated a number of causes. They concluded that possible aetiological factors responsible may be one or more of microalgal toxicosis, hypoxia, smothering by suspended solids, or hydrogen sulphide toxicity.

2. *EGGS AND LARVAE*

2.1 *Egg Collection and Treatment*

Eggs collected from captive broodstock are subjected to a variety of physical and biological influences during the process of spawning, collection, embryogenesis and hatching. They are collected under intensive conditions, and are also susceptible to a number of fungal, viral and bacterial diseases which may at times be derived from the parental fish.

2.1.1 *Physical Damage*

Fertilised eggs are usually collected after broodstock spawning by a skimmer overflow and held submersed in a tank until they are placed into tanks for development and hatching. They may also be transported to other hatcheries for ongrowing. During this process they may vary in their sensitivity to damage at different times of embryological development. This degree of sensitivity and stage of sensitivity depends upon the species. For example, sterilised eggs of milkfish, *Chanos chanos* were most sensitive to physical shock both at the beginning and end of larval development (Hilomen-Garcia, 1998), while eggs of grouper, *Epinephelus coioides* (Caberoy and Quinitio, 1998), and coho salmon (Jensen, and Alderdice, 1983) were much less sensitive to handling stress at the later stages of development.

2.1.2 *Sterilisation*

A number of different procedures have been examined to sterilise eggs to prevent transmission of disease. Eggs of chinook salmon *Oncorhynchus tshawytscha* were disinfected with buffered free-iodine for 10 minutes, then treated daily with formalin until the eyed stage of development in an attempt to eliminate mortality due to infection with the fungus *Saprolegnia parasitica.* This produced a significantly lower mortality from hatch to swim-up compared with daily hand picking of dead eggs and fry (Barnes, Cordes and Sayler, 1997). Iodophores are generally considered to be effective as antimicrobial agents, however their effectiveness is reduced by their toxic effects on the fish themselves. The effectiveness of Povidon-iodine varied on the time of its application to eggs, and concentrations for its use in spotted halibut, *Verasper variegatus* and Red Sea bream (Hirazawa, Hara, Mitsuboshi, Okazaki, and Hata, 1999). Bufodine, when tested on Atlantic halibut significantly affected the eggs, the survival and the development by both the developmental stage at which the eggs were treated and the concentration of disinfectant used (Bergh and Jelmert, 1996). Surface disinfection of the eggs of the Atlantic halibut with ozonised seawater was shown to be effective in inactivating a notovirus (VER) when eggs were exposed to this pathogen. However delayed hatching or non-hatching occurred in some eggs of all groups that were treated (Grotmol and Totland, 2000). The toxicological effect on larvae from use of the disinfectants benzalkonium chloride, formalin, hydrogen peroxide, thimerosal (merthiolate), polyvinylpyrrolidone iodine and sodium hyperchlorite on eggs of the red drum, *Sciaenops ocellatus* were tested independently by Douillet and Holt (1994). They concluded that the early tail-free stage of development was the most resistant to all germicides but that successful disinfection of eggs was achieved after a 5-min exposure to hydrogen peroxide at 3% concentration. However, the other two species tested (yellowtail snapper, *Ocyurus chrysurus*, and spotted seatrout, *Cynoscion nebulosus*) were more sensitive to hydrogen peroxide.

2.2 *Larval growth and development*

The growth rates of larvae of marine fish species are highly variable, and appears to be dependant on the species concerned, the water temperature, and the availability of food. The growth rates of early larval stages of wild tunas described to date appear to be relatively slow for tropical species, but it remains to be seen if the intensive conditions found in hatchery conditions will change this situation.

2.2.1 *Wild SBT Larvae*

The growth rates of larval SBT were determined by the use of daily growth increments in the otoliths of larvae collected from the "Oka' spawning grounds in January-February 1987 (Jenkins and Davis, 1990). Daily increments were marked, however subdaily increments were also apparent. The optically dense part of each increment occurred at about dusk. Most of the larvae sampled came from a single cohort from fish which spawned over about 2 days. The daily progression of otolith increment number for this cohort was further evidence that increments were formed daily. Larvae ranged from about 7 to 18 days old, the larval stage appeared to last about 20 days. Growth curves could not be fitted to plots of size-at-age due to a large variance in size-at-age but the growth trajectories of larvae were approximately

linear. The distribution of individual growth rates was approximately normal, ranging from 0.20 to 0.47 mm standard length (SL) per day and a mean of 0.32 per day. Over the range examined, the growth of larvae was relatively slow and linear, although the growth trajectories of oldest larvae 14 to 15 days old showed an apparently sudden increase in growth rate and exhibited a curvilinear increase in the days immediately preceding collection. The fast growth often attributed to young stages of tuna was assumed to occur in the late larval/early juvenile stage rather than the early larval stage of this species. They assumed that, like tropical tunas, development from fertilization to hatching is between 1 and 1.5 days, and 2-3 days between fertilization and yolk absorption, making about 3 days between fertilisation and the first daily otolith increment. The otoliths had a zone of optically dense increments spanning increments 15 to 30. This was thought to be the time of metamorphosis and fast growth and corresponding to the outer layers of the otoliths in the biggest larvae in their collection.

Larval growth was density dependent, individual larvae between 3.4 and 7.5 mm in length had between 4 and 13 daily growth increments in their otoliths and the average individual growth rate was between .28 and .37 mm per day. This differed between locations but the mean growth rate was not correlated with temperature, which did not vary much. Mean growth rate was negatively correlated with larval abundance and positively correlated with the feeding indices, stomach fullness, mean prey number, and feeding incidence (Jenkins, Young and Davis, 1991).

2.2.2 *Captive Populations of Tuna spp.*

Fertilised eggs were obtained from spontaneous spawning of broodstock of northern bluefin tuna in net pens. Newly hatched larvae were transported on 9 July by air and installed as 2-day yolk sac larvae in a 5,000l rectangular reinforced plastic tank 5x1x1m deep at a density of 6 larvae per litre. The water was maintained at 25°C, and the photoperiod was natural and between 100 and 1000 lux. Larvae grew from 3.7 mm total length (TL) on day 2 to 15.6 mm at day 30. On day 14 the flexon phase was recorded at 6.5 mm TL. Metamorphosis occurred by day 25 at about 11 mm TL. First feeding occurred on the third day post hatching at 3.3 mm TL. The primitive digestive system differentiated on day 4, the gastric gland and pyloric caecae appeared on days 11 and 14 respectively. The number of pyloric caecae and gastric glands increased markedly by the juvenile stage at day 30. Survival was very low, 0.19% at 35 days post hatching (Kaji, Tanaka, Takahashi, Oka and Ishibashi, 1996). Higher growth rates have been reported. Larvae have been described as reaching 40mm TL after 30 days (Kumai, 1998).

Assays from eggs of northern bluefin tuna demonstrated that at the early cleavage stage, eggs were composed almost entirely of protein, free fatty acids, triglycerides and phospholipids. The eggs were obtained from spontaneous spawning at 27°C between 1700 and 2000 in July in Japan of about 250 seven-year-old northern bluefin tuna kept in a net cage. Because of the declining levels of triglycerides with embryogenesis, this was considered a main endogenous energy fuel (Takii, Miyashita, Seoka, Tanaka, Kubo and Kumai, 1997)

Yellowfin tuna larvae were reared from hatching to beyond metamorphosis in May and June 1996. They were 2.65 mm SL just after hatching and 27.68 mm SL on Day 37. Transformation to juveniles occurred around 30 days after hatching at about 13 mm

SL. The primitive digestive system differentiated on Day 4. The gastric gland and pyloric caeca first appeared on Day 14 and 16, respectively. The pharyngeal and jaw teeth became fully functional synchronised with gastric gland differentiation. The number of gastric glands and pyloric caeca and volume of the gastric blind sac increased markedly toward the juvenile stage. (Kaji, Tanaka, Oka, Takeuchi, Ohsumi, Teruya and Hirokawa, 1999)

2.3 *Larval Diet*

Larvae of most marine fish currently undergoing aquaculture trials or farmed commercially have copepods as an essential part of their wild diet. SBT are no exception. Copepods, like many other zooplankton, utilise microalgae as a primary food source, and transfer important algal nutrients to higher trophic levels on the food web.

Microalgae vary considerably in their biochemical composition. A review of about 40 species from seven classes showed that they varied in their proportions of protein (6-52%), carbohydrate (5-23%) and lipid (7-23%). All species had similar amino acid composition, and were rich in the essential amino acids. Microalgal polysaccharides were variable in sugar composition, but most had high proportions of glucose (21-87%). Diatoms, prymnesiophytes, cryptomonads and eustigmatophytes were rich in one or both of the 20:5(n - 3) and 22:6(n - 3) polyunsaturated fatty acids (5-35% total fatty acids). All species had relatively high concentrations of ascorbic acid and riboflavin. (Brown, Jeffrey, Volkman, Dunstan, 1997).

Although batch and continuous cultures of the harpacticoid copepod *Tisbe holothuriae* have been run for numerous generations in shallow trays or in continuous bioreactors (Stottrup and Norsker, 1997), copepods are generally considered to be difficult to grow in sufficient numbers for commercial use. Consequently the starting diets used for larvae of farmed marine fish are usually rotifers followed by *Artemia* nauplii, usually enriched by the addition of either live microalgae, microencapsulated diets, or yeast based diets (Coutteau and Sorgeloos, 1997).

To develop a suitable diet for larval SBT their feeding behaviour and the nutritional characteristics of their natural diet should be understood together with a knowledge of existing successful marine fish larval diets, including the results of feeding larval tuna.

2.3.1 *Diet of wild populations of SBT*

The diet of larval SBT consists mainly of copepod nauplii, cyclopoid copepods, *Corycaeus* spp, calanoid copepods, *Clausocalanus* spp and cladocerans, *Evadne* spp (Uotani, Matsuzaki, Makino, Noda, Inamura and Horikawa, 1981). More recently Young and Davis (1990) confirmed this range of diet items. They determined that the time of maximum feeding was just prior to dusk and larval stomachs were empty by 2200. The larvae that they examined were between 2.69 and 9.84 mm in standard length and aged between 2 and 14 days old. Most were 4-5 mm and 5-9 days old. Of the larvae caught, 52.7 % that were caught during daylight (0600-1800) had food in their stomachs. Incidence of stomachs with food increased with fish size. Feeding only occurred in daylight, peaking after sunrise and in mid afternoon after a decline about 1100. Fish larvae were a principal diet of post-flexon larvae. Overall mouth width was correlated with prey width. Fish smaller than 4.75 mm (less than 5 days

old) fed mostly on copepod nauplii, prey width was 0.08-0.16 mm, mean 0.087 for fish less than 5 mm SL. The prey was gradually replaced by cyclopoids as they grew bigger (prey width 0.06-0.30 mm, mean 0.171 and then calanoids (prey width 0.08-0.32 mm, mean 0.20. Fish larvae greater than 7 mm SL fed on fish larvae, including SBT larvae.

They suggested that larvae smaller or 5 mm long selected for copepod nauplii and corycaeid copepods and against calanid copepods. A similar pattern occurred in larvae greater than 5 mm. Here there were less copepod nauplii and more cyclopods. Cladocerans were rare in the plankton, but appeared to be positively selected. Larvae needed to eat 30% of their body weight as food per day.

2.3.2 *Diet of Larvae in Captivity*

Feeding of marine fish larvae in captivity is, in most cases, limited to two types of live prey, rotifers and *Artemia.* This reduction in the range of food available for the cultured larvae may lead to nutritional imbalances or deficiencies. A large amount of research has been recently devoted to the study of their dietary requirements, especially of their essential fatty acid needs. Studies on the biochemical composition of developing eggs and larvae, as well as the comparison of the patterns of loss and conservation during starvation, shows the importance of n-3 HUFAs and arachidonic acid for larvae of marine fish (Izquierdo, 1996).

Copepod-based diets versus enriched live diets: Comparison of copepod-based diets with enriched *Artemia* nauplii have generally shown better results from the use of copepods, and these have been related to the ratios and level of HUFAs in their tissues. When the effects of enrichment of *Artemia franciscana* by emulsions of either fatty acid ethyle esters diluted with olive oil, or triacylglycerol were examined, this resulted in an increase of total lipid content from 20.0% to 28.2-28.7% of dry matter mainly due to the accumulation of neutral lipid. The level of n-3 HUFA increased drastically during enrichment from 6.3% of total fatty acids in freshly hatched nauplii to between 20.4% and 21.8% in 24-h enriched *Artemia* and was not significantly affected by the source of n-3 HUFA. Enrichment with either of the emulsions resulted in an increase of the neutral lipid fraction which concentrated greater than 64% and 91% of the EPA and total DHA present. However, the major polar lipids, phosphatidylethanolamine and phosphatidylcholine, of freshly hatched *Artemia* showed very low levels of DHA (22:6n-3), and carried about 45% of the total EPA (20:5n-3) present. This was in sharp contrast with the high levels of n-3 HUFA, in particular DHA, in the polar lipid fraction reported for wild copepods. (Coutteau and Mourente, 1997).

Marine copepods were shown to be superior to enriched *Artemia* as food for Atlantic halibut larvae, and this superiority was related to the level of DHA in the feed. Survival of the halibut larvae and percentage of larvae undergoing successful metamorphosis (complete eye migration and dorsal pigmentation) was higher in larvae fed the marine copepod, *Eurytemora velox* (40%) than in larvae fed *Artemia* nauplii (4%) doubly enriched with *Schizochytrium* sp., (Algamac 2000) and a commercial oil emulsion (SuperSelco). Larval specific growth rates did not differ, nor were indices of eye migration significantly different between larvae fed the two diets. The DHA:EPA ratios in enriched *Artemia* nauplii were 0.4 and 1.0, for SuperSelco and Algamac The DHA / EPA ratios for two size ranges of copepods were

2.0 for those 125-250µm and 0.9, for those 250-400 µm (the smaller size range containing the highest level of n-3 HUFA). The total lipids of eyes, brains and livers of larvae fed copepods had higher levels of DHA and lower levels of EPA than those of larvae fed enriched *Artemia.* Histological examination of the livers and intestines of the larvae were consistent with better assimilation of lipid from copepods than lipid from *Artemia* nauplii up to 46 days post-first feeding (Shields, Bell, Luizi, Gara, Bromage and Sargent, 1999).

Similar results were obtained by (Naess and Lie, 1998) who offered six groups of Atlantic halibut, calanoid copepods at different periods from days 11 to 25 after first feeding to establish at which stage normal pigmentation was determined. *Artemia* nauplii enriched with an oil emulsion were used prior to and after the copepod period. Control groups were fed on copepods or *Artemia* only. While the *Artemia* diet initiated an earlier intake of food and higher initial growth compared to the copepod diet, the lowest frequency of normally pigmented juveniles was found in the *Artemia*-fed group (66.4%), while the copepod group showed almost 100% normal pigmentation. A high degree of eye migration was found in all groups, but was lowest in the *Artemia*-fed group. The initial stage of eye migration was found to occur at a larger body size in fish given *Artemia* and copepods, or a copepod diet than in fish fed on *Artemia* alone (Naess and Lie, 1998).

When Atlantic halibut larvae were fed either SuperSelco enriched *Artemia* nauplii or extensively grown copepods, pigmentation rates were found to be higher in the copepod-fed fish: 99.2% compared to 66.4%. Copepod-fed fry showed significantly higher levels of DHA than their *Artemia*-fed counterparts in eye phosphatidylethanolamine. Ratios of DHA: EPA were also higher in the copepod-fed fish. Arachidonic acid ratios were also higher in zooplankton-fed fish but there was no significant difference in these ratios between normal and malpigmented *Artemia*-fed halibut. (McEvoy, Naess, Bell and Lie, 1998).

Enrichment of live diets: The beneficial effects of feeding larvae diets enriched with HUFAs, are now well recorded: eg growth and survival in palmetto bass larvae fed enriched *Artemia* (Tuncer, Harrell and Chai, 1993); growth of milkfish fed with rotifers and *Artemia* enriched with HUFA and Vitamin C (Gapasin, Bombeo, Lavens, Sorgeloos and Nelis, 1998), growth and metamorphosis in summer flounder (Baker, Alves and Bengtson, 1998), and growth and survival of Red Sea bream fed with the rotifer *Brachionus plicatilis* cultured with *T. tetrathele* (Fukusho, Okauchi, Nuraini, Tsujigado and Watanabe, 1984). Indeed, when adequate levels of n-3 HUFA was supplied in rotifers fed to early larvae of Red Sea bream this was associated with a growth acceleration of 250% when compared with those given a low dietary supply. Moreover, like other marine fish, seabream has a preference for DHA over EPA for both growth and survival (Tandler, Harel, Koven and Kolkovski, 1995).

Interestingly, while diets containing enriched *Artemia* increased successful metamorphosis in larval and juvenile barramundi, *Lates calcarifer* (Dhert, Lavens, Duray and Sorgeloos, 1990) and also their survival and growth rate (Rimmer, Reed, Levitt and Lisle, 1994), enrichment of rotifers in the diet did not.

The functions of DHA and EPA during early stages of marine fish larvae are apparently different. High amounts of EPA in relation to DHA may create an imbalance in the structural composition of the phospholipids. Turbot larvae tended

to exhibit lower pigmentation success with lower DHA:EPA ratio in the total lipid fraction of the larvae, especially when the absolute amounts of EPA were high compared to those of DHA in the total lipid and phospholipid fraction. (Rainuzzo, Reitan and Olsen, 1997)

A 20 mg ascorbic acid / kg diet is sufficient for normal growth and survival in the early post-weaning stage of European sea bass and turbot (Merchie, Lavens and Sorgeloos, 1997). The levels of ascorbic acid in larval turbot fed live food enriched with various levels of ascorbic acid were correlated with the ascorbic acid content of the live food administered. However, a saturation of the body reserves occurred and differences in neither growth nor overall survival were detected (Merchie, Lavens, Dhert, Gomez, Nelis, DeLeenheer and Sorgeloos, 1996). Seabass larvae fed on ascorbyle palmitate-enriched rotifers (days 4-12) and *Artemia* nauplii (days 13-46) also showed no significant differences in production characteristics nor in stress resistance. Catfish, *Clarias gariepinus* larvae fed the same diets showed no improvements in survival, but growth may have been improved, (Merchie, Lavens, Dhert, Pector, Soni, Nelis, Ollevier, DeLeenheer and Sorgeloos, 1995).

When larval black sea bream (*Acanthopagrus schlegeli*) were fed for 15 days fed on rotifers cultured with different algal species, differences in survival rate, total length, and body weight was considered to probably be due to difference in the contents of n-3 HUFA rotifers following enrichment. (Fukusho, Okauchi, Tanaka, Kraisingdecha, Wahyuni and Watanabe, 1985).

Green water feeding: Microalgal addition to first-feeding tanks along with the rotifers usually improves growth and survival in larval turbot and halibut, whereas short-term enrichment of rotifers with algae does not (Reitan, Rainuzzo, Oie and Olsen, 1997).

The algae in larval tanks tended to modify and stabilize the nutritional quality of the rotifers in the period before they were consumed by the larvae. The lipid content and fatty acid composition of the rotifers reflected the composition of the algal diets, and the algal species used may be an effective tool to control the fatty acid content. Larvae reared in green water consumed higher numbers of rotifers during the stagnant period than larvae kept in clear water conditions, while analysis of the larval gut contents showed lower rotifer numbers in the gut of larvae reared in green water conditions. This was thought to mean longer residence time of the food in the larval gut, and presumably also higher digestion and assimilation efficiencies of larvae maintained without algae than in larvae maintained with algae (Oie, Makridis, Reitan, Olsen and Inge, 1997).

Inert microdiets Microbound and micro-encapsulated diets have been designed to supplement live feed in the culture of fish larvae, to substitute for live food during the early stages of rearing The growth rate and survival of larval gilthead bream fed a microencapsulated diet following 4 days feeding on rotifers were similar to that of larvae fed on rotifers. (Yufera, Pascual and Fernandez, 1999).

The addition of *Artemia* to microdiet-fed seabass larvae positively affected rates of assimilation and growth, regardless of the age of the fish (Kolkovski, Tandler and Izquierdo, 1997)

Ingestion rates were also markedly improved when inert microdiets were co-fed to larval seabream *Sparus aurata* together with live *Artemia* nauplii. This was considered to be due to one or both of visual and chemical stimuli of the live diet to ingestion, or the direct influence of the biochemical composition of nauplii on larval digestion and assimilation (Kolkovski, Koven and Tandler, 1997). Survival of larvae of this species fed only microcapsules ranged from 11%, when the capsules were added from first feeding, to over 50% when pre-fed rotifers. The addition of live prey (5% of the total food supplied on dry weight basis) improved the survival (42%) when the microcapsules were supplied from the start of feeding. Feeding incidence on microcapsules was similar to that obtained with rotifers (Fernandez-Diaz and Yufera, 1997).

2.3.3 *Diet of Larval Tuna in Captivity*

Newly hatched larvae of northern bluefin tuna were fed rotifers enriched by "Super Rotifer II" were from day 3, *Artemia* nauplii and coral trout eggs and larvae were supplied from day 12, *Artemia* larvae and an unspecified artificial diet was added several days before metamorphosis at 30 days. Survival was very low, 0.19% at 35 days post hatching (Kaji, Tanaka, Takahashi, Oka and Ishibashi, 1996). Higher growth rates have been reported. Larvae at were described as reaching 40 mm TL after feeding rotifers from day 2 to 20, *Artemia* from day 13 to 28, live fish larvae from day 16 to 29, anchovy from day 20 to day 30 and sand lance from day 25 to day 30 (Kumai, 1998).

Assays from eggs of SBT demonstrated that at the early cleavage stage, eggs were composed almost entirely of protein, free fatty acids, triglycerides and phospholipids. Because of the declining levels of triglycerides with embryogenesis, this was considered a main endogenous energy fuel (Takii, Miyashita, Seoka, Tanaka, Kubo and Kumai, 1997)

Yellowfin tuna larvae were reared from hatching beyond metamorphosis were fed on rotifers from day 4, on fish larvae and *Artemia* nauplii from Day 16, and then on frozen fish and minced fish meat. Survival rates were not given (Kaji, Tanaka, Oka, Takeuchi, Ohsumi, Teruya and Hirokawa, 1999).

2.4 *Environmental stimuli and larvae*

The larval stages of tuna have been reported to inhabit the surface mixed layer of tropical oceans. In this location variability in salinity is likely to be low, however during cyclonic events temperatures may well vary due to upwelling of cooler waters from deeper down. Behavioural studies on captive larval fish suggest that most need a minimal threshold light intensity to be able to develop normally and grow, probably related to the aptitude to localise, catch and ingest prey. Light is also indispensable for body pigmentation, an important phenomenon involved in early development and growth. Too intense light can be stressful or even lethal. A few species are able to develop and grow at very low intensities or, sometimes, in the absence of light. Generally, long day length and availability of food improves larval rearing quality (Boeuf and Le Bail, 1999).

2.4.1 *Wild SBT Larvae*

Surface temperatures in December and January in the spawning grounds vary between 27° and 28.6°C and salinities between 34.6-35.0 ppt. In January, the bottom of the mixed layer lay between 16 and 22m. Three days after a cyclonic event the mixed layer reached 48m. In December, a pycnocline was not clearly apparent (Davis, Jenkins and Young 1990a). Temperature profiles with depth are given in Davis and Clementson (1989). In the absence of a pycnocline, few larvae were found below 35m. In its presence, all larvae were in the mixed layer. Far more larvae were in the surface samples (0-2 m) during the day than at night. Horizontal distribution of larvae suggested that older larvae occurred at low densities in patches of 1-3 km in diameter. while 7-8 day old larvae occurred in high densities in patches between to 5-15 km diameter. The highest densities were greater than 22 larvae per cubic metre. Spawning adults were considered to occur in 5-15 km diameter patches (Davis, Jenkins and Young, 1990b).

Diffusion models and drogued buoys, suggested that ocean currents are not sufficient to account for the movement of larvae to the Leeuwin or South Equatorial current, so it was concluded that movement must occur during the post larval stage (Davis, Lyne and Jenkins, 1991).

2.4.2 *Larval Fish in Captivity*

Studies on growth and survival of marine fish under differing conditions of light and temperature are scarce. After 35 days, growth and survival of haddock (*Melanogrammus aeglefinus*) larvae were not significantly different between photoperiod treatments of 24L : 0D and 15L : 9D. Overall larval survival was poor, but greater in white versus black tanks after 41 days (2% versus 1%, respectively). Growth of larvae was impaired in black tanks at low light intensity. Transmission and reflection of light was low in black tanks at low incident light, and there was very little upwelling light. The resultant poor prey to background contrast was thought to result in larvae being unable to consume sufficient food to sustain a level of growth comparable to that in other treatments, (Downing and Litvak, 2000).

Barramundi larvae 2-10 days old grew progressively faster under conditions of 8, 16 and 24 h light per day; survival rates did not differ between the treatments. Larvae 8-20 days old also grew significantly faster in 16 and 24 h light than in 8 h light; survival rates did not differ between the treatments. Juveniles, initially 11-12 mm total length, showed no significant difference in growth or survival rates in either 12, 18, or 24 h light (Barlow, Pearce, Rodgers and Clayton, 1995).

3. *ISSUES OF RESEEDING*

There is some scepticism that reseeding of marine populations of wild fish produces any tangible benefits either to the wild populations, or to the economics of fisheries that they are purported to assist (Hilborn, 1998). However an increasing number of studies suggest that populations of wild fish stock may be augmented by a carefully planned program of releases of cultivated juveniles. For hatchery releases to increase overall population size the released cultured fish must survive, grow and contribute to natural recruitment, and they should not outcompete wild stocks.

Blankenship and Leber (1995) discussed a responsible approach to marine fish stock enhancement, and laid out ten issues which should be addressed before any such enhancement is attempted. These were 1) prioritise and select target species, 2) develop a management plan that identifies harvest opportunities, stock rebuilding goals, and genetic objectives, 3) define quantitative measures of success, 4) use genetic resource management to avoid deleterious genetic effects, 5) use disease and health management, 6) consider ecological and life history characteristics when forming enhancement objectives and tactics, 7) identify released fish and assess stocking effects, 8) use an empirical process for defining an optimum release strategy, 9) identify economic and policy guidelines, and 10) use adaptive management.

3.1 *Survival and economic benefits of releases*

A study of the recruitment patterns of cultured juvenile pacific threadfin (*Polydactylus sexfilis*) suggested that in one location released cultured fish made a major contribution (greater than 50%) to the recruitment of juveniles. However the effectiveness of releases was generally determined by the size at release, release season, and the release site (Leber, Brennan and Arce, 1998).

The importance of size at release was also emphasised by an examination of releases of juvenile Atlantic cod (*Gaddus morhua*). Here it was concluded that although a size dependent effect on natural mortality was small in fish greater than 12 cm long on release, fewer than 2% of cod released at 8 cm survived their first 3 months at sea. In the same location 75% of those released at 12 cm survived during the same period of time (Kristiansen, Ottera and Svasand, 2000).

Following pilot hatchery releases from 1990 to 1993, striped mullet (*Mugil cephalus*) fisheries in Kaneohe Bay, Hawaii, comprised about 13.0% of cultured fish by autumn 1994. By refining release protocols over a 3-year period, the proportions of cultured striped mullet in nursery habitats 10 months after release increased from 3% to 10% and finally to 50% of the total striped mullet (wild and cultured) collected in net samples (Leber and Lee, 1997). The recapture rate of different sized juveniles was directly related to the seasonal timing of releases. Greatest recovery of the smallest fish occurred when they were released at the same time as peak recruitment of similar sized wild juveniles (Leber, Blankenship, Arce and Brennan, 1997).

The economic benefits of reseeding programs have not been generally examined. However the enhancement program for Japanese flounder (*Paralichthys olivaceus*) was examined in Japan by analysis of the proportion of cultured individuals released at about 10 cm long with characteristic pigmentation, and wild flounders landed in the commercial catch at five markets. Released flounders comprised between 19.5% and 61.1% of the total landed catch, and averaged 28.5% over all markets. Cost benefit analysis concluded that the stock enhancement program was economically profitable (Kitada, Taga and Kishino, 1992).

3.2 *Genetic Mixing*

The majority of attention in the genetic management plans of stock enhancement programs are that the genetic identities of wild fish populations may be eroded by the transplantation of non native hatchery derived populations, or that hatchery fish

may be deficient in overall genetic variability. This deficiency ultimately reducing the genetic variability in the population into which they are released.

Modelling the effects of stocking on the effective size of fish populations and accompanying genetic risks for red drum and gulf sturgeon, *Acipenser oxyrinchus desotoi* Tringali and Bert (1998). concluded that the effects on each species was an intrinsic function of the life history characteristics and population demography of each. The stocking program for gulf sturgeon could reduce their effective population sizes well below suggested threshold values, however the red drum population was unlikely to undergo significant loss of single locus or polygenic variability.

King, Ward, Blandon, Colura and Gold (1995) conducted genetic surveys on red drum and spotted seatrout, to detect baseline levels of genetic variation, delineate population structure, and identify rate genes useful as genetic markers. The results of the surveys showed that biological and physical processes necessary for formation of discrete stocks were not present, and gene flow was sufficient to prevent genetic partitioning. Nevertheless clinal genetic variation was detected in wild stocks of spotted seatrout and so this allowed modification to the stocking program to develop broodstocks representative of the various locations along the coast.

Experience with Atlantic salmon in Norway suggests that in some streams are dominated with escapees from fish farms. Because of the restricted number of parental fish in broodstocks, these often differ from naturally produced fish in the river. However a review of interactions between wild and cultured Atlantic salmon did not demonstrate serious genetic effects, although life history characteristics between captive and wild salmon were said to often differ, but the most serious effect detected was the introduction of diseases (Heggberget, Johnsen, Hindar, Jonsson, Hansen, Hvidsten and Jensen, 1993).

3.3 *Genetic Relationships Between Tuna Species*

Although Conover (1998) suggests that local adaptation in the Atlantic silverside, *Menidia menidia* may happen, in pelagic marine species, where geographic barriers to gene flow are usually absent, and larval life stages are usually highly dispersive or migratory, the opportunities for local adaptation are comparatively few. Consequently the degree of genetic separation between stocks or populations of tunas are likely to be low. Both allozyme and mtDNA approaches show tunas in the genus *Thunnus* to be very closely related to one another, but also indicate that difference between the two presently recognised subspecies of northern bluefin tuna, *Thunnus thynnus thynnus* from the North Atlantic, and *T. t. orientalis*, from the North Pacific, may be worthy of species status (Ward, 1995). An analysis of all eight species of the genus *Thunnus* by mitochondrial and nuclear genes confirmed that the Pacific northern bluefin tuna had mtDNA distinct from that of the Atlantic subspecies, but very similar to that of the albacore. In contrast, no difference in nuclear genome was found between the Pacific and Atlantic northern bluefin tunas. The Atlantic northern bluefin and southern bluefin possessed mtDNA sequences which were very similar to those of species in the yellowfin tuna group, but not so similar to those of albacore or bigeye tuna. These relationships are inconsistent with phylogenies based on morphology (Chow and Kishino, 1995)

Assays based on PCR-RFLP demonstrated interoceanic subdivision of bigeye tuna, in which individuals from the Atlantic Ocean were differentiated from those found in the Indian Ocean and Pacific Ocean (Bremer, Stequert, Robertson, and Ely, 1998).

The five *Thunnus* species of the Pacific tunas, albacore, bigeye, northern bluefin, southern bluefin, yellowfin, and frigate tuna, kawakawa (*Euthynnus affinis*) and skipjack have been shown by allozyme analysis to have a high genetic identity in their phylogenetic relationships (Elliott and Ward, 1995). Albacore was the most divergent of the *Thunnus* species. There was little differentiation between yellowfin, southern bluefin and northern bluefin tunas and phylogenetic analyses failed to resolve the branch order among the *Thunnus* species. The non-*Thunnus* tunas were quite divergent both from one another and from *Thunnus* species.

Examination of the population structure of yellowfin tuna from 5 locations in the Pacific Ocean and one from the Atlantic ocean with RFLP analysis of mitochondrial DNA showed no evidence of differentiation between samples (Scoles and Graves, 1993). However inter- and intra-oceanic differences in the genetic identity of yellowfin tuna have been shown (Ward, Elliott, Grewe and Smolenski, 1994). Here an examination of samples from the western, central and eastern regions of the Pacific Ocean showed that two eastern samples (from southern California and northern Mexico) were not significantly different from each other but they were significantly different from the five western / central samples (Philippines, Coral Sea, Kiribati, Hawaii-91 and Hawaii-92). A more extensive genetic study of this species suggested from allozyme analysis, the existence of at least four yellowfin tuna stocks: Atlantic Ocean, Indian Ocean, west-central Pacific Ocean, and east Pacific Ocean. Mitochondrial DNA analysis did not differentiate west-central Pacific Ocean collections from east Pacific Ocean collections but did support the separation of Atlantic Ocean, Indian Ocean, and Pacific Ocean stocks. (Ward, Elliott, Innes, Smolenski, and Grewe, 1997)

Mitochondrial DNA analysis provides a reliable discrimination between northern and SBT, although they are morphologically similar and can be easily misidentified. Use of this method confirmed that only six of 12 individual tuna from Australia originally considered to be northern bluefin tuna were in fact northern bluefin tuna The presence of northern bluefin tuna as far south as south-western Tasmania and New Zealand was also confirmed by this method (Ward, Elliott and Grewe, 1995).

3.4 *Genetic Relationships Within SBT*

Proctor, Thresher, Gunn, Mills, Harrowfield and Sie (1995) examined stock structure by chemical analysis of the sagittal otoliths of juvenile and adult SBT collected from off South Africa, South East Australia, southern Australia and Western Australia. They showed that variation in the composition of the primordium was unimodal and usually normally distributed. This composition varied between different sized individuals. Individuals collected from widely separated locations did not differ in the composition of most recently deposited sections of their otoliths, and all variation in the composition of adult otoliths was encompassed in the range of variation of juveniles collected from all known major migration routes. These results are consistent with the hypothesis of a single spawning area for this species, but also showed that the range of environmentally correlated variation in composition was too low to provide a robust test of the diversity of migration routes.

In juvenile northern bluefin tuna (*T. thynnus*) a chemical analysis of whole otoliths showed that differences in chemical composition could be seen between individuals caught from the Mediterranean Sea and the Northern Pacific Ocean (Secor and Zdanowicz, 1998). This suggests that Procter *et al's* , 1995 observations may be taken to strongly indicate a single stock of SBT.

Further confirmation is indicated by Grewe, Elliott, Innes and Ward (1997) who examined polymorphic allozyme loci and mitochondrial DNA variants from fish collected from the same areas. They detected no significant spatial heterogeneity, nor did they find any significant differences in juveniles caught off Western Australia from what appeared two temporally separated spawning peaks.

Appendix II

The Consultation Process

Sixty-eight individuals were questioned regarding the SBT propagation project. Interviews were conducted on a face-to face basis and were guided by a questionnaire. Not all questions were asked of any particular interviewee, nor were questions always answered. Opinions were sought about the best way to run the business of the project, and on the most appropriate research to achieve the propagation of the SBT.

Individuals interviewed came from 8 occupational categories (Table 1).

Table 1 Occupational category of people interviewed

Government Research Scientist
Government Research Manager
Tuna Farmer
Salmon farmer
Industry Association
Government policy advisor
Hatchery biologist
R &D Funder

As may be expected, most people interviewed were either tuna farmers (20) or government research scientists (20). The next most frequent two categories were government research manager (9) and hatchery biologist (8) (Figure 1).

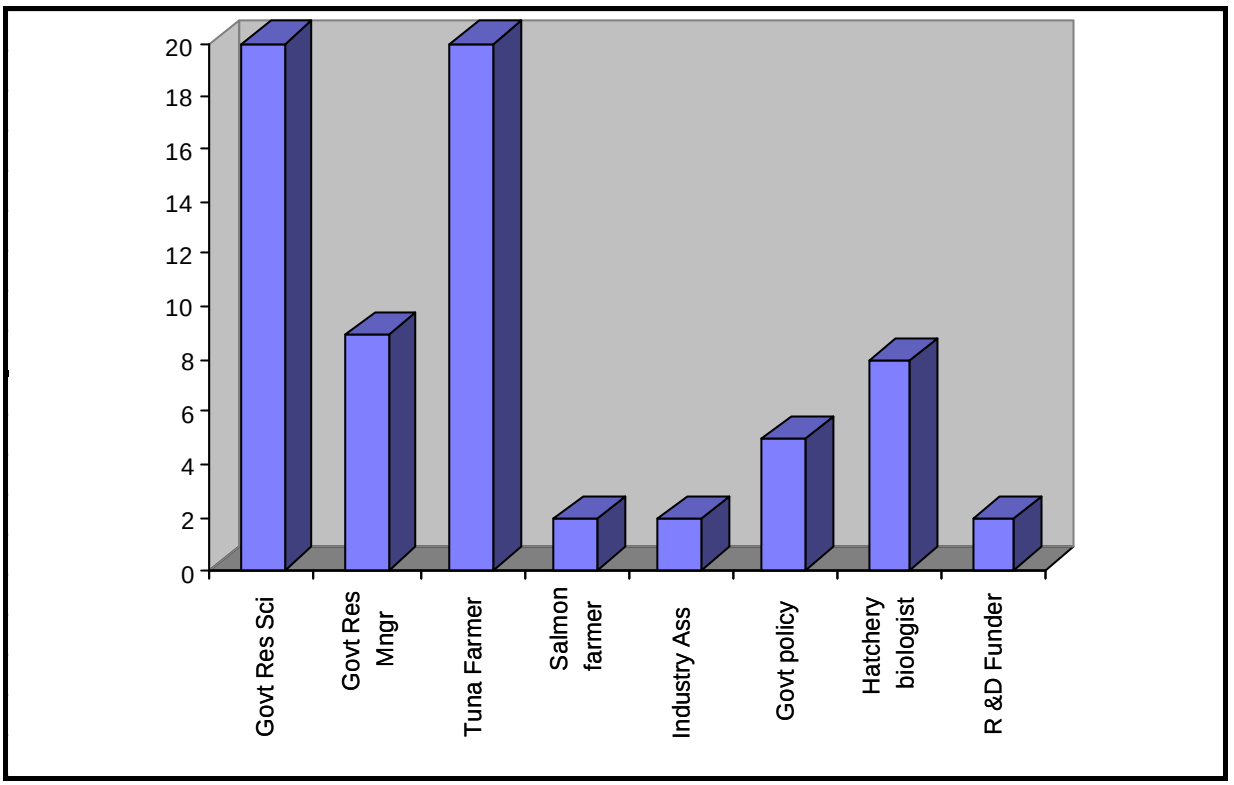


Figure 1 Occupations of those interviewed

The views given to business questions mostly reflected the policies of the employing entities. This was not so for the questions related to research, where there appeared to be common agreement on the issues to be examined, and the best ways of succeeding. Overall, most government employed individuals, whether in the category of scientists, science managers, or policy had not evaluated the benefits, the need, or the desirability of propagating SBT for either farming or reseeding. Of the respondents from twenty-one business or government entities that had, 37% were supportive, and 29% were undecided (Figure 2). Fourteen percent considered that no action should take place until all the alternative options had been financially evaluated.

Table 2 Industry and Government Managers Interviewed

Industry Associations

Aquaculture Council of Western Australia
Simon Bennison

Tuna Boat Owners Association of South Australia
Brian Jeffriess — President

Marine Fish Farmers

Stehr Group
Hagan Stehr — Managing Director
Glyn Chillingworth — General Manager
Tassal
Mike Ginnivan — Managing Director
Ajka Pty Ltd
Greg Honeychurch — General Manager
M G Kalis Group
Ken Palmer — Managing Director
Alex Kailis — Chief Executive Officer, Seafood Division
Secol Farmed Tuna Pty Ltd
Rick Kolega — General Manager
Blaslov Fishing Group
Justin Nelligan — General Manager
Rick Lehman
Lukin & Sons
Dinko Lukin — Chairman
Makaira Pty Ltd
Steve Nel
DI Fishing Co. Ptl. Ltd.
Robin Pike — Managing Director, *Australian Bluefin Pty Ltd.*
Joe Puglesi — President
Patrick von Stieglitz
Kinkawooka Pty Ltd
Bob Puglisi — Managing Director
Andrew Puglisi
Tony's Tuna International Pty Ltd
Tony Santic — Director
Saron Marine Farms
Mark Thyer
Marnikol Fisheries Pty Ltd
Mario Valcic — Managing Director
Australian Fishing Enterprises Pty. Ltd
Daryl Woods — Tuna Farm Manager

Government Fisheries & Aquaculture Managers

Fisheries WA
Peter Rogers — Director
Dan Machin — Aquaculture Development Officer
Greg Paust — Program Manager Pearling & Aquaculture
DPIWE Tasmania
Darby Ross — Manager, Marine Farming Branch
Fisheries Victoria
Richard McLoughlin — Director
Australian Fisheries Management Authority
Bob Stanley — Senior Observer AFZ Observer Program
Fisheries & Aquaculture Branch, AFFA
Glenn Hurry — Assistant Secretary
Paula Shoulder
Matthew Dadswell — Policy Advisor
Primary Industries and Resources South Australia
Ian Nightingale — General Manager, Aquaculture SA

Financial Managers

Capital Strategies
Paul Robinson — Director

Government R&D Managers

South Australian Research and Development Institute
Rob Lewis — Chief Executive Officer
Anthony Cheshire — Chief Scientist, South Australian Aquatic Sciences centre
CRC For Finfish Aquaculture
Peter Shelley — Chairman, Interim Board
Aquaculture CRC
Peter Montague — Chief Executive Officer
Jim Baker — Executive Officer
Lee Ridge — Business Development Manager
Tasmanian Aquaculture and Fisheries Institute
Colin Buxton — Director
CSIRO Marine Division
Ian Poiner — Program Manager Tropical and Pelagic Ecosystems
Peter Rothlisberg — Program Manager Aquaculture and Biotechnology
Bernard Bowen Fisheries Research Laboratory, Fisheries WA
Jim Penn — Director
Fisheries Research and Development Corporation
Peter Dundas-Smith — Executive Director
Patrick Hone — Programs Manager

Industry R&D Managers

M G Kailis Group
Richard McCulloch — Research Director
Tassal
Pheroze Jungalwalla — Manager, R&D

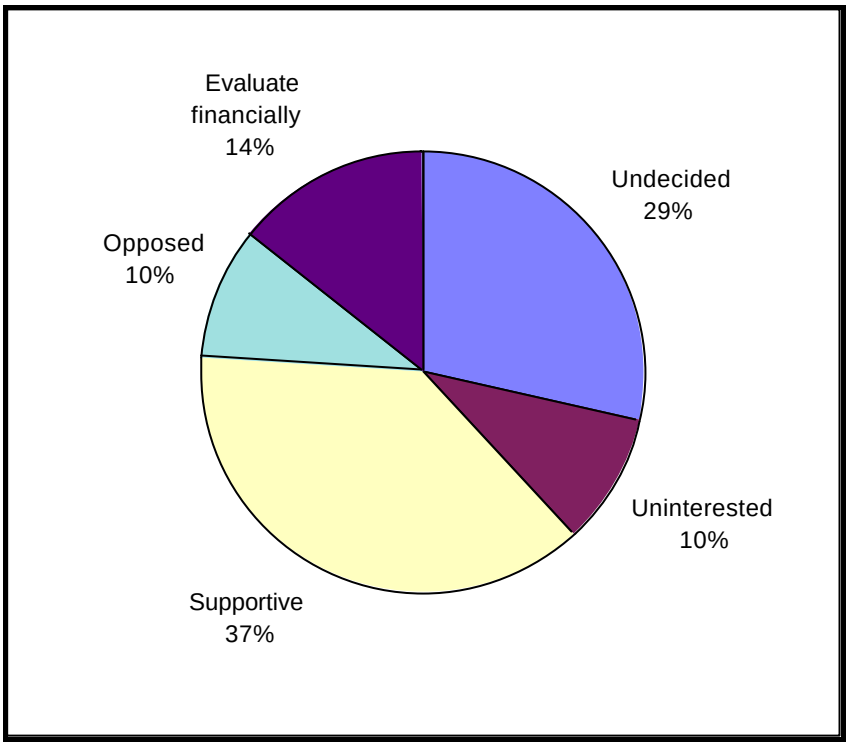


Figure 2 Support for the SBT Propagation Project

Views about Business Arrangements for the Propagation Project

Discussions were conducted with representatives from 2 industry associations, and 18 companies involved with tuna farming. Managers and policy advisers from 12 government agencies, including 6 R&D providers were also interviewed (Table 2). Views were sought in regard to the best business arrangements for the propagation project. Representatives of only 18 entities had views regarding the best way in which the propagation project might best be managed. Of these, 3 considered that the controlling entity should be FRDC and 4 that it should be the proposed CRC, while 1 was in favour of a CRC/FRDC combination, and one in favour of a FRDC/CCSBT combination. Nine believed that some form of startup business should be formed to encompass the interests of prospective participants. In regard to overseas participation in the project, views were fairly divergent. Most interviewees that responded (41%) held no view. Other views were fairly evenly divided (Figure 3).

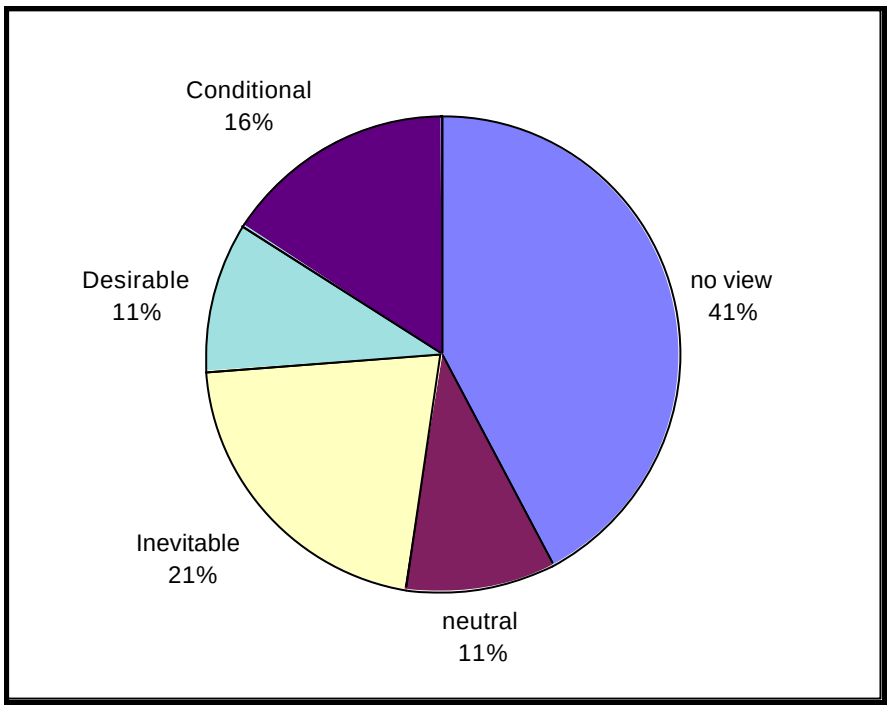


Figure 3 Support for overseas participation in the project

Of the respondents from the 16 entities that replied to questions regarding the financing of the project, 4 held no firm views, seven were in favour of direct cash contributions, and 9 were in favour of some type of levy based funding. In regard to the ownership of the intellectual property arising from the research, most (60%) were of the opinion that the benefits should be proportional to the contribution. Only 15% were of the view that the benefits should be held by the governing entity (Figure 4). This appeared to reflect a lack of knowledge about the governance of incorporated business entities, rather than an extreme view that benefits should all be repatriated.

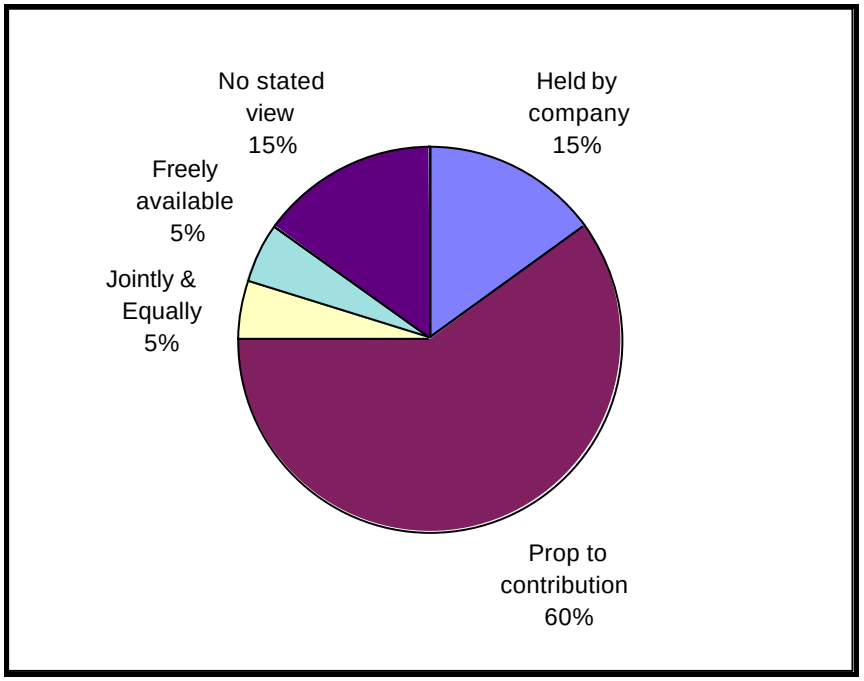


Figure 4 Support for the ownership of the results

Views about The Research & Development Plan for the Propagation Project

A successful research and development program for closing the life cycle of SBT will entail solving a number of issues relating to different parts of the species' life cycle. There are a number of critical issues that will need to be solved. Scientists were questioned to determine their views on the best of a number of options on how to do this. In most cases there was agreement as to the best approaches, however some differences were present.

Scientists interviewed came from 9 government research agencies and 5 industry hatcheries or farms (Table 3).

Table 3. Industry and Government Scientists Interviewed

Government Research Scientists	
South Australian Research and Development Institute	
Steven Clarke	Program Leader Aquaculture
Wayne Hutchinson	Scientist
NSW Fisheries	
Geoff Allen	Aquaculture Program Leader
Tasmanian Aquaculture and Fisheries Institute	
Ned Pankhurst	Head of School of Aquaculture, UTAS
Steve Battaglene	Aquaculture Program Leader
Chris Carter	Research Scientist
CSIRO Marine Division	
Tim Davis	Principal Research Scientist
Vince Lyne	Principal Research Scientist
John Gunn	Senior Research Scientist
Bribie Island Aquaculture Research Centre, Qld DPI	
Wayne Knibb	Principal Research Scientist
Abegail Elizar	Principal Research Scientist
Michael Bourke	Senior Technician
Agency For Food & Fibre Science, Qld DPI	
Bruce Goodrick	Research Scientist
NSW Fisheries	
Geoff Allan	Program Leader, Aquaculture
Bureau of Rural Sciences	
John Kalish	Senior Research Scientist
Fisheries Western Australia	
Sagiv Kolkovski	Research Scientist
Chan Lee	Research Scientist
Greg Maguire	Research Scientist
ADU TAFE, Western Australia	
Ken Frankish	Hatchery Manager
Industry Scientists	
Clean Seas Hatchery	
Shawn Whittiker	
Brendan Spillman	
B & S Aquaculture Technology	
Jason Aurhop	
Steven Shotten	
Navahoe Pty Lty	
Terry Bryant	Managing Director
Tuna Boat Owners Association of Australia	
Kirsten Rough	Research Officer
Spencer Gulf Aquaculture Pty Ltd.	
Andrew Tindale	Hatchery Manager

Obtaining larvae for experiments from wild fish.

Those that expressed a view about the feasibility of obtaining freshly hatched larvae for larval growth experiments by stripping at sea and airfreighting to experimental facilities all agreed that this would be very difficult. Although the eggs and larvae obtained from wild caught fish are likely to be of better quality than those held in tanks, there are many difficulties with doing so. The proportion of fish that are caught when running ripe is unknown, their capacity for being stripped is untested and their size and thick body wall may well produce difficulties with manual expression of the eggs. Harvesting gonads and maturing them *in vitro* was suggested as one way of getting around this problem.

If stripping at sea were to be successful, it would be critical to have finished transporting the eggs either before they hatched (less than one day), or immediately after hatching. The early yolk stage before first feeding would probably be the best time (day 2-3). Apart from these technical difficulties, any political sensitivities would also have to be overcome, as the best chance of obtaining ripe adults would probably be by utilisation of fish caught in the Indonesian fishery, and permits to operate in Indonesian waters would need to be obtained. If this approach were to be taken then it would probably be best achieved by a joint project with an Indonesian research institute.

Obtaining broodstock from the wild

Maturing SBT are found in the southern fishing grounds (40-50°S) off the southeast and south of Australia but spawning does not take place there. These individuals all have small gonads and it is believed that they move to an area based at about 40-50°S, 105°E. where maturation continues and thereafter they move northwards to spawn in a restricted area in the Eastern Indian Ocean between the Sunda Islands and Australia. The spent fish then move south with the Leeuwin current, at least some of them reappearing in the Tasman Sea from November onwards, dominating the catch between February and March.

Although the fish are spawning in tropical water, possibly within 1 metre of the surface, they are usually caught between 50 and 220m depth and even down to 350m deep. Larger fish are caught in shallower water. Water temperatures in this region in January are strongly stratified, varying from around 27-29°C on the surface, to 10C at 350m. In the southern fishing grounds they are fished between 40-160m depth in water temperatures of 10-12°C. Data retrieved from archival tags suggests that they are capable of, and frequently do, move vertically through these water depths during the course of their daily swimming activity.

There was a general belief that it would be possible to capture immature fish in the usual way from the Great Australian Bight and hold these for a number of years until they became mature. Fish around 40 kg are seen there and these fish would be between 6 and 7 years old, and are usually encountered deeper and below those currently used for the current fattening activity.

Collection of spawning fish from the northern spawning grounds was considered to be impracticable by most, but not all of those consulted. Most that had not worked with wild tuna had not considered the stratification of temperature with depth in that region, and so had assumed that the adults were in the same water temperature as the

larvae, ie 27-28°C. When this was drawn to their attention, most scientists agreed that this method would not be feasible because of the high surface water temperatures there.

One scientist suggested that mature adults might be taken by long-line from the southern feeding grounds, and towed to southern Australia by the current methodology for juveniles. Wherever the fish might be caught, the structure of the towing cage is such that the maximum speed that fish can be transported is 1 knot.

Induction of maturation and spawning

It was recognised that holding and maturing broodstock will entail the development of a suitable diet to produce maturation and quality eggs and larvae. Most felt that induction of maturation and spawning would be best done by holding adults in tanks on land and manipulating temperature and photoperiod. One scientist suggested that this could be done at sea by the use of liners and heat exchangers. Several suggested that adults should be both held in tanks and also in sea-based cages. Most believed that photoperiod and temperature manipulation, should be used together with hormonal manipulation. The measurement of gonad condition during this process was recognised as a problem, however several scientists noted that there is now an immunological method available for measuring the level of vitellogenin in the fish by the use of mucous smears.

It was recognised that sufficient generic heterogeneity should be retained if about 200 individuals were held for broodstock purposes, however there was uncertainty as to how many schools these should be used when taking fish. It was felt that between 300 and 1000 fish may be needed to perform statistically rigorous experiments.

It was noted that fish in an experimental net cage had difficulty in turning in a net with a diameter of 11 m, and the size of holding tanks was identified as a critical issue. Tanks of 30m diameter and 10 m depth were suggested, this size was based on that known to work for existing net cages. It was recognised that the cost of tanks was closely associated with their depth and the engineering strength needed. Few people would hazard a guess as to the minimum number of fish that could be held together without interfering with their normal behaviour, however one felt that the fish numbers per tank could probably be reduced to as low as 25 fish**.**

Growth of larvae to metamorphosis

Egg hygiene was only mentioned as an issue by one person who identified sterilisation as an issue needing treatment. The larval stage is believed to last for about 20 days in the wild. Everyone identified nutrition as a critical issue for larval growth and survival. It was suggested that their nutrient requirements might be relatively easily approximated by an analysis of the nutrient profiles of eggs from wild caught fish. Overall there was a recognition that pelagic species such as this feed on copepods in the wild, and that if rotifers and *Artemia* are going to be used, these should be enriched. There was agreement that there was not yet a suitable copepod feed for commercial use, however there was no clear consensus as to if enrichment would be better done using microparticles or algae. The use of green water was generally felt to enhance the passage of the enrichment medium through to the larval fish. There was a general recognition that for a commercial industry, the dependence on live food was a presently unavoidable complication, especially the difficulties associated with a reliable *Artemia* supply.

There was a recognition that air bladder inflation was important, and a general belief that the use of surface skimmers had solved most of the problems. One scientist pointed out the need to be careful with illumination levels due to the increased swimming activity associated with phototaxic larvae. There was a recognition that it would be best to wean larvae from live feed on to a pelleted formula, but no strong views on how to do it.

Growth of post-metamorphosis fish to fingerlings

In the wild juvenile fish are found in the Leeuwin current up to 50 cm fork length. Juveniles are found in 13°C water and dive to 8-9°C water to feed. Post-metamorphosis fish are believed to be out of tropical waters by 100 days old. Yellowtail Kingfish are removed from the nursery environment into sea cages by the time they are 5 gm in weight. There was concern of the reported deaths by hitting walls of northern bluefin tuna in Japan, however there was a general consensus that the use of appropriate animal husbandry methods such as circular tanks, directional water flow or raceways, and appropriate illumination was likely to go far in overcoming this problem.

The need for a suitable juvenile feed was observed, and although it was not believed that the methodology for sedation and transport of fingerlings would be an insurmountable problem, this would need to be investigated.

Appendix III

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Appendix IV

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