FINAL REPORT



Aquatic Animal Health Subprogram: Detection and management of health issues in yellowtail kingfish (YTK, *Seriola lalandi*) – the foundation for a health program for Australian finfish aquaculture.

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May 31, 2005

FRDC Project No. 2003/216









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Table of Contents

Non-technical	summary	5
Acknowledgm	ents	7
Background		7
Need		8
Objectives		9
Methods and re	esults	9
Discussion		11
Risk analysis a	and literature review	18
	7 Tables 1 & 2	23
	doption	46
	pment	47
	mes	47
		48
		51
Appendix 1.	Intellectual property	66
Appendix 2.	Staff	66
Appendix 3.	Australia questionnaire	67
Appendix 4.	Japan questionnaire and results	71
Appendix 5.	Guidelines to managing fish health of YTK	74
Sub-appe	endix A-Gfh. Australian National list	99
Sub-appe	endix B-Gfh. Legislation	100
Appendix 6.	Parasites identified in S.lalandi	101
Appendix 7A.	Stakeholder feedback, Workshop	102
Appendix 7B.	Stakeholder feedback, SAMFFA	103
Appendix 7C.	Stakeholder feedback, Research	104
Appendix 7D.	Stakeholder feedback, Interviews	105

2003/216 Detection and management of health issues in yellowtail kingfish (YTK, *Seriola lalandi*) – the foundation for a health program for Australian finfish aquaculture.

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OBJECTIVES:

1. Review and compile yellowtail kingfish (YTK, *Seriola lalandi*) health information from the industry, scientific literature and research organisations.

2. Determine the objectives and needs of a generic farm-level YTK disease identification programme.

3. Identify the obstacles and opportunities for the recognition and diagnostic confirmation of YTK diseases.

4. Provide the YTK industry with a qualitative and relative fish health risk assessment, and propose generic health management control measures.

5. Develop a photographic handbook on disease recognition for the YTK farmers and others.

NON-TECHNICAL SUMMARY:

Since 1999, the kingfish (YTK, *Seriola lalandi*) aquaculture industry has been a significant investor in South Australia's regional economy. Currently, the annual YTK production is 2,000 tonnes and is anticipated to approach 7,000 tonnes by 2008 with a retail value of \$55 million. The industry provides both direct and indirect jobs for up to 1050 workers in regional communities. Although still in its infancy, this aquaculture industry has been a major success story in the expansion of the Australian seafood and food-animal industries, and there is tremendous potential for the continued development of a sustainable and economically successful Australian finfish aquaculture industry. However, international experience has shown that one of the most pervasive and persistent limits to the growth and viability of a new finfish sector is disease. To date, the YTK industry of South Australia has enjoyed limited losses due to disease, nevertheless, anticipating, preventing and dealing with future diseases is a priority of industry, government and researchers. Consequently, disease identification, risk analysis and health management were deemed key approaches for this FRDC project.

Interviews and questionnaires in Australia and Japan elicited important information from four (4) main groups of experts: fish culturists, government officers, researchers and laboratory service professionals. Little is known or published about the infectious disease aspects of *Seriola lalandi*, specifically, so an extensive literature search was performed, looking for diseases and related topics reported in fishes of the same genus, *Seriola*. Pathology reports and papers published in Japanese (with English abstracts or translated with assistance) were also included. A substantial bibliography, literature review, a list of 41 plausible hazards to YTK health, and a risk analysis developed as a result. These outputs of the project will provide researchers and professionals with a useful information about YTK health, diagnostic and husbandry information and they will better identify research needs, funding priorities and facilitate more informed health management decisions. The lack of published information about YTK is, in itself, a noteworthy risk to the health of kingfish and supports proposals to enhance data collection, monitoring and surveillance of kingfish.

(...continued)

Training, diagnostic services and ongoing financial support from the State's lead agency (PIRSA) were identified as top-priority issues by those interviewed. Fish culturists and divers have exhibited a keen willingness to be trained and conduct routine disease assessments of YTK as basic "Level 1" fish health specialists. The YTK industry recognizes that fish health and diagnostics begin at the enterprise level. As such, a generic document summarizing guidelines for managing fish health has been authored and submitted to the YTK industry for consideration as part of a proposed best management practice (BMP) endeavour. That document may also serve to facilitate any future industry or government surveillance programs. A great need is also evident for a cost-effective private (or state government joint venture) mobile diagnostic service.

Australian stakeholders expressed concerns about: a) potential diseases of YTK, b) fish health and diagnostic services (i.e. what is available, essential, affordable and timely?), c) sustained fish health versus economic (and market) sustainability, and d) communication and differing mandates of industry versus government. It is clear that industry and government agencies must work in synergy to anticipate, prevent and deal with any potential future infections that might arise in cultured YTK. Yet a lack of public funds and resource personnel remain key inhibitors to disease prevention in South Australia. A view, widely shared by fish farmers, is that public benefactors of healthy, wholesome seafood and marine ecologies should allot public funds (via PIRSA) to subsidize the diagnostic and preventative surveillance measures taken by fish culturists. Consumers of other food-animals raised in South Australia benefit from this significant diagnostic support, yet aquaculture is treated somewhat differently.

Very little documentation or peer-reviewed information about the health hazards of *S.lalandi* exists. Consequently, the YTK risk analysis is subjective and qualitative. The overall health risks ranged from negligible to high yet the majority (27 of 41, or 66%) have been ranked as negligible, very low and low risks. Eleven (11, or 27%) of 41 were calculated as moderate overall risks, and three (3, or 7%) as high-risk hazards. Any pre-emptive and applied R&D efforts that arise from this report should be targeted at the fourteen (14) moderate and high risk hazards; ten (10) of which have already been identified in South Australian kingfish facilities. Tools of prevention and control of disease should be considered a priority.

A 64-page guidebook entitled "A Photographic Guide to the Diseases of Yellowtail (Seriola) Fish" (ISBN 0-920225-14-4) has been published as an adjunct component of this project to facilitate the training, recognition and identification of relevant diseases. The book is designed as an immediate diagnostic field guide for farm staff, fish health specialists and students.

OUTCOMES ACHIEVED TO DATE:

The literature review, risk analysis, guidelines to management and photographic pathology book have stimulated a much greater awareness of potential and future needs, opportunities and safeguards for the YTK aquaculture industry of South Australia for PIRSA, researchers and diagnostic service personnel. This project may form the basis for (or at least stimulate) further applied R&D and the development of tools to control diseases. The development of best management practices, integrated health management procedures, disease surveillance programs or amendments to current fish health regulations may also arise, provided industry and government bodies work as partners with mutual intentions.

KEY WORDS: aquaculture, yellowtail, kingfish, *Seriola lalandi*, disease, fish health, risk analysis.

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Background

The culture of kingfish (YTK, *Seriola lalandi*) in sea net pens is a primary industry and resource of South Australia with tremendous potential for growth, sustainability, economic success. It also offers significant employment opportunity to rural coastal communities. The kingfish aquaculture industry began in 1999 and has been a major success story in the expansion of the Australian seafood and food-animal industries. However, international experience has shown that one of the most pervasive and persistent limits to the growth and viability of a new finfish sector is disease. This project was undertaken because it was widely acknowledged that too little is known about the infectious disease aspects of kingfish and that anticipating, preventing and dealing with future diseases were among the highest priorities identified by the YTK R&D steering Committee in 2003.

The kingfish sector has been a significant investor in the South Australia's regional economy and production of these fish has now reached 2,000 tonnes. The annual production is anticipated to reach 7,000 tonnes by 2008 with a retail value approaching \$55 million. In addition, the industry estimates it will provide both direct and indirect jobs for 1050 workers in regional communities. The 'spin-off' jobs will have been created from the multiplier effect on ancillary industries, such as: processing, transport, engineering, boat and cage manufacturing, marketing, retail, building, financial and other services. Markets requiring quality sashimi (raw fish) and 'white tablecloth' products are currently being targeted in Asia, Europe, Australia and the USA. These markets continue to expand and the Asian sushi markets are largely considered to be insatiable. International and national markets demand everincreasing rigorous environmental monitoring and recording standards, and although South Australian tonnages are small in world terms (2,000 tonnes vs 6,000-8,000 tonnes in Japan), over the next decade the consumer demand for high quality seafood is expected to increase as shortages arise in global markets.

Diseases, especially infectious and parasitic diseases, are a constant concern for the aquaculturist. Our limited ability to control the natural environment and stressors inherent to captivity, tend to impose an increased susceptibility to disease within farmed fish populations. This situation coupled with opportunities for exposure of fish to indigenous natural pathogens from the wild fauna creates the potential for disease problems inside pens. Currently, the kingfish industry of South Australia has experienced only low-level mortality and it has not been significantly affected by bacterial or viral diseases. However, it is conceivable that diseases will become a significant problem as the industry's production expands. It can be further anticipated that most of these disease challenges will arise from opportunistic indigenous microorganisms that already exist in local marine waters, yet they remain

undetected as pathogens to date. Therefore, disease management strategies cannot rely solely only on the principles of exclusion of foreign 'exotic' pathogens and must rely more upon surveillance, recognition and early diagnoses.

Disease can limit aquaculture productivity in several ways. Firstly, the direct cost due to disease-induced fish death. Secondly, this loss is magnified by an elevated costof-production due to sub-optimal growth and poor feed conversion. Thirdly, some disease agents can affect seafood quality and affect marketing; pathogens are frequently used as non-tariff barriers to trade in seafood products. Finally. international experience has shown that the public expresses deep concern about disease in semi-open aquaculture settings. Concerns about the use of drugs and chemicals to control diseases, the perception of food safety, and worries about loading the environment with farm-magnified pathogens have all plagued the international fish farm industry. Australia enjoys the enviable position that its sea net pens are isolated, its waters are pristine, and its aquaculture industry is relatively free of significant infectious diseases. One exception to this is the presence of indigenous and problematic external parasites. Maintaining this 'limited disease' status provides tremendous market advantages as well as enhancing the economic performance and environmental sustainability of the industry, so disease identification, a qualitative risk analysis and health management strategies were deemed key approaches within this project.

Need

The kingfish R&D committee of 2003 was comprised of representatives of industry, State and Commonwealth governments and the scientific community. They recognised disease management and disease risk identification as high priorities in their 2003-2008 Strategic R&D Plan. These priorities are in line with the research and development priorities for aquaculture identified by the South Australian Fisheries Research Advisory Board. In addition, the targeted priorities that this project addressed within industrial development plans included: fish health, farm husbandry, and public perception. With regard to concerns about natural resource sustainability, this project supports and facilitates environmental protection and will contribute to the development of best environmental practices.

Prevention is the best and most cost-effective strategy for managing disease, yet the relatively small size of South Australia's YTK industry has yet to attract the attention of manufacturers of fish vaccines and drugs. So Australia's current lack of licensed, labelled and available control tools for aquaculture makes other forms of prevention the only viable option.

There are four requirements that must be addressed in order to have an effective preventive program. Firstly, one must be able to anticipate the problems one may face. A preliminary assessment of the risks of disease development and amplification is an important first step. Secondly, the knowledge of, and accurate identification of clinical symptoms and pathology by trained farm staff allows a mechanism for early detection and categorization of potential fish health problems. Thirdly, that mechanism is an ongoing system of observation and recording in the form of action-based detection of emerging changes. Finally, one must identify actions that should be taken in response to laboratory and disease-related findings. In other words, a guideline to managing fish health through best management practices is required. However, before recommending the best disease management practices, one must first conduct an inventory of the industry needs

and identify the gaps in knowledge and infrastructure that limit the capacity to achieve disease control objectives.

Objectives of Project 2003/216:

- 1. To review and compile kingfish (YTK) health information from the industry, scientific literature and both national and international research organisations.
- 2. Determine the objectives and needs of a generic farm-level kingfish disease identification programme, with acknowledgement that surveillance may, in the future, be expanded to industry and government levels.
- 3. Identify the obstacles and opportunities for the recognition and diagnostic confirmation of kingfish diseases.
- 4. Provide the kingfish industry with a qualitative and relative fish health risk analysis, and propose generic health management control measures.
- 5. Develop a photographic farm handbook on disease recognition for the kingfish industry and others.

Methods

An assessment of the present opportunities, needs and obstacles facing the kingfish (YTK) industry in South Australia was an essential first step to identify and clarify the issues related to fish health and disease. Numerous other topics of concern about industry development, markets, economics and aquaculture sustainability were also identified. The preliminary information from Australia was communicated and gathered in two ways by the Principal Investigator. A questionnaire was circulated to three stakeholder groups and follow-up interviews were conducted.

A similar questionnaire and interview approach was taken to gather information from Japanese *Seriola* farmers and researchers. In meeting with Japanese biologists and researchers, numerous unpublished case reports and annual summary disease reports were volunteered as documentation relevant to the diseases of *Seriola* fishes raised in Japanese waters. In addition, the Principal Investigator found opportunity while working in Japan over the past 10 years to conduct diagnostic assessments of many diseases commonly afflicting *Seriola* fishes.

A literature search and review was undertaken to investigate the quality and availability of published information about *Seriola* biology, culture, disease-causing agents of *Seriola*. Subsequently, a qualitative risk analysis was undertaken to address the relative importance of hazards to the health of kingfish in South Australia.

Finally, a disease recognition guidebook was compiled and published by the Principal Investigator, and a 2-day workshop was offered to members of the yellowtail kingfish industry, veterinarians and government personnel to disseminate the information and findings of this project.

Results

The Australian questionnaire was circulated to three main groups of people: aquaculturists, government officers, and laboratory services personnel. The questionnaire (Appendix 3) is best described as a subjective, value-minded approach to elicit stakeholder responses in terms of importance and depth of awareness. Follow-up interviews were conducted with the same individuals to discuss specific fish health and diagnostic experiences and to identify relevant yet broader topics of fish health, service needs, policy, and aspects of industry sustainability. Information gathered from stakeholders is vital for projects such as this to educate and inform the Principal Investigator about historical and ongoing conditions related to the culture of kingfish in South Australia. Yet we must accept that the information offered by stakeholders is subjective and biased. The representatives surveyed in Australia and Japan are certainly knowledgeable and leaders in their respective fields of expertise. However their numbers are quite limited. This is the reality and limitation of gathering grass-root information about YTK farming and disease, and it highlights one of the inherent limitations of this study. It is hoped that the information within this report will be corroborated (or disputed) in the near future through research and peer-reviewed publications.

With regard to knowledge gleaned from Japan, it is inappropriate to make direct comparisons of Japanese Seriola aquaculture to that of kingfish culture in South Australia due to vastly different farming practices and regulatory environments. The respective marine and political environments are also too different to draw meaningful comparisons. Nonetheless, the information from Japan remains very important. In order to transfer lessons of experience from elsewhere to develop a qualitative and relative risk analysis of kingfish diseases in South Australia a number of approaches were required in soliciting information from Japan. Firstly, the Principal Investigator has worked in Japan regularly since 1995 so he has created ample opportunity to conduct diagnostic assessments of many diseases of Seriola fish in Japan. These observations have been helpful in adding perspective to the interviews, questionnaires and the literature. Secondly, a questionnaire, translated into Japanese, was used to gather information about a number of hiramasa (YTK, S.lalandi) diseases relative to the diseases of other Seriola species (Appendix 4). The questionnaire was circulated to six representative Japanese aquaculturists, three pharmaceutical and vaccine distributors, and several government research pathologists. Thirdly, the Principal Investigator has established relationships within the aquaculture and academic communities in many areas of Japan, consequently interviews were kindly granted from Japanese fish farmers and research biologists to discuss the major disease and control events confronting cultured Seriola fish. Fortuitously, printed summary data (written in Japanese) was shared by the network of prefectural Fish Marine Research Centres. Annual summary reports (Nagasaki, 1994, 1998, 2002, www.marinelabo.nagasaki.nagasaki.jp/) reflect the prefectural laboratory submissions and disease diagnoses of all fish types and, of particular interest here, include those of hiramasa (YTK, S.lalandi). These data reports proved very enlightening to corroborate anecdotal experiences.

A literature search and review was undertaken to investigate the quality and availability of published information about *Seriola* biology, culture, disease-causing agents of *Seriola*, and papers reflecting specific diagnostic methodologies and husbandry practices. The search for diseases of *Seriola* fish then focused to review documents (published in English or Japanese) more specific to infections and diseases of kingfish, *Seriola lalandi*. The review of printed material is confusing in that the historical taxonomy of the *Seriola* fish species is complicated by the use of numerous synonyms. Common and scientific names for yellowtail kingfish include: kingfish, hiramasa, goldstriped amberjack, Japanese amberjack, purple yellowtail, yellowtail amberjack, *Seriola lalandi, S.aureovittata, S.lalandi lalandi, S.lalandi aureovittata, S.dorsalis, S.grandis, S.banisteri, S.fonki, S.mazatlana* and *S.pappei*. In many dated papers it is not clear whether *S.aureovittata* and *S.dumerili* refer to amberjack (now *S.dumerili* (Risso)), or the goldstriped amberjack (now *S.lalandi*), or the purple yellowtail. To confuse matters further, Japan has since created a *Seriola*

hybrid of amberjack and goldstriped amberjack (*S.dumerili* and *S.aureovittata* respectively) called "burihira". The species nomenclature is clarified somewhat by Pootenaar et al (2001), as well as Ho et al. (2001), Fishbase (<u>www.fishbase.org</u>) and Tachihara et al. (1997), each of whom cites many of these species and subspecies synonyms of kingfish simultaneously within their respective publications. For clarity, in the bulk of this report I have tried to refer to *Seriola* fishes as either yellowtail (or *S.quinqueradiata*), kingfish (or *S.lalandi*) and amberjack (or *S.dumerili*).

The literature search also included a brief review of Australian legislation and any documents related to aquaculture and fish health appear in sub-Appendix B-Gfh of the Guidelines to Managing Fish Health of YTK (Appendix 5). It is beyond the scope of this report to summarise, critique, and interpret the acts and regulations of the Australian Commonwealth and State governments.

A qualitative risk analysis was undertaken to address the relative importance of hazards to the health of kingfish in South Australia. The analysis integrates information about the magnitude of impact of specific diseases based on pathogenesis, other potential impacts in terms of loss of market, increased mortality rates or reduced productivity, as well as current National and International trade implications. The analysis lists the most plausible hazards (disease-causing agents) likely to affect kingfish of South Australia and it assigns overall estimates of risk to each hazard based on veterinary expertise, disease case reports, published literature, and the responses from Japanese and Australian interviews. This risk analysis is perhaps the most significant component of the project; as such, it forms the main body and discussion of this report.

The project objectives: to educate, train, recognise disease and to disseminate information to stakeholders, have been met through the delivery of a 2-day workshop and the creation of a disease handbook. The Principal Investigator's 10-year collection of *Seriola* pathology photographs provided the material to publish a 64-page guidebook as an adjunct component of this YTK health project. The book is entitled: *"A Photographic Guide to the Diseases of Yellowtail (Seriola) Fish"* (ISBN 0-920225-14-4). It is comprised of high resolution, detailed pathology photographs of disease-related topics most typically observed in *Seriola* fish types. Each of its 30 topics includes one page of easy-to-read information, descriptions, and diagnostic collection recommendations. The topics range from farm-hygiene to bacterial, viral, parasitic diseases, in addition to some speculative disease syndromes. The book is designed as a basic, hands-on, "what am I looking at now" diagnostic field guide for farm staff, divers, fish health specialists and students.

A fish health workshop was conducted for members of the South Australian Marine Finfish Farmers Association (SAMFFA) and other stakeholders at Arno Bay, South Australia on July 15/16, 2004. The workshop served to disseminate information arising from the project and deliver conceptual overviews on many health-related topics. Various theories, insights to ongoing R&D projects, and a wet lab session (to practice necropsy, collection and submission techniques for Level 1 diagnostic competency) were also delivered.

Discussion

> In finfish aquaculture industries on a number of continents the Principal Investigator has witnessed a decline in mortality rates from initial levels approximating 30-40% per production cycle to more acceptable levels approaching

5%. Much of this decline seems attributable to the implementation of disease management. Once the 90-95% survival targets are achieved the farmers and health professionals tend to focus more of their attention on production-based health medicine to address hazards influencing sub-clinical infectious diseases that affect the optimal performance of the animals. Sub-clinical conditions are often unrecognised and the risk (or loss) is not immediately apparent to the average observer. Awareness is enhanced by performing routine diagnostic activity, or surveillance procedures to measure the sub-clinical performance of risks (other than disease and death), or applying measurements that depict performance changes once a husbandry or environmental parameter is altered. These measures are even more difficult in aquaculture settings due to the nature of the production (FAO/NACA, 2000; Stephen, 1995).

In contemplating a 'state of health' versus 'a state of disease', it becomes obvious that people have different notions of these two concepts. The task of defining the two states illuminates subtle, yet complex and significant differences. We often refer to health and disease as if the two words are directly interchangeable opposites of one another but this is misleading. Disease is certainly a lack of health, yet health is not simply the absence of disease. Stephen (1995, 1997) offers the following definition of "disease": changes in the structure or function of a body that presents a particular set of signs and symptoms that are distinctly different from what is considered a normal state. Moreover, Stephen considers "health" to be the extent to which an individual or group is able to satisfy needs and cope with changes in the environment. The latter is a modified version of the W.H.O. definition of health (Nielsen 1992). The detection of specific pathogens or the detection of disease will often provide an incomplete and potentially misleading measure of the population's health (Stephen, 1997). In addition, the presence of a microorganism in (or on) a fish should not be mistaken as a disease, although an infection can potentially lead to disease given the appropriate environmental and host conditions. An infection can remain asymptomatic, it can result in shedding of the microorganism to others, and it can remain undetected as either a detriment to the host or as a benign commensal. The point is many factors lend to the causation of disease, some of which do not involve infectious agents. The complexity of the aquatic ecosystem often obscures the distinctions between health, sub-optimal performance and disease (FAO/NACA 402/2, 2001).

In the early stages of development of this project its title suggested health issues would be addressed, yet it soon became clear that the focus of the stakeholders of the kingfish industry is on the potentially infectious pathogens and husbandry practices that may result in a measurable biological loss in fish populations. In other words, stakeholders indicated the specific and immediate need for information about diseases, diagnosis, prevention and management. Hence the project adjusted accordingly.

> One of the main areas of concern of the YTK stakeholders is mortality and morbidity of fish due to diseases (infectious and others) that will affect fish survival to market, meat quality, and the saleability of product to consumers. These issues are typically the initial concerns of every developing aquaculture industry, so it is important to first identify the hazards and plan protocols to mitigate, minimise or control death due to diseases. In addition, the recurring themes arising from interviews (Appendix 3) fall into four main categories:

a) Diseases evident in kingfish (in Australia and other countries),

- b) Fish health and diagnostic services; what is available and affordable?
- c) Communication, and different mandates of industry versus government,
- d) Sustained fish health versus the economic sustainability of the industry.

A number of diseases are already evident in cultured kingfish of South Australia yet the mortality rate associated with each of those diseases remains relatively low. More detail about Seriola diseases is discussed within the literature review and risk analysis (see pages 18-45). Aquatic environments are not sterile and it is likely that opportunistic disease-causing microorganisms (pathogens) already exist naturally as indigenous organisms in the gulfs of South Australia. Currently, there is no active surveillance or affordable plan to identify those pathogens or diseases amongst wild fish populations, so it is impossible to say with certainty what may exist beyond the Consequently, the government policies tend toward net pen enclosures. precautionary principles to managing the industry. During interviews fish culturists expressed that are frustrated with this restrictive approach and that communication between industry and government groups tends to remain somewhat guarded as a result. Government officers acknowledge this sentiment and concede that their job descriptions are largely focused on precautionary policy development, cost-recovery, and enforcement if necessary. There is little indication of plans to allot more manhours or more funds to realise the opportunities related to field extension work or to support the industry. Cultured kingfish, reared inside sea pens, continue to serve as sentinel animals of natural infections within the waters of South Australia. The question that begs an answer is: Will the aquaculture industry be held responsible and accountable for the inevitable discovery of indigenous marine pathogens? Surveillance of what already exists would be helpful, but where is the money, the willingness, and the diagnostic support for such an activity?

The top three priorities of industry stakeholders are: 1) education and training, 2) government subsidies for diagnostic work, and 3) field extension services by PIRSA's aquaculture personnel. Industry personnel understand that each enterprise should monitor and identify infectious diseases inside its own net pens to recognize potential In-house monitoring will also enhance evidence-based health problems early. management actions. However, the fish farmers feel that expanding the availability and efficiency of diagnostic services is also essential in South Australia. This can be achieved by implementing or subsidizing an affordable, mobile veterinary service, or an extension service by PIRSA, in addition to introducing more cost-beneficial laboratory services. Government case-confidentiality, surveillance objectives, and money are the obstacles to further growth, cooperation and success of YTK aguaculture. The industry and government have a willingness to work together, but a new paradigm must develop. Industry members in South Australia prefer a fish health extension service similar to that offered by Tasmania's Department of Primary Industries Water and Environment (DPIWE). A cohesive 50/50 extension service is envisioned to facilitate the long-term sustainability of kingfish aquaculture as a 'primary industry' of the state. Two main obstacles to these priority issues are: the government's current policy and enforcement mandate over the industry, and the shortfall of public funds allotted to optimising production of wholesome animal Health services personnel, who assist farmers in need with regard to protein. diagnostic work and integrated health management recommendations, would be helpful to government, industry and the public.

Communication and cooperation of stakeholder groups toward mutual goals is of key importance to those interviewed, and measurable support from aquaculture's lead

state agency is important to farmers. The mutual goals include: working and expanding within progressive (not restrictive) policies, sustainable economics, markets and growth, and the maintenance of ecologies with minimal, shallow and reversible environmental foot printing. The industry is concerned that PIRSA will remain reactive and restrictive to the industry rather than offering proactive support to limit losses, sustain growth, secure markets and assist in the health management of troublesome events. These are not people or personality issues. Rather, the differences of opinion stem from a mismatch of mandates, objectives, job descriptions and allocation of public money to food industries. An environmental non-government organisation, the World Wildlife Fund (WWF), recently recognised that aquaculture is definitely here to stay. WWF has acknowledged that as key impacts of aquaculture become evident and relative to other lesser impacts, the precautionary approach should flex toward better management practices (personal communication J.Clay, VP WWF, Vancouver 2003). In other words, all progressive farming activity has some effect on the local environment and once that effect is determined, it can (and will) be managed by the farmers and governments in the best interests of the fish in order to sustain a clean rearing environment.

Issues of financial and market sustainability are largely beyond the scope of this report but these topics are noted here to illustrate the critical and integral role that fish health plays in markets and industry sustainability. It was clear from all respondents that the cultured kingfish industry appears to be caught in a perpetual "chicken and egg" argument. That is, if the fish don't stay healthy, wholesome and alive to harvest then there is little need for a marketplace. Ironically, the opposite is also true and equally important; if a sustained marketplace is not developed first, there is little need to strive for optimal health, growth and survival of the fish. So where is a private company or government to allocate its time, support and money...on consumer confidence, in best management practices, on marketing products, or preventative population health?

The kingfish industry of South Australia has great potential for growth and development, but its infancy is somewhat self-defeating at this point. The YTK industry needs assistance to attain a critical mass for continued growth, sustainability and access to tools of prevention. The economics of securing expensive tools of health maintenance (vaccines, diagnostics and treatments) to a small industry (with its present status and low requirements) is too costly for product manufacturers, farmers and service consultants. The products and services are cost-prohibitive, if available at all.

> Other significant obstacles identified by stakeholders of kingfish include a lack of training with respect to meaningful history and sample collections, no relevant disease information or service expertise and no photographs of fish lesions. The viewpoint of most fish farmers is that regional laboratories offer slow and vague results, yet the labs are performing to the best of their ability despite limitations of diagnostic tests (i.e. no virology, no molecular diagnostics, no serology or toxicology) and low numbers of fish submissions. One other fair comment from laboratory recipients of fish samples suggests that many farm personnel are relatively untrained and ill equipped to collect and submit precise samples, including complete and insightful history sheets, when unexplained fish deaths occur. As a result, the diagnostic labs often face the "garbage-in, garbage-out" phenomenon in terms of quality of information and communication.

Diagnostic laboratories have supply and demand issues as well. They receive only intermittent fish submissions, likely due to cost-prohibitive fee structures and lack of government subsidy for fish diagnostics. The limited fish expertise at laboratories or in the field is another concern of the farmers. Consequently, no immediate tentative diagnoses or recommendations are offered so initial decisions and actions are somewhat delayed. The limited diagnostic tools at regional laboratories are potential hazards to YTK health and this may require further attention from State agencies and the private contract labs. The technical guidelines on health management (FAO/NACA, 2001b) suggests that the accurate diagnosis of aquatic animal diseases require different levels of disease surveillance and data collection, ranging from farm-site observations through to the application of state-of-the-art diagnostic technology. Three standard levels of disease diagnosis are defined by NACA: Level I activities include farm-site monitoring and supplying information essential for making presumptive diagnoses. Level I diagnosis is an essential starting point for

making presumptive diagnoses. Level I diagnosis is an essential starting point for reducing the risk of pathogen transfer via movement of fish and for detecting emerging clinical infections (AQIS, 1999). FAO/NACA recommends that all countries should ensure that Level I capabilities are well-established in addition to obtaining access to and/or developing Level II and III resources within and outside the farming region. Level I diagnostic (or farm-level) training is generally appropriate for:

- macroscopic ecto-parasites that are easily identified,
- diseases with specific and consistent gross pathology, and
- farm sites with an established history and/or susceptibility to specific diseases.

Level II diagnostic (or basic lab level) capability is required for diseases whose clinical signs could be caused by a variety of infectious (and non-infectious) agents. These pathogens are not readily recognized by gross examination using the naked eye. In these cases, bacteriology, mycology, cytology and histology are commonly required. Level III diagnostic capabilities (not to be confused with Level 3 contagion containment facilities) are required for more problematic pathogens and those that are difficult or impossible to identify at Levels II and I. Tests such as virology, immuno-fluorescence, serology, toxicology, immunological and molecular techniques, and even electron microscopy are required.

Australia certainly has highly capable Level III diagnostic laboratories. The DPIWE laboratory in Tasmania and the Fish Diseases Laboratory at the Australian Animal Health Laboratory (AAHL) undoubtedly have the means and capability to offer more in-depth diagnostic testing. Unfortunately, when Level III labs are located such great distances from the YTK farm sites, costly delays in sample submission, transportation and communication are inevitable. For example, if fish samples emerge that pique Gribbles' and PIRSA's diagnostic interests, those samples likely require re-collection at the original farm, then forwarding to the AAH or Tasmania labs for more extensive diagnostic confirmation. In reality, 3-10 days are likely to pass for the completion of the entire transportation and diagnostic process.

All three levels of diagnostic expertise, including fish culturists and extension officers, are necessary to detect emerging, rare or truly exotic diseases. Furthermore, in many developed countries, Level III capabilities at regional laboratories have proven to be extremely useful and timely in the face of notifiable animal epizootics (Drs. Lewis and Ritchie, personal communication, Canada, 2004.).

Objectives to identify opportunities for the recognition and diagnostic confirmation of diseases of kingfish and to train stakeholders were addressed by means of a 2-day

workshop and the creation of a photographic disease guidebook. FRDC received 20 copies of the guidebook and 100 copies were delivered to SAMFFA. Most of industry's 100 books were immediately distributed to delegates of the kingfish health workshop held in July 2004, so the guidebook may now serve as a reference text for all future YTK disease matters. The workshop event created networking opportunities for veterinarians, laboratory personnel, divers, aquaculturists, researchers, regulators, and Chief Executive Officers of fish farming enterprises. Seven (7) speakers (veterinarians, microbiologists, researchers, government officers, and service personnel) presented information to 35 delegates. It was the first workshop of its kind to offer such a wide array of tangible information and activity for stakeholders of the YTK industry and it is hoped that more hands-on workshops of this nature will continue on an annually basis. The stakeholders' feedback on the workshop is included as Appendices 7A and 7B.

> With regard to the literature review and risk analysis, Dr. Ernst of the University of Adelaide kindly provided valuable feedback and recommendations on the original draft analysis. His comments have been carefully considered and numerous amendments have been made as a result. Details of the literature review are largely embodied within the risk analysis below. There are few publications about kingfish, and even fewer pathology case reports or controlled comparative studies involving *Seriola lalandi*. Conversely, there is a substantial volume of information about two other predominantly cultured fishes of Japan, yellowtail (*Seriola quinqueradiata*) and amberjack (*Seriola dumerili*).

Background information on Japanese aquaculture is offered by Ho et al. (2001). He states that the culture of *S.quinqueradiata* began in 1927, yet *Seriola* culture did not develop into a significant production industry until the 1950s. On average, in the past 10 years, the total annual production of *Seriola* fish has approximated 150,000 MT (Nakajima et al., 1998 citing Fisheries data of 1995). In 2003, yellowtail (*Seriola quinqueradiata*) production biomass from 23 Prefectural regions was estimated at 46,000MT, whereas the wild-caught (ranched) *Seriola lalandi* production in Japan is estimated as a small percentage of that: approximately 6,000-8,000 metric tonnes (Fisheries Association, personal communication, Japan, 2004).

The principal losses of *Seriola* species to disease in Japan reached a total biomass of 8,651 MT (Japanese Fisheries Agency report 1996, using 1993 data and summarised by Sano 1998). This compares with 15,893 MT lost in 1984 (Sano and Fukuda 1987, from 1984 Fisheries Agency data). The 1993 epizootics were ranked in pathogen categories: bacterial diseases attributing to 97.5% of the deaths, parasitic diseases were responsible for 2.2% of deaths, and viral diseases for 0.3%. The losses have been converted to percentage of tonnage and are ranked by pathogen below. A reminder here that these percentages approximate the annual tonnage or biomass of fish lost, whereas the number or inventory of fish dying from each disease would reflect a very different ranking. For example, diseases caused by YTAV, RSIV and Vibrio normally generate high mortality rates at a time when the yellowtail are still fingerlings or juveniles so the farm biomass is relatively low. By comparison the mortality associated with Lactococcosis is generally seen in larger fish so loss of fish tonnage is significant for this disease.

	% MT	% MT
Pathogen/disease (from Sano, 1998)	1993	<u>1984</u>
Bacteria:		
Lactococcosis (previously Enterococcosis)	67%	67%

Photobacteriosis (previously Pasteurellosis)		25%	14%
Haemolytic jaundice	5%		
Vibriosis		0.2%	1.8%
Parasites:			
Gill 'flukes' (Heteraxiniasis)		1.4% of toni	nage
Skin flukes (Benedeniosis)		0.7%	
Trichodiniasis		0.2%	
Blood flukes		0.05%	
Viruses:			
Red Sea Bream Iridovirus (RSIV)		0.3% of toni	nage
Aquabirnavirus, Yellowtail Ascites Virus (YTA)	V)	0.08%	

It is also noteworthy that the epizootics noted above do not include other known yellowtail diseases, such as: flexibacteriosis, lymphocystis, epitheliocystis, streptococcosis (caused by *Streptococcus iniae*), pseudomoniasis, nocardiosis and mycobacteriosis, yet Kusuda and Kawai (1998) mention many of these diseases as problematic to *Seriola* fish. This discrepancy within published information illustrates that the mere mention of a pathogen in the literature does not usually reflect disease probability or the prevalence in the population. Most scientific reports reflect academic, incidental, or experimental findings. Studies often confirm that a disease-causing microorganism can indeed infect a host fish under defined or specific challenges and environmental conditions, and although reports of this nature are very important to this project, the conditions leading to disease must also be considered.

According to Sharp et al. (2003), kingfish are distributed in temperate waters around New Zealand, Australia, Japan, South Africa and western USA; *S.lalandi dorsalis* off California USA, *S.lalandi aureovittata* in Asian waters, and *S.lalandi lalandi* in the southern hemisphere (Fisheries Research Institute, 1998). Sharp et al. (2003) also cited a recent study that observed significant genetic divergence between kingfish populations from Japan compared to those from Australia-New Zealand (Nugroho et al., 2001). Yet, for the purpose of this kingfish report and to summarise the published reports from various researchers, all references to *Seriola aureovittata, S.lalandi, S.lalandi, S.dorsalis,* and *S.grandis* are considered synonymous with yellowtail kingfish, *Seriola lalandi*, the Carangid fish cultured in the waters of South Australia.

Risk analysis

Introduction: In terms of risk, the primary concern of industry stakeholders is the occurrence of any adverse effect on the future viability of the kingfish industry that might be realized in the form of loss; loss due to 'hazards to fish health'. Simply stated then, what disease-causing microorganisms and subsequent diseases are most likely to arise and become problematic to the kingfish industry of South Australia, and what are the relative likelihoods of those diseases arising there? Beyond that, this risk analysis attempts to characterize the impact of loss, or 'consequence' of diseases, by considering contributing factors and the certainty of evidence.

This kingfish risk analysis is a **subjective and gualitative** assessment. Currently there is insufficient documented evidence to analyse the risks in a quantitative Consequently, the qualitative analysis emerged based on stakeholder fashion. interviews, questionnaires, farmer and veterinary experiences, and Japanese diagnostic laboratory summaries corroborated by published literature whenever possible. The dearth of documented peer-reviewed information about the health hazards of S.lalandi relative to the substantial volume of published data regarding other Seriola fish types (S. quinqueradiata, S. dumerili) is, in itself, a noteworthy risk to the health of kingfish. This fact supports proposals targeting the collection of data, monitoring and surveillance of kingfish. Only twenty-two (22) reports were discovered that mentioned kingfish; two (2) of which are S.lalandi pathology case reports, and two (2) others reflecting comparative, infectious susceptibility experiments relative to other fish species. The remainder are largely review documents and/or reflect the incidental presence of ecto-parasites or incidental pathogens found in kingfish.

Stakeholder input and consultation is an important component of the analytical process, particularly in this kingfish study since the industry is relatively new, highly scrutinized by consumer groups and the general public, plus the hazards themselves seem somewhat novel to South Australia. In that respect, the hazard list and risk analysis will hopefully prove enlightening and educational for stakeholders of various expertise.

Hazard identification: The hazards to kingfish were selected by observation and reports in the literature of plausible infectious and non-infectious agents capable of inducing disease in *Seriola* fish species. Two (2) additional hazards are listed here due to their presence on national and international disease lists (AFFA, 2003b; OIE, 2004; as shown in sub-appendix A-Gfh of Appendix 5), and because of their potential for extreme impact on the industry. The two additional hazards are "striped-jack" viral nervous necrosis (SJVNN^{*}) and viral haemorrhagic septicaemia (VHS).

FAO/NACA (2001) suggests that Australia may have more than 1700 known transmissible agents reported from aquatic animals, however only a few are considered to have major pathogenic or socio-economic importance. Forty-one (41) of the most plausible hazards to health have been identified and listed in this risk analysis for *Seriola lalandi*. The list was generated from interviews with industry and government personnel, experienced biological and veterinary opinion, pathology case reports and published literature.

^{*} a nodaviral infection synonymous with viral encephalopathy and retinopathy, VER, VEN, VNN.

Many of the health hazards identified for kingfish are microorganisms. That is not to say that the mere presence of the potential pathogen alone is sufficient to adversely affect fish health, or warrant an overzealous action that may then adversely affect farm economics, markets or the viability of industry. Of course there are some exceptions to this premise, such as the discovery of microorganisms listed on national or OIE (Office International des Epizooties) lists of pathogens and diseases of concern. The discovery of these microorganisms, even prior to symptoms of clinical disease, must be reported, investigated and acted upon.

Methods (risk analysis): In basic terms, the process of risk analysis used here is as follows:

- the concerns of stakeholders are determined,
- information is gathered from interviews, expert experiences and the published literature,
- hazards are identified, and
- the estimates of likelihood and consequence for each hazard (or outcome of each hazard) are then assigned, within the context of available control measures and other contributing factors.

The estimate of 'overall risk of loss' for each hazard arises from combining estimates of likelihood and consequence using a risk matrix as shown in Figure 1 (AS/NZS 4360,1999; AFFA, 2003a).

This risk analysis is largely summarized in Tables 1 and 2 below, but for clarity I offer some conceptual guidelines and definitions of the assessment:

- 1) <u>Hazards to fish health</u> include plausible infectious pathogens and noninfectious conditions that may result in disease of kingfish.
- 2) The <u>outcome of a hazard</u> is typically the disease (see definition below).
- 3) Outcomes ultimately lead to <u>adverse consequences</u>, which vary in both number and severity. Five (5) key consequences were considered in this analysis, and each was affected by two (2) main contributing factors.
- Quality of information and the <u>certainty of evidence</u> are important to consider when assigning estimates of likelihood.
- 5) The <u>degree of likelihood</u> is assigned. It estimates the probability of entry, establishment and spread of a hazard.
- 6) The <u>magnitude of impact</u> is assigned which estimates the severity of consequence of entry, establishment and spread of a hazard.
- 7) An estimate of <u>overall risk of loss</u> is the product of likelihood and consequence.

In many cases, the outcome of a hazard may eventually take the form of disease, and it is generally the risk of disease that is assessed in this report in terms of likelihood and magnitude of impact. **Disease is defined here** as: *changes in the structure or function of a body that presents a particular set of signs and symptoms that are different from what is considered a normal state* (Stephen and Iwama, 1997).

The five (5) key adverse consequences include:

- i. acute and significant mortality,
- ii. lower, yet persistent morbidity and mortality rates,
- iii. unfavourable growth performance and feed conversion (performance),
- iv. reduced product quality and saleability to market, and
- v. notifiable conditions and potential barriers to movement.

Two (2) main contributing factors (A & B) are shown in Table 1 below. They tend to influence the degree of likelihood or the severity of consequence and, consequently, the overall risk rating. The factors are:

- a) lack of control tools (vaccines, effective chemo-therapeutants), and
- b) lack of early detection tests, and/or the limited application of timely level II to III diagnostic services at local or regional laboratories, which may exacerbate the accumulation of pathogens, disease duration and outcome.

A third contributing factor of sorts is the lack of routine monitoring and surveillance for hazards by farm and government personnel. This activity perhaps delves into the area of *risk management* as opposed to being considered a specific *hazard to fish health*, since in the absence of any other hazard the lack of monitoring in itself will not cause loss. Nevertheless, as a 'non-event', the lack of monitoring and surveillance for hazards may influence the overall risk ratings by delaying disease recognition thus enabling the accumulation of pathogens and subsequent disease outbreaks.

Generally speaking, estimates of impact for a particular hazard have been rated higher if the adverse consequences are numerous. So, a hazard with four (4) adverse consequences tends to warrant a higher impact rating than a hazard associated with only three (3) adverse consequences.

Certainty of evidence: Due to the absence of quantitative data and peer-reviewed information about kingfish, the information gathered for each hazard was further assessed in terms of its 'certainty of evidence' (see Table 2 below). This was approached in three (3) ways:

- **Firstly**, it was established that expert experience (and/or documentation) confirmed that the hazards affecting *Seriola lalandi* had already occurred in at least one of three countries: Japan, New Zealand or Australia. Thirty-three (33) of the 41 hazards were confirmed with certainty, by interview and/or literature, to already affect kingfish in at least one country under favourable environmental conditions.
- Secondly, a subjective ranking system depicting certainty (zero to 5) was assigned to each hazard based on the following criteria:
 5 = the disease has already been positively diagnosed (documented or at least observed and personally communicated to the Principal Investigator) in *S.lalandi* of South Australia.

4 = *S.lalandi* fish have been shown to contract the disease within peer-reviewed scientific literature,

3 = Japanese government case reports and/or grey literature reports the occurrence of the disease in *S.lalandi*,

2 = the disease in *S.lalandi* is not documented, yet it has been documented in other *Seriola* fish,

1 = the disease is not identified in *S.lalandi*, yet it has been documented in numerous NON-*Seriola* warm-water marine fishes, and

0^{*} = no evidence was found for the hazard in any marine warm-water fish.

*Note: the 'zero' ranking did not apply to any of the *plausible* hazards listed here. Implausible hazards found no value within this risk analysis however a more extensive list of possible parasites is included as Appendix 3. For rankings of 1 and 2, an 'unknown' estimate of likelihood could have been

applied; instead, a subjective judgment (based upon probability definitions below, as well as veterinary observation and Japanese experience) enabled the estimate of likelihood to be assigned as 'low' in most cases. Two exceptions to this standard are the 'high' likelihoods of occurrence to natural seasonal hazards like phytoplankton blooms and environmental low oxygen conditions (i.e. during very warm sea water temperatures) due to their unpredictable yet inevitable occurrences in global aquaculture settings.

• The **third** facet of certainty of evidence reflects the results of the Japanese survey (Appendix 4). The significance of a disease amongst sea pen cultured hiramasa, *S.lalandi*, was compared to the significance of that same disease amongst other cultured *Seriola* fish, based on the experience of Japanese experts. This information affects both the probability of occurrence as well as relative significance of impact, species resilience and/or the susceptibility of *S.lalandi* to each hazard. The values of 'disease significance' amongst ranched Japanese *S.lalandi* were assigned as: unknown, very low, low, moderate and high.

Estimates of overall risk: The estimation of overall risk was determined by applying the information outlined above to **estimates of likelihood and consequence** of entry, establishment and spread within the sea pens of kingfish of South Australia using the risk estimation matrix (Figure 1). The six (6) descriptors for likelihood and consequence axes are the same: negligible, very low, low, moderate, high and extreme. For the purpose of this report, each **descriptor of consequence** is defined as follows:

- **Negligible** = unknown or no significant pathological or biological changes, with low or no measurable economic effect at the enterprise (farm) level.
- **Very low** = very limited pathological effect or morbidity and mortality, with minor economic effect at the enterprise level and negligible effect on the industry.
- **Low** = pathology is evident and accompanied by low morbidity and mortality rates, with manageable economic effect to the enterprise yet low economic significance to the industry.
- **Moderate** = significant obvious pathology, substantial seasonal morbidity and mortality rates, with significant cost to the farmer to warrant intermittent concern by the industry.
- **High** = serious biological consequences, prolonged high mortality rates, enterprise survival is questioned, significant economic concern to the industry.
- **Extreme** = catastrophic consequences to the entire industry, total mortality or eradication of fish is considered, trade implications at the national level.

Terms used for **likelihood of occurrence** in <u>South Australia</u> are:

- **Negligible** = the hazard is not known to occur in *Seriola* fish.
- Very low = the hazard is extremely unlikely to occur in S.lalandi.
- **Low** = the hazard is unlikely to occur in *S.lalandi* with any certainty.
- **Moderate** = the hazard may occur with even probability (50/50).
- **High** = the hazard has already emerged, or is likely to emerge, in *S.lalandi* of South Australia, yet its establishment or spread remains minimal or undetermined.

• **Extreme** = the hazard has already emerged with significant prevalence in *S.lalandi* of South Australia with high certainty.

Again, both 'negligible' and 'very low' likelihoods are not very compatible with this risk analysis of most *plausible* hazards. That said, an exception was made in assigning a 'negligible' status to two important OIE reportable diseases: namely, nodaviral encephalopathy/retinopathy (or SJVNN) and viral haemorrhagic septicaemia (VHS).

Probability:						
Extreme	Negligible	Very low	Low	Moderate	High	Extreme
High	Negligible	Very low	Low	Moderate	High	Extreme
Moderate	Negligible	Negligible	Very low	Low	Moderate	High
Low	Negligible	Negligible	Negligible	Very low	Low	Moderate
Very low	Negligible	Negligible	Negligible	Negligible	Very low	Low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Very low
	Negligible	Very low	Low	Moderate	High	Extreme
	Consequence					

Figure 1.	Risk estimation	matrix (from	AFFA, 2001)
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Discussion - risk analysis: The overall health risks to *Seriola lalandi* of South Australia ranged from negligible to high, yet the majority (27 of 41, 66%) have been ranked as negligible, very low and low risks. Eleven (11) of 41 (27%) were calculated as moderate overall risks, and three (3, or 7%) as high-risk hazards. The selection and risk rationale of the 41 hazards is offered here, but an emphasis is placed on the 14 hazards estimated to be moderate and high risks to the kingfish industry; ten (10) of which have already been identified in South Australian YTK culture facilities.

The hazards and factors influencing the 'estimation of consequence' are summarized in Table 1, whereas aspects of 'certainty of evidence' and the overall risks are presented within Table 2:

Table 1. Summary of yellowtail kingfish risk analysis: hazards and contributing factors influencing 5 key consequences.

Five (5) key consequences	i	II		iv	<u> </u>	Contributing A	Contributing B
	Acute and	Lower persist-	Reduced fish	Reduced	Possible barrier	No control tools	No early detection, or Level II or III
	significant mortality	ent mortality or morbidity	performance	product quality or saleability	to movement. Reportable	no vaccines or chemotherapy	diagnostics req'd
Plausible hazards to fish health	mortanty	of morbially	performance	of saleability	Reportable	chemotherapy	diagnostics req d
Viral hazards:	1						
Red sea bream iridovirus (RSIV)	Y		I Y		Y		X
Yellowtail ascites aquabirnavirus (YAV)	Y		Y			x	×
Viral deformity aquabirnavirus (VDV)	Ŷ		Y			×	x
Lymphocystis iridovirus				Y		x	x
Nodaviral enceph. & retinop. (VER/SJVNN)				-	Y	X	x
Viral haemorrhagic septicaemia (VHS)					Y	×	x
Bacterial hazards:					-		
Photobacterium damsela piscicida	Y		Y				x
Listonella anguillarum	Y		Ŷ				
Lactococcus garvieae	•	Y	Ý	Y			
Streptococcus dysgalactiae		Ý	Ý	Y			x
Nocardia seriolae		Y	Y	Y		х	x
Mycobacterium marinum		Ý	Ý	Y		X	X
Tenacibaculum maritimum		Y	Y	Y			<u>^</u>
Chlamydial epitheliocystis		Y	Y	•		x	x
Pseudomonas spp. dermatitis		Y	Y	Y		X	X
Seedling bacterial over-growth	Y	I	Y	T		^	^
Parasitic hazards:	T		T				
			l Y		1		x
Heteraxine heterocerca (gill)	Y		Y				^
Zeuxapta seriolae (gill)	Y	Y	Y	Y			
Benedenia seriolae (skin)		Y	Y				X
Neobenedenia spp.			× ×	Y		N N	X
Caligus aesopus & spinosis (gill arches)			Y			X	
Caligus spp. (4+ on skin)				Y		X	
Cryptocaryon spp. (skin)		Y	Y	Y		X	
Myxobolus buri (brain)		Y	Y	Y		X	Х
Other myxsporeans (brain)		Y	Y	Y		X	x
Blood fluke (Paradeontacylix-like sp.)		Y	Y			Х	x
Microsporidium seriolae (meat)		Y	Y	Y		X	x
Kudoa spp. (meat and heart)			Y	Y		X	x
Unicapsula seriolae (meat)			Y	Y		Х	x
Non-infectious complex syndromes:							
Necrotic enteritis (+/- bacterial)		Y	Y			X	X
Green-liver syndrome (+/- parasitic)			Y			X	x
Haemolytic anemia syndrome (+/- bacterial)	Y		Y			x	X
Cataracts (+/- nutritional)			Y				x
Seedling nutritional syndrome(s)		Y	Y				X
Growout nutritional syndrome(s)			Y				x
Broodstock nutritional syndrome(s)			Y				X
Swim-bladder over-inflation / deformity	Y		Y	Y			X
Head & jaw deformity		Y	Y	Y			
Phytoplankton & micro-algal blooms	Y			Y			
Hypoxia (oxygen deprivation) syndrome	Y		Y				
Predation		Y	Y	Y			X
					•		•

Table 2. Summary of yellowtail kingfish risk analysis: overall risk estimates and certainty of evidence.

	"Likelihood" or	"Magnitude of impact"		Evident now in	Certainty of	Disease significance
	Estimate of	Estimate of	Estimate of	S. lalandi	evidence	relative to other
	probability of entry,	consequence of entry,	overall	of NZ, Austr.	ranking	Seriola spp. in Japan
Plausible hazards to fish health	establishment & spread	establishment & spread	risk	&/or Japan	(5 to 0)	(Japanese survey)
/iral hazards: Pg 25						
Red sea bream iridovirus (RSIV)	high	high	HIGH	Yes (in Japan)	3	low
fellowtail ascites aquabirnavirus (YAV)	moderate	moderate	low	Yes (in Japan)	3	very low
/iral deformity aquabirnavirus (VDV)	low	low	negligible	r co (in capan)	2	unknown
_ymphocystis iridovirus	extreme	very low	very low	Yes	5	unknown
Nodaviral enceph. & retinop. (VER/SJVNN)	negligible	extreme	very low		1	unknown
/iral haemorrhagic septicaemia (VHS)	negligible	extreme	very low		1	unknown
Bacterial hazards: Pg 29			,			
Photobacterium damsela piscicida	high	moderate	moderate	Yes (Japan)	4	low
istonella anguillarum	extreme	moderate	moderate	Yes	5	low
actococcus garvieae	extreme	moderate	moderate	Yes	5	moderate
Streptococcus dysgalactiae	low	low	negligible		2	unknown
Nocardia seriolae	low	high	low	Yes (Japan)	3	low
Mycobacterium marinum	low	high	low	Yes (Japan)	3	very low
Fenacibaculum maritimum	moderate	low	very low		2	unknown
Chlamydial epitheliocystis	extreme	moderate	moderate	Yes	5	moderate
Pseudomonas spp. dermatitis	moderate	low	very low	Yes (Japan)	3	unknown
Seedling bacterial over-growth	extreme	moderate	moderate	Yes	5	moderate
Parasitic hazards: Pg 37						
Heteraxine heterocerca (gill)	low	high	low	Yes (Japan)	4	unknown
Zeuxapta seriolae (gill)	extreme	moderate	moderate	Yes	5	moderate
Benedenia seriolae (skin)	extreme	high	HIGH	Yes	5	moderate
Veobenedenia spp.	low	moderate	very low	100	2	unknown
Caligus aesopus & spinosis (gill arches)	extreme	very low	very low	Yes	5	unknown
Caligus spp. (4+ on skin)	extreme	very low	very low	Yes	5	unknown
Cryptocaryon spp. (skin)	low	moderate	very low	105	2	unknown
Myxobolus buri (brain)	low	moderate	very low	Yes (Japan)	3	low
Other myxsporeans (brain)	low	moderate	very low	103 (000001)	2	unknown
Blood fluke (Paradeontacylix-like sp.)	low	moderate	very low	Yes (Japan)	3	unknown
Microsporidium seriolae (meat)	low	high	low	Yes (Japan)	3	low
(udoa spp. (meat and heart)	low	high	low	Yes	2	unknown
Jnicapsula seriolae (meat)	low	high	low	Yes	4	unknown
Non-infectious complex syndromes:Pg 41		i iigii	1011	165	, -	dinknown
Necrotic enteritis (+/- bacterial)	extreme	moderate	moderate	Yes	5	very low
Green-liver syndrome (+/- parasitic)	high	negligible	negligible	Yes	5	low
Haemolytic anemia syndrome (+/- bacterial)	low	high	low	Yes (Japan)	2	low
Cataracts (+/- nutritional)	high	very low	very low	Yes	5	unknown
Seedling nutritional syndrome(s)	extreme	moderate	moderate	Yes	5	moderate
Growout nutritional syndrome(s)	high	low	low	Yes	5	unknown
Broodstock nutritional syndrome(s)	high	low	low	Yes	5	unknown
Swim-bladder over-inflation / deformity	extreme	moderate	moderate	Yes	5	high
Head & jaw deformity	extreme	moderate	moderate	Yes	5	high
Phytoplankton & micro-algal blooms	high	high	HIGH	Yes (Japan)	2	moderate
Typoxia (oxygen deprivation) syndrome	high	mign	moderate	Yes (Japan)	2	moderate
Predation		low	low	Yes	5	unknown
	extreme	IOW	IOW	res	5	UNKNOWN
		1	1		I I	
			1			

Viral Hazards

Red sea bream-like iridovirus (RSIV): Yellowtail ascites aquabirnavirus (YTAV): Viral deformity aquabirnavirus (VDV): Lymphocystis iridovirus: Nodaviral encephalitis & retinopathy (SJVNN): Viral haemorrhagic septicaemia (VHS):

Overall risk (from Table 2)

high risk hazard to YTK. low negligible very low very low very low

High risk - Red sea bream-like iridovirus (RSIV):

In this risk analysis RSIV is deemed to have a high likelihood of entry and establishment, most likely by means of migrating wild fish. This viral pathogen is listed on both the Office International des Epizooties (OIE, 2004) and Australian national list of aquatic animal diseases (sub-appendix A-Gfh of Appendix 5), and a red sea bream-like iridovirus is frequently associated with high mortality rates in numerous species of fish including *Seriola* fish, and more specifically *S.lalandi* of Japan (Anon, 1994, 1998, 2002). The consequence of this virus clearly has a high impact, particularly with contributing factors of no diagnostic tool of early detection of infections, and no vaccine available in Australia; thus leaving the overall risk to cultured kingfish and the industry as high.

Sixty (60) or more different viruses have been investigated in finfish species, yet less than 10 are thought to be the cause of severe epizootics in aquaculture (Ahne, 1994). Red sea bream iridovirus (RSIV), although not yet identified within Australia, is a ubiquitous pathogen in Japanese aquaculture where marine temperatures and wild fishes are common to marine environments of South Australia. Nakajima et al. (1998) reported that the same type of iridoviral disease has caused serious damage to yellowtail fish stocks of Japan and afflicted 20 cultured marine fish species in 17 prefectures of western Japan. Furthermore, he reported that the RSIV isolates from various fish species in Japan are closely related to one another by reaction patterns against antibodies and virion polypeptide profiles (Nakajima et al., 1998).

RSIV-like disease has been diagnosed and reported within government prefectural lab submission summaries as the cause of outbreaks in juvenile (100-200g) *S.quinqueradiata, S. dumerili* and *S.lalandi* in Japan (Anon. 1994, 1998, 2002). Interviews with Japanese aquaculturists and diagnosticians (2003) revealed that at least one population of older, larger kingfish (1.5 to 2kg in weight) had experienced epizootic iridoviral-like mortality during their second summer in net pens. Presumably, these Japanese kingfish were immunologically naïve at the time of natural viral challenge since they had not received the injectable RSIV vaccine as fry (commercially available to *Seriola* fishes since 2003), nor had the kingfish exhibited any natural RSIV-like mortality as juveniles the previous summer.

RSIV-like mortality is a common seasonal event in Japanese aquaculture and it has been investigated, diagnosed and reported by numerous researchers using many fish species (Nakajima et al. 1995a, 1998, 1999, Jung et al. 1997). In Japan, iridovirus seems to induce outbreaks in young cultured fish when sea temperatures reach 23°C and/or following typhoons, during which fish tend to become crowded, physically traumatized and likely stressed within net pens subjected to violent sea conditions. One other contributing factor to iridovirus-like epizootics may relate to fish size and the immunological status of the population. The susceptibility of *S.quinqueradiata* to red sea bream-like iridovirus seems greatest amongst juveniles

less than 200 grams in weight. The Kagoshima Experimental Research and Disease Centre for Fisheries (Anon., 1994, 1996) documented summary laboratory submission data indicating that iridovirus (of undisclosed type) affected amberjack, *S.dumerili,* and yellowtail, *S.quinqueradiata*. Certainly, peak submission data was documented during the summer months (June, July and August) particularly as water temperatures exceeded 23°C.

The development of a highly sensitive and specific method for detecting RSIV in fish at the early stage of disease is most desirable and numerous researchers continue to investigate this aspect of iridoviral disease (Kurita et al., 1998; Nakajima et al., 1995a, 1999). Caipang et al. (2003) has published a real-time PCR assay for the detection and quantification of RSIV without the need for cell culture. This could prove helpful for surveillance of RSIV in wild and cultured fish.

Low and negligible risks - Aquabirnaviruses YTAV and VDV:

The overall risk from the aquabirnaviruses, yellowtail ascites virus (YTAV) and viral deformity virus (VDV), is deemed low and negligible, respectively, in this analysis. Experience from Japan indicates that S.lalandi fry, although affected by yellowtail ascites virus (YTAV), tend not to exhibit severe clinical disease and mortality when compared with S. guingueradiata fry (Isshiki and Kusuda, 1987). The consequence of these diseases is assigned values of low and moderate because the losses tend to occur in very young fry before population biomass and investment costs accumulate. In addition, Isshiki et al. (1989) suggests that YTAV within Japanese aquaculture is due mainly to the use of wild-caught fingerlings so perhaps using cultured fry originating from hatcheries would minimize the incidence of carriers and disease. In Australia, no wild kingfish fry are captured for ranching, which is the norm in Japan. Isshiki et al. (1989) assessed wild-caught yellowtail fingerlings for YTAV titres and found that the incidence of infection ranged from 8.57% to 14.9% (depending on the invitro cell lines used). Furthermore, 47.1% of the carrier fingerlings developed intra-peritoneal fluid (ascites) and these fish exhibited very high viral titres, whereas fingerlings with lower viral titres did not develop ascites.

Nakajima et al. (1998) and Muroga (2001) cite transmission studies by Isshiki et al. (1993) that indicate YTAV is evident in yellowtail brood, eggs, ovarian fluid and seminal fluid, suggesting a vertical transmission mechanism by the virus. Horizontal viral transmission apparently remains questionable or at least not easily demonstrated experimentally. However, Isshiki and Kusuda (1987) showed that YTAV immersion challenges of Seriola fish can lead to viral-related mortality rates of 13-20%. These researchers also compared the susceptibility to infection and survival of fingerlings of five species of marine fish, including S. guingueradiata and 'burihira' (a hybrid of S.aureovittata (now S.lalandi) and S.dumerili following injections of yellowtail ascites virus. The research found that yellowtail are most susceptible to YTAV, exhibiting an overall mortality of 40% post-injection, whereas the hybrid fish 'burihira' suffered only 21% mortality. This suggests that Seriola fish other than S.quingueradiata may be less susceptible to YTAV disease. Regardless, both Seriola fish types exhibited similar gross pathology. Isshiki et al. (2001) found that the host range of marine aquabirnaviruses is broad and it is generally accepted that marine aquabirnaviruses are acutely pathogenic in yellowtail and related Seriola species. Furthermore, the vast host range of marine aguabirnaviruses is thought to include S. quinqueradiata, S. dumerili, and shellfish, which may have implications at finfish operations in terms of reservoir and transmission epidemiology. The literature corroborates this speculation in that an isolate of marine aquabirnavirus from Japanese pearl oyster was neutralized by antiserum obtained from *S.quinqueradiata* (Suzuki and Kusuda, 1997: International symposium of disease in marine aquaculture).

YTAV disease tends to manifest itself as ascites and cardiac effusion (the release and accumulation of straw-colour fluid to the heart and body cavities). It is a disease that causes relatively high mortality rates in both hatchery reared and sea pencultured fry and fingerling S. quinqueradiata in several prefectures of Japan (Miyazaki, 1985; Egusa and Sorimachi, 1986). The disease pathology indicates viral invasion and destruction mainly of the acinar tissues of the pancreas and parenchyma in the liver, hence the disease is considered IPN-like (Egusa and Maeno et al. (1995) describes the systemic pathology of Sorimachi. 1986). He notes that, although both YTAV and VDV (viral deformity aquabirnaviruses. virus) are serologically and virologically similar, there appears to be quite noticeable differences in clinical symptoms: abnormal swimming, convulsion and scoliosis with VDV, yet marked swelling and ascites with YTAV. VDV and YTAV were determined to be very close to IPNV (infectious pancreatic necrosis virus) by virus neutralization tests and polyclonal antibody tests, yet distinguishable by monoclonal antibody (MA) testing (Nakajima and Sorimachi, 1995b). Epizootics of YTAV in S. quinqueradiata cultured in Japan tend to arise at water temperatures 18-22°C and particularly in fingerlings less than 10g in weight (Nakajima et al., 1998).

Viral deformity virus (VDV) is considered a negligible risk in this analysis. VDV is a pathogen known to elicit erratic swimming and nervous behaviour in *Seriola* fry. Nakajima et al. (1993) identified an aquabirnavirus in *Seriola quinqueradiata* fingerlings showing whirling and erratic swimming behaviour, scoliosis and high mortality in a Japanese hatchery. The disease was diagnosed as viral deformity and yellowtail fingerlings tend to exhibit the disease from 17-23°C but not at 26°C. In 1999, I observed ranchers of wild amberjack fry (*Seriola dumerili*) who routinely culled 2-3 cm fry that exhibited erratic, whirling swimming behaviour. In that area of China, diagnostic laboratory services are not readily available nor, at that time, did the aquaculturists have the incentive, inclination or means to pursue a diagnosis of that disorder of the central nervous system. Various pathogens can elicit erratic swimming behaviour in fry and VDV should be considered on the differential diagnostic list.

Very low risk – Lymphocystis virus, SJVNN and VHS:

The other viral hazards that may affect kingfish are lymphocystosis (an iridovirus), viral nervous necrosis (SJVNN) and viral haemorrhagic septicaemia (VHS), and each has been assigned a very low overall risk in this analysis. SJVNN and VHS are listed on the OIE and Australian national lists of aquatic animal diseases (OIE, 2004; AFFA, 2003b) and although the consequences of SJVNN and VHS might be devastating to the kingfish industry, I am unaware of any scientific report or experience that indicates that Seriola, and specifically S.lalandi, are susceptible to these two viruses. On the other hand, lymphocystis disease or 'black spot' has already been observed in captured broodstock of South Australia likely related to fin abrasion and stress (personal communication, 2002; Durham et al., 1996), yet the infection appears to be self-limiting and offers little or no consequence, clinically. Lymphocystis virus is an iridovirus of sorts and it elicits a chronic and benian hypertrophic epithelial disease in numerous finfish, including Seriola lalandi (Yoshimizu and Kimura, 1990). It is not considered a disease of serious consequence in cultured S.guingueradiata (Nakajima et al., 1998).

Other iridovirus-like, systematic, haemorrhagic syndromes (i.e EHN, epizootic haematopoietic necrosis) have been reported in Australian fish (Hedrick et al. 1992). He speculates that iridovirus-like agents that exist in numerous amphibians and finfish may have the potential to adapt and evolve amongst varied hosts, to the point that the geographic distribution and appearance of iridovirus-like agents in Australia may occur as ornamental finfish and amphibians are transported. That would further complicate programs aimed at preventing or controlling the global spread of fish viruses.

Striped-jack viral nervous necrosis (SJVNN, sometimes referred to as nodaviral encephalopathy and retinopathy, VER, VNN, or VEN) is the cause of epizootic disease in numerous marine finfish fry in many countries. Arimoto (1993) applied cohabitation protocols to investigate the susceptibility of *Seriola lalandi* to SJVNN. He concluded that *S.quinqueradiata* and *S.lalandi* were not susceptible to SJVNN. No reports of yellowtail susceptibility to the nervous necrosis virus were found. Although Curtis et al. (2001) cites Munday and Nakai (1997) that VNN has been reported among many cultured populations of marine fish worldwide with the number of susceptible host species continuing to grow.

As for viral haemorrhagic septicaemia (VHS), at least 45 species of marine and fresh water fishes have tested positive for VHS virus, yet it has only been isolated from fish living in regions with water temperatures 15°C or less. I am not aware of VHS ever being documented in *Seriola* fish, nor has the virus been isolated in Australia to date (Anon., 2001; AFFA, 2003b; personal communication, Hardy-Smith 2004).

Bacterial hazards

Photobacterium damsela piscicida: Listonella anguillarum: Lactococcus garvieae: Chlamydial epitheliocystis: Seedling bacterial over-growth: Nocardia seriolae: Mycobacterium marinum: Tenacibaculum maritimum: Pseudomonas spp. dermatitis: Streptococcus dysgalactiae:

Overall risk (from Table 2)

moderate risk hazard to YTK. moderate moderate moderate low low very low very low negligible

Moderate risk – Photobacteriosis:

Photobacterium damsela piscicida, formerly **Pasteurella piscicida** (Gauthier et al., 1995), is the causative bacterium of 'pseudotuberculosis' of young cultured *S.quinqueradiata* and the disease has been associated with serious economic losses in yellowtail culture of Japan since 1969 (Kawahara et al., 1998). It is perhaps the bacterial disease of greatest consequence to yellowtail farmers as it typically results in severe, acute and persistent mortality rates. To date, there is no early detection of this infection and no vaccine is available. Chemo-therapeutants are available in some countries to control outbreaks but their effect is relatively short-lived. Photobacteriosis is known to affect *S.lalandi* of Japan in a limited manner, and the disease has yet to emerge in Australia. For these reasons, photobacteriosis has been deemed a moderate risk to fish health in South Australia.

Five (5) bacterial pathogens of kingfish have been assigned moderate overall risks in this risk analysis. Responses from the Principal Investigator's interviews of stakeholders within South Australia (2003) indicate that at least four (4) of these five (5) diseases have already been recognised and diagnosed in dead and moribund cultured kingfish of South Australia.

Kawakami et al. (1999, 2000) experimentally infected several fishes, including *S.quinqueradiata* and gold striped amberjack (also known now as kingfish, previously *S.aureovittata*) with *Photobacterium damsela piscicida* to compare mortality rates and various immunological activities. Whereas both fish species were found to be highly sensitive to *Photobacterium* (challenged by $1.2x10^2$ CFU/fish), the 10-day cumulative mortality of *S.quinqueradiata* and *S.aureovittata* was 70% and 10% respectively. The difference was attributed to both a greater production of superoxide anion in kidney leucocytes of *S.aureovittata* as well as a complement factor within the serum of the kingfish. Studies such as this, offering direct species-to-species comparisons, remind us that fishes differ despite a common genus.

Noya et al. (1995) describes the development of 'Pasteurella disease' in gilthead seabream, *Sparus aurata*. He shows the haemolytic and destructive nature of *Photobacterium damsela piscicida's* extracellular products as well as the accumulation of intact bacteria within phagocytes in numerous organs. Degenerate macrophages and granulomata that harbour, then release, viable bacteria may play an important role in disseminating the pathogen systemically throughout the fish. Matsuoka and Kamada (1995) confirmed and documented discharge rates of *Photobacterium* from experimentally diseased *S.quinqueradiata* before and after death, supporting evidence of pathogen transmission and the importance of

removing dead and dying fish from populations to minimize bacteria loading of the environment.

More recently Yoshida et al. (1997b) and Zorrilla et al. (1999) both acknowledge that, beyond the experience of Japanese researchers and aquaculturists culturing *Seriola* fish, *Photobacterium* had become and increasing disease problem in warm-water fishes of different species in numerous countries, and that bacterial attachment to host cells, in addition to external toxins, plays an important role in bacterial invasiveness. Optimal water temperatures for the multiplication of *Photobacterium damsela piscicida* are reported to range from 20 to 26°C (Ishioka, 1990; Noga, 1995; Kitao, 1993; Sano and Fukuda, 1987) and this appears to be borne out in commercial farming situations in Japan. Ishioka's (1990) graphic representation of pathology submissions and diagnosis of diseases in laboratories does coincide largely with typical seasonal patterns of mortality rates observed at Japanese *Seriola* farms. The incidence of disease tends to increase when water temperatures rise above 20°C, and the sequence of disease is, generally: yellowtail ascites virus, then Listonellosis, then Photobacteriosis, Iridovirus (RSIV) and eventually an increasing incidence of more chronic diseases such as Lactococcosis.

Moderate risk – Listonellosis:

Listonella anguillarum (formerly vibriosis) is another pathogen of moderate overall risk to kingfish. Diagnostic personnel of Japan and Australia tend to agree that *S.lalandi* exhibit clinical signs compatible with this disease which manifests itself in numerous ways depending on the age, size and immunological status of the fish (personal communications, 2003; Anon., 2002a; Ishioka, 1990). The mortality can become significant and problematic as juveniles and yearlings if the fish are not vaccinated as fingerlings. The YTK industry of Australia may be particularly vulnerable to this disease in that, to date, there are no readily available tools of prevention or control for systemic listonellosis in South Australia.

Listonella disease arises in recurring seasonal pattern and tends to affect fish of all age classes and sizes by infecting the intestine, the skin, brain or eye. Given the vast array of marine *Vibrio* species and their ubiquitous nature, it is generally accepted that *Vibrio* and *Listonella* species should be considered opportunistic, if not primary, pathogens of *Seriola* fish. Yet within the available literature, there is surprisingly little mention of this bacterium causing disease and mortality amongst *Seriola* fish, perhaps because of its typical association with other concurrent infections. Fish farmers of Japan believe 'vibriosis' has become a disease of little significance in *S.lalandi* and *S.quinqueradiata*. The latter are now routinely injection-vaccinated against *Listonella anguillarum*.

Tajima et al. (1985) demonstrated 12 of 13 bacterial isolates from *S.quinqueradiata* were confirmed as *Vibrio anguillarum* (now *Listonella*). Diagnostic lab reports from Kagoshima prefecture of Japan (1995) indicate that yellowtail fish are susceptible to infections by other marine *Vibrio* species, including: *V.alginolyticus*, *V.parahemolyticus*, and *V.damsela*. Alcaide et al. (2000) added *V.vulnificus* to the list of potential pathogens of amberjack, *S.dumerili*, in Spain.

Moderate risk – Lactococcosis:

Another moderate risk to cultured kingfish may be the infection and disease caused by *Lactococcus garvieae* (formerly Streptococcus and *Enterococcus*

seriolicida). This bacterium appears to be an insidious pathogen that significantly affects survival, fish performance and meat quality if the fish are not vaccinated as fingerlings. The cumulative mortality however is perhaps not as significant as the sub-clinical effect on loss of production. This infection is chronic and the disease tends to kill *Seriola* fish, including *S.lalandi*, once the fish approach one- and two-years of age. The YTK industry of Australia may be particularly vulnerable to this disease in that, to date, there are no readily available tools of prevention or control for Lactococcal and Streptococcal infections in South Australia.

The diagnostic records of the Japanese government prefectural labs (Anon., 2002a) indicate that *Lactococcus* has been isolated from dead hiramasa, *Seriola lalandi*. Personal communications (2003) in Australia suggest that lesions consistent with this disease have been observed in cultured kingfish of South Australia yet diagnostic case reports were not produced or volunteered at the time of interviews. Akhlaghi (1996) and Carson et al. (1993) each made reference to *Enterococcus* (now *Lactococcus garvieae*) being present within the fresh water and marine waters of Australia. In a recent risk assessment of southern bluefin tuna an estimate of moderate to extreme likelihood of presence or entry of *Lactococcus* (and aquabirnaviruses) to Australian waters was assigned (Nowak et al., 2003).

Information from Japan indicates that Enterococcus (Lactococcus garvieae) has been observed and studied since 1974 in S. guingueradiata and the disease had spread to all areas in Japan within years (Kusuda and Kawai, 1982; Aoki, 2000). However one must acknowledge that the Japanese ranching/aquaculture industry is far different than the kingfish industry of South Australia. Nakai et al. (1999) and Ooyama et al. (1999) reiterate Kitaa et al. (1979): that Lactococcus garvieae is considered ubiguitous in fishes and culture environments of Japan, and the bacterium is an opportunist requiring control by numerous husbandry techniques, including vaccination. In 1989, before the widespread use of commercial vaccines, farming companies of Japan reported losses of >8000MT of S. quinqueradiata due to enterococcal bacteria and exotoxicosis (Sano, 1998; Alim, 1996). 1993 losses totalled 5784 MT (Fisheries Agency 1996). More recently, the government prefectural data summaries (Anon., 2002a) indicate that *Enterococcus* is an ongoing disease of concern in that it is implicated in 48 of the 95 lab submissions. Enterococcal intracellular and extracellular toxins relate to the pathogenicity and highly virulent nature of the microorganism (Sako, 1990), whereby sixty-four (64) percent of yellowtail mortality was attributable to lactococcosis, and 21 percent to photobacteriosis.

The bacterium is apparently capable of surviving outside of the fish host. Kusuda and Kawai (1982) found that prolonged survivability of *Lactococcus garvieae* up to 42 days at 25°C was evident in sea water obtained from pen-culturing areas, and the infection of yellowtail is apparently exacerbated by low dissolved oxygen, as well as by blood fluke infestation (Fukuda, 1997; Kumon et al., 2002). Enterococcal epizootic periods span from summer to winter, yet the mortality sharply declines once sea water temperatures drop to 15°C (Sano and Fukuda, 1987).

Since 1999, the antibiotic control of lactococcosis disease in Japan has largely been replaced by the widespread and effective application of a commercially available vaccine. However, the vaccine appears to be ineffective to prevent *Streptococcus iniae;* one of three streptococcal infections observed in *S.quinqueradiata. S.iniae* is

quite frequently found as an infection of the brain of yellowtail. Kaige et al. (1984) found that lordosis, kyphosis and scoliosis was due to meningo-encephalitis caused by *S.iniae* rather than the aquabirnavirus VDV or a myxosporean parasites described by Sakaguchi et al. (1987), and Maeno and Sorimachi (1990). Nervous behaviour and skeletal deformities have many etiologies: nutritional, genetic, developmental, toxins, bacterial, viral and parasitic, and in the experience of the Principal Investigator, *Lactococcus* species are commonly associated with vertebral osteitis and meningo-encephalitis of *Seriola* fishes.

Clinically, *Lactococcus garvieae* can be confused with *Streptococcus dysgalactiae* which has similar tail-abscess lesions. *S.dysgalactiae* is a relatively uncommon infection to date but it has apparently been emerging in selected yellowtail populations of Japan since 2001 (personal communication, Yoshida 2003). The commercial lactococcal vaccine in Japan is not believed to provide cross-protection against *S.dysgalactiae* infections.

Researchers (Iwata, 1982; Taniguchi, 1982b; Yasunaga, 1982) have speculated that one of the sources and factors of 'streptococcal' pathogens to cultured *Seriola* fish in Japan is from raw baitfish being fed to the cultured yellowtail. Yasunaga discovered that *Streptococcus* sp. was isolated from each of the selected ten frozen, wild sardines destined to be yellowtail feed. This supports the notion that fish pathogens often survive the process of freezing and that wild, uncooked sources of feed may very well be a high risk to cultured fish. Fortunately, feeding raw or frozen bait-fish is not a typical practice of the sea-pen kingfish industry within Australia, yet it is still practiced in parts of Japan and within tuna industries, including that of Australia. Consequently, *Lactococcus* and *Streptococcus* may be a relevant risk factor to kingfish depending on their proximity to tuna farms and rearing areas.

Moderate risk – Epitheliocystis:

Chlamydial epitheliocystis disease has already emerged amongst kingfish of Australia and Japan as a problem (Japanese survey, Appendix 4) and it has been assigned as a moderate risk to fish health. Pathology lab results of Japanese prefectural labs suggest that *Seriola lalandi* are indeed affected by, and succumb to, epitheliocystis branchitis (Anon., 2002a). The infection (and disease) is difficult to diagnose and there are few if any effective control tools for the aquaculturist to rely upon to help mitigate the losses.

In some places the disease seems to exhibit a seasonal limitation but mortality rates can become quite significant, despite the general lack of gross lesions. The environmental and epidemiological factors initiating mortality remain largely unknown. *Chlamydia* organisms have been found in numerous fresh water and marine fishes and some of the susceptible finfish species include: blue gill, trout, American plaice, channel catfish, carp, bass, and mullets and amberjack (Miyazaki et al., 1986; Crespo et al., 1990). To my knowledge, it has yet to be documented within the scientific literature that *S.quinqueradiata* and *S.lalandi* are indeed susceptible finfish hosts affected by this opportunistic microorganism, although epitheliocystis was mentioned by one author in grey literature as an important disease of larvae during early developmental stages (Benetti, 2000).

Grau and Crespo (1991, 1992) described the disease in amberjack (*S. dumerili*) in Spain. They found 7 of 15 wild juvenile amberjack (*S. dumerili*) in the coastal waters of Europe were infected with *Chlamydia* and they concluded that epitheliocystis

infection occurs as a chronic, non-pathogenic condition in wild fish populations. Crespo also found that 50% of the amberjack diseased with epitheliocystis were also infected with blood flukes, yet he determined that the microorganism associated with epitheliocystis can certainly act as the primary pathogen and causative agent to mortality. Kobayashi et al. (2004) reported populations of amberjack (*S.dumerili*) weighing 750 to 850 grams experienced 20 to 50% mortality in each of four farming areas of Japan at water temperatures ranging from 20 to 25°C.

Moderate risk – Seedling bacterial over-growth:

Bacterial over-growth of seedling hatchery rearing tanks is deemed a moderate risk. 'Tank crashes' due to excessive bacterial accumulations tend to be commonplace in marine hatcheries of Australia and Japan, and entire larval populations (potential grow-out fish representing hundreds of thousands of animals) are routinely culled as a result. The control of bacteria within seedproduction tanks and inside the larvae is difficult. To date, I am unaware of any labelled chemo-therapeutic tools available to combat the bacteria, but cleaner live-feed products are becoming more widely available, and environmentally-friendly sanitizers are on the commercial horizon in many countries.

Providing clean live-feed to marine larvae is a complex science (and art). Undoubtedly, many types of bacterial microflora are likely necessary to facilitate and maintain live-feeds, as well as meet the nutritional needs of fish larvae. The proliferation of pathogens and opportunistic bacteria within live-feed tanks may also overwhelm larvae and create serious challenges to fish health and survival.

Other incidental and low-risk larval diseases are discussed in a review paper by Muroga (2001). He summarizes viral and bacterial disease in marine hatcheries of Japan, yet there is little or no mention of disease related to *Seriola* seed production with the exception of the aquabirnaviruses yellowtail ascites virus (YTAV) and viral deformity virus (VDV). The lack of *Seriola*-related information in the literature may indicate that either *Seriola* fry are quite resilient to specific infections, or perhaps the number of *Seriola* seed-culture facilities is significantly fewer than those of other fish species. Olafsen (2001) also offers an overall review of literature in which he discusses various interactions and facets of microflora associated with fish larvae production. He too makes no specific reference to *Seriola lalandi* production or development.

Other researchers specifically investigated the survival rates of *S.lalandi* during seed production to find initial mortality rates of 30-35%. The cause was unknown. These losses however were followed by significant cannibalism, reducing survival to 5% by day 35 post-hatch (approximately seed size was 10mm). The researchers speculated that the survival rate would have been improved had the juveniles been size-sorted prior to the cannibalism period (Ebisu and Tachihara, 1993). Similarly, Imaizumi (1993) reported a typical Japanese hatchery rearing survival of *S.quinqueradiata* to be 5–10% when grown at 20 to 22°C and at densities of 300 to 500 seed per cubic meter (at 20mm size).

Tachihara et al. (1997) continued to investigate *Seriola lalandi* seed survival (under university tank rearing conditions) and he reported the survival to range from 0.13–1.0% when raised under low densities of 44 to 191 seeds per cubic meter. He suggested the high mortality was due to undiagnosed losses during early-

development stages. The low survival rate was subsequently alleviated four fold by applying a prophylactic triiodo-thyronine supplement to *S.lalandi* broodstock.

Low and very low risk bacterial hazards - Nocardiosis, Mycobacteriosis, *Tenacibaculum* and *Pseudomonas*:

These opportunistic marine bacteria can be problematic to *Seriola* fish of Japan and the incidence of these diseases has risen dramatically in the past eight (8) years (Anon., 1998, 2002). These diseases are not known to be a significant problem to *S.lalandi* of Japan but some mortality has been experienced and documented in laboratory case reports. *Nocardia and Mycobacterium* have yet to emerge as infections amongst cultured kingfish populations of South Australia. The likelihood of establishment and spread in the Australian aquaculture industry may be low since farm sites are located great distances from one another in remote locations, and there is no import of live carrier fish from other countries. In addition, one of the main sources of *Nocardia* and *Mycobacterium* in other Asian countries is thought to be raw or frozen fish feed made from wild bait fish, yet there is little or no use of raw or frozen bait fish feed in the culture of kingfish raised in Australia.

The index or first case of nocardial disease at a fish culture facility always leads one to question the source of the pathogen. Early case reports of the pathology and microbiology of nocardiosis in Japanese yellowtail and amberjack have been documented by many researchers (Kusuda and Nakagawa, 1978; Matsuzato, 1978; Kariya et al., 1967, 1968). The reports describe a ubiquitous microorganism in terrestrial and marine environments that arises as a disease concern in S.quingueradiata and S.dumerili. Nocardiosis is now seen as a re-emerging disease and economic problem in aquaculture (Miyoshi and Suzuki, 2003). In Australia, Bransden et al. (2000) reported nocardiosis (of undisclosed species) in Atlantic salmon of Tasmania that had been exposed to both leaf litter and unpasteurized feed made from pilchards. Although the prevalence of infection was only 3%, it illustrates that the opportunistic pathogen can easily and inadvertently be introduced to cultured fish from feed ingredients and the environment. A similar index case in Taiwan was reported by Chen et al. (2000) when sea bass, Lateolabrax japonicus, succumbed to Nocardia seriolae disease with an incidence of 17.5% mortality within the first month of the epizootic.

The survival (and transmission) of the bacteria inside and outside of the host fish is another area of speculation. Viability studies of Nocardia in sea water by Kusuda and Nakagawa (1978) show the bacterium to survive more than 90 days provided the seawater contained organic fish extracts. It was speculated that nocardial survival, viability and horizontal transmission is strengthened in 'polluted' waters containing free and organic particles. The same authors also report positive results using an experimental vaccine, yet in 2003 there is still no effective, commercially available vaccine to offer protection against fish nocardiosis. Sako (1998) conducted similar bacterial survival studies in SW using Vibrio (Listonella) anguillarum, Streptococcus species, Edwardsiella tarda, Photobacterium damsela piscicida, and Nocardia kampachi. His results indicate that nocardial survival was not different among filtered and unfiltered sea water and that, in general, the fish pathogens tested often survived weeks in the experimental environment lending important insight to the potential for horizontal environmental transmission amongst fish groups. Shellfish reservoirs of Nocardia and mycobacterium also come to question in that shellfish have been known to be carriers of these two (and many other) marine pathogens.

Nocardial abscesses in fish tissues create a physical and immunological barrier to antibiotics so the control of chronic nocardiosis by chemotherapy is not likely tangible or efficient (Kusuda and Nakagawa, 1978; Miyoshi and Suzuki, 2003). Other researchers have investigated *in vitro* antibiotic sensitivities to find mixed efficacy depending on *in vitro* techniques (Itano and Kawakami, 2002: Fukuda, 2001; Kurogi unpublished data, 2001). On *Seriola* farms of Japan in the past ten years, the incidence of nocardiosis appears to be increasing and *in vivo* antibiotic control has not proven cost-effective once clinical lesions and fish mortality is evident. So prevention, early detection of lesions and disease management is considered by most to be the only means by which to minimize the losses due to nocardiosis. A PCR method (directly from carcass samples) was recently developed by Miyoshi and Suzuki (2003) for the early identification of infections, yet there remains no early test for nocardial infections.

Mycobacterium species are difficult to study and relatively little is known about the disease in *Seriola*. In particular, how and when the infection arises or how does one control the infection (and disease) once it is diagnosed. The Japanese literature prior to 1990 suggests 'tuberculoidosis' of *Seriola* presents quite confusing microbiological results including descriptions of disease caused by *Corynebacterium* (Kimura and Kitao, 1971) and *Mycobacterium* species (Kusuda et al., 1987). That, coupled with the difficulty in isolating and working with the microorganism *invitro*, leaves the details and management of mycobacteriosis a mystery. This disease is chronic, complex and seems to be quite variable in its gross pathological presentation, presumably due to concurrent chronic infections in the same carcass. Clinical mycobacteriosis is more evident in cultured *S.quinqueradiata* in the cool autumn and winter months and diseased fish are commonly concurrently infected with *Lactococcus garvieae* and *Listonella anguillarum* (Kusuda et al. 1987; Kusuda and Kawai, 1998) presumably due to the chronic and debilitating nature of the primary mycobacteriosis.

Colorni et al. (1998) has investigated *Mycobacterium marinum in vitro* as well as *in vivo* in sea bass (*Lates calcarifer*). He concluded that, although injectable antibiotics may provide some delay or reduction in the development of internal lesions, the eradication of *Mycobacterium* was not achieved. In general, other research reports indicate that *Mycobacterium* leads to a chronic debilitating disease with little or no effective antibiotic control (Kusuda et al., 1987).

Tenacibaculum maritimum (formerly Flexibacter maritimus) is often considered a secondary infection of fish, as is Pseudomonas dermatitis. Very little information is available in the literature. The infection is thought to be common amongst slower and bottom-dwelling fish due to the fishes' more sedentary behaviour and frequent physical contact with other fish and benthic surfaces. S. lalandi does exhibit slower behaviour relative to other Seriola fishes and Ishioka's (1990) epidemiological analysis of Japanese lab submissions indicates kingfish (formerly known as S.aureovittata of Japan) were diagnosed with 'gliding bacteria', more so than S.quingueradiata and S.dumerili; at least based on the lack of laboratory submissions related to the latter two fish species. During interviews however, Japanese hiramasa farmers said that skin conditions of S. lalandi were not given much diagnostic attention and they did not seem to develop into diseases-ofconcern. In other Seriola fish, skin and fin erosions are accompanied by redness, necrosis, secondary vibriosis and other opportunistic bacterial infections that easily arise in fish affected by gliding filamentous bacteria (Kusuda and Kawai, 1998;

Kusuda and Kimura, 1982; Miyazaki et al., 1975). These mixed infections are commonly seen in *S.quinqueradiata* and Miyazaki found that the bacteria migrate along loose connective tissue planes resulting in a severe necrosis and extensive dermal ulceration (Miyazaki et al., 1975). The large, open skin lesions are suggestive of a predominant and virulent *Tenacibaculum* dermatitis, as opposed to the typical bacterial invasion secondary to ulcerative listonellosis.

Parasitic hazards

Benedenia seriolae (skin):	high risk haza
Zeuxapta seriolae (gill):	moderate
Heteraxine heterocerca (gill):	low
Microsporidium seriolae (meat):	low
Kudoa spp. (meat and heart):	low
Unicapsula seriolae (meat):	low
Blood flukes:	very low
Neobenedenia spp. (skin):	very low
<i>Caligus</i> spp. (gill & skin):	very low
Cryptocaryon spp:	very low
Myxobolus buri and myxosporeans (brain):	very low

Overall risk (from Table 2)

high risk hazard to YTK. moderate low low low low very low very low very low very low very low

<u>High and moderate risks – Skin flukes (Benedenia seriolae) and gill flukes</u> (Zeuxapta):

Monogenean gill and skin fluke infestations continue to be highly problematic to South Australian producers of kingfish. The farmers know too well the cost of these parasites, particularly Zeuxapta seriolae; in terms of mortality, lost growth and performance, and the high labour cost to manage this disease (Weaver, 2000; Whittington and Ernst, 2002). In this risk analysis Zeuxapta has been deemed a higher risk than Heteraxine to S. lalandi because Zeuxapta infestations are already well established within S.lalandi sea net pens of South Australia and Japan, whereas *Heteraxine heterocerca* appears, so far, to remain largely host-specific to S.guingueradiata, despite some lab case reports from Japan and the Mediterranean (Sharp et al., 2003; Anon., 2002a; personal communications Ernst and Ogawa, 2004). Heteraxiniasis is capable of creating significant pathology amongst Seriola fishes but more hostparasitic challenge tests are required to determine if Heteraxine species are adaptable to S.lalandi. Regardless, to date there is no early detection of Heteraxine species, and diagnostic training is necessary to distinguish between gill fluke species for definitive identification and then surveillance of S.lalandi. Although chemo-therapeutants are available and occasionally used in South Australia to control ecto-parasites, the control is relatively short-lived even when accompanied by an integrated pest management approach. Scholz (1999) suggested the control of many important parasitic diseases is still far from satisfactory and further research is needed. The use of chemotherapy has biological and environmental limitations so other control approaches will be important.

Gill flukes are common irritants and pathogens of *Seriola* fish in Japan and Australia. They are considered key contributing factors to fish stress, sub-optimal feed conversion rates, plus hypoxic- and osmotic-related mortality. Farmers, biologists and researchers in Japan and Australia expend significant effort investigating the biology, taxonomy and various management techniques to control these parasites and to minimize the clinical and sub-clinical effects on fish. The literature on Carangid (*Seriola*) fish is teeming with case reports and experiments related to parasitic infestations. Researchers have compiled lists and reports reflecting numerous ecto-parasites of *Seriola* species, including *S.lalandi* (Ogawa and Yokoyama, 1998; Whittington et al., 2001, 2002; Diggles, 2002). See Appendix 6. Weaver (2001a) cited dermal ulceration of cultured kingfish in South Australia due to a monogenean skin fluke infestation. In wild kingfish, Sharp et al. (2003) assessed

forty-six (46) wild fish and he noted that both *B.seriolae* and *Z.seriolae* infected all kingfish examined.

With regard to **Neobenedenia** species, incidental findings on *S.lalandi* have been reported in Japan (Appendix 6), hence this organism is listed as a plausible and potential hazard in this risk analysis. The likelihood of its establishment is considered low at this time. Ogawa et al. (1995) speculated that the potential threat of *N.girellae* to the health of cultured Japanese fishes is indicated by its known low host specificity (as is the case with many monogeneans), its wide distribution, and ability to cause mortality during heavy infestation.

Integrated pest management of ecto-parasitic diseases of *Seriola* species is an active area of research in Australia, and it is perhaps a topic better left to the experts in the field. Infestations are numerous and still complicated by gaps in knowledge of biology and life cycles, as well as complicated taxonomy.

Low and very low risks – Internal parasites, *Kudoa spp., Unicapsula seriolae,* <u>*Microsporidium seriolae* and Blood flukes:</u>

Internal microscopic parasites are certainly capable of infecting Seriola lalandi raised in areas of consistently warm water (>18°C) but the likelihood of establishment of these parasites is considered low in South Australia. Although these parasites are evident in Japan, the 'low' estimate for South Australia is mainly justified due to the cool, deeper, flowing marine environments of the Australian farm sites compared to the rearing environments and high concentrations of sea net pens typical of Japanese aquaculture. The Japanese Seriola industry has similar ranching procedures to the tuna industry in that wild-caught juvenile fish (already with a complement of various symbiotic and perhaps pathogenic viral, bacterial and parasitic infections) are placed into sea pens for further growth and cultivation. That, coupled with high cage densities and shallow low current bays, leaves Japanese Seriola more susceptible to disease than are the kingfish of South Australia. In addition, I speculate that a number of the parasites listed here may require intermediate hosts that thrive in more tropical waters, although life-cycle details remain largely unknown. Furthermore, unlike Japan, Australia does not import live wild fish that may be carriers of exotic pathogens.

The myxosporean *Kudoa amamiensis* has been reported to infect the skeletal musculature of *S.quinqueradiata* grown in a limited geographic area of Southern Japan (Egusa and Nakajima, 1978; Yokoyama et al., 2000). The complete life cycle of myxosporeans has yet to be elucidated and the risk to kingfish of South Australia is more than likely low. Egusa and Nakajima (1978, 1980) hypothesized that the source of infection in southern Japan may be coral fishes in the vicinity of aquaculture net pens. *Kudoa pericardialis* on the other hand has been observed and reported as a parasite of the heart of Japanese cultured *S.quinqueradiata* raised in cooler waters (Nakajima and Egusa, 1978). The organism has been described extensively yet no literature was found documenting reduced productivity, morbidity or mortality related to this infection of the heart.

In 1982, Lester reported finding the causative agent of post-mortem muscle lysis, or 'soft flesh', as the myxosporean parasite **Unicapsula seriolae** from wild Australian *S.lalandi*. The prevalence of infection was 16 of 26 fish gathered near Brisbane. I am unaware of any identification of *Unicapsula* spp. amongst kingfish of South Australia. Egusa (1985) describes the cysts and spores of *Myxobolus buri* sp.n. (see

below). He also summarised that three other myxosporean species had been reported with *S.quinqueradiata*: *Kudoa pericardialis* from the heart cavity, *Kudoa amamiensis* from skeletal muscles and an unclassified Multivalvulida from the brain.

No information on the parasite *Cryptocaryon* and kingfish was discovered in the literature but the Principal Investigator has observed 'white spot' on the skin of *S.dumerili* and *S.quinqueradiata* reared in shallow bays of Japan with limited exchange of water. The proximity of the nets to the ocean floor may also be a factor in the cycling of this parasite between the benthos and hosts.

Another internal parasite that infects the meat and has a profound effect on product quality is *Microsporidium seriolae*. It was reported as a major obstacle in non-filtered sea pen seed production of *S.quinqueradiata* fry weighing 0.5-32.8g in one area of Japan (Sano et al., 1998). Heavy infestations by the parasite to skeletal muscle leads to emaciation of the host fish and death ensues in those with severe infections. Lightly infected fish tend to survive (Egusa, 1982). Areas of Japan where microsporidiosis is common typically have warm shallow bays and nets from the rearing pens hang within meters of the ocean floor. The mode of transmission of this parasite remains unknown but temperature and the benthos seem to influence the prevalence and infestation rate of this pathogen. Cysts have been shown by Sano et al. (1998) to arise in cultured fry as early as 10 days post-entry to sea pens moored in shallow, warm environments.

With regard to blood flukes of Seriola fish, Japanese amberjack farmers certainly face significant fish mortality and husbandry challenges with this parasite. The early identification of affected fish and a safe means to control adult blood flukes remains to be discovered. In general, eggs tend to be released to gills during the winter and spring months (Ogawa et al., 1986, 1989, 1993, 1994) often subsequent to the arrival of imported amberjack fry from China. Presumably, the source of the recurring infection is both the imported amberjack fry that are transported from China annually, and possibly the local magnification of fluke populations already established in Japanese bays. Smith (1997) recorded that the number of blood fluke species and fish hosts is increasing, although I found no published scientific literature indicating blood flukes affecting S.lalandi. That said, an infestation was noted in hiramasa within Japan's Prefectural disease summaries (Anon., 1994, 2002). In addition, S.dumerili and S.lalandi have been listed by Cribb (Appendix 6) as being affected by Paradeontacylix sanguinicoloides.

Montero et al. (1999) and Crespo et al. (1992) both suggested that blood fluke eggs accumulate in amberjack gills in spring and summer months, and Crespo et al. (1992) also found that 50% of the amberjack diseased with epitheliocystis were also infected with blood flukes. Although amberjack, Seriola dumerili, of Japan and the Mediterranean die in massive numbers due to Paradeontacylix species (such as P.grandispinus and *P.kampachi*) mass mortality is rarely reported in S. guingueradiata. Rather, the presence of an un-named blood fluke, thought to be a lone species different than the flukes of amberjack, has been observed in routine diagnostic cases in Japan (Kumon et al., 2002). More research is needed in this area.

Colquitt et al. (2001) reported the presence of flukes in [ranched] southern bluefin tuna and also in several other marine cultured finfish, such as sea bass (*Lates calcarifer*), and amberjack (*S.dumerili*). Yet a lack of detection of flukes in wild-

caught tuna led Colquitt et al. (2001) to speculate that blood fluke infections may be a post-capture phenomenon. Although this notion is possible, such reasoning fails to acknowledge other possible explanations as to why wild, robust tuna did not reveal the parasite when examined.

Information pertinent to other ecto-parasites, deemed as very low risk pathogens to kingfish, is reflected and summarised by the following excerpts from the literature: Caligus is the largest genus of parasitic copepods containing more than 250 species, and it is thought that the majority of the species remain unknown. Ho et al. (2001) wrote that S.quinqueradiata of Japan and wild S.lalandi in Korea, as well as S.lalandi cultured in Japan, have been reported to be infested with Caligus lalandei. *C.lalandei* is somewhat unique in that it is four fold greater in length than in width. Ho et al. (2001) provided an overview that the culture of *S. guingueradiata* began in 1927, yet it did not become a major industry until 1950. Furthermore, he stated that we know nothing (little) about the migration of S.lalandi and S.dumerili in the western North Pacific, so it is impossible to say whether the occurrence of *C.lalandei* in Japan or Korea is due to a natural phenomenon (i.e. a recent migration of amberjack (S.dumerili) or an 'artificial activity' such as the active importation of live juvenile fish from one country to the next. C.lalandei has not yet caused serious problems to vellowtail culture in Japan, unlike C.spinosis which has been problematic in the past (Ho et al., 2001).

Sharp et al. (2003) noted that *Benedenia seriolae* and *Caligus lalandei* infect the skin, whereas *Caligus aesopus* and *Lernanthropus* species were located on the gills or gill arch of *S.lalandi*. It is difficult to say with certainty whether these two species will pose a potential threat to kingfish under sea pen conditions. In addition, *Caligus seriolae* and *C.spinosis* have been associated with mortality of *S.quinqueradiata* in Japan (Grau et al. 1999 via Sharp et al., 2003).

Finally, a brain myxosporean, *Myxobolus buri*, has been deemed a low risk hazard for kingfish reared in South Australia due to the stark differences in sea net pen environments and husbandry of Australia compared to Japan. The use of extruded, pasteurised pellets and the location of sea net pens in deep and flowing waters does not appear conducive to Myxobolus disease. Upon investigating the epidemiology of Myxobolus-related scoliosis in yellowtail Sakaguchi et al. (1987) showed that scoliosis was significantly reduced in populations fed intensively and frequently during periods of initial captivity of the wild yellowtail fry. The authors suggest then that the parasitic load in the brain is minimised if fish do not consume the Myxobolus buri parasite from wild or raw feed types. Egusa (1985) reported that the incidence of Myxobolus encephalitis in S. guingueradiata (since 1970) to be as high as 30% amongst various fish populations. Equsa also cited Furukawa et al. (1981) as detecting the myxosporean, the most probable cause of scoliosis, in the brains of both normal and diseased fish, yet the parasite was most abundant in the cerebral cavities or ventricles of the fish exhibiting scoliosis. There is a correlation between scoliosis deformity in yellowtail and myxosporean parasitism in the brain, and it is strongly suggested that the skeletal abnormalities occur when parasitic cysts infect particular regions of the brain such as the fourth ventricle (Maeno and Sorimachi, 1990).

Non-infectious hazards / syndromes

Phytoplankton and algal blooms: Hypoxia (low oxygen) syndrome: Necrotising enteritis (winter): Seedling nutritional syndromes: Swim-bladder deformity: Head/jaw deformity: Haemolytic anemia: Grow-out nutritional syndromes: Broodstock nutritional syndromes: Predation: Cataracts: Green livers:

Overall risk (from Table 2)

high risk hazard to YTK. moderate moderate moderate moderate low low low low very low negligible

High risk – Phytoplankton and micro-algal blooms:

The Japanese coastal regions where aquaculture is practiced are highly populated by humans. In addition, numerous rivers in Japan deliver nitrogenous and phosphorous nutrients to these same coastal areas. Consequently, an organic loading of the local aquaculture environment is a result (Ishioka, 1990). These factors can influence the frequent magnifications of marine algae, diatoms and phytoplankton, or 'blooms'. Although this is not the *current* situation in South Australia, blooms are natural environmental phenomena and, similar to other natural events, they are determined by environmental factors that we are largely unaware of. As such, bloom events are highly unpredictable with regard to where and when they may arise; near populated areas or not. In many countries where aquaculture has the advantage of decades of experience, the surveillance for problematic pathogenic phytoplankton both locally and regionally is scheduled at least once daily. Emergency contingency plans are arranged far in advance in light of the potentially catastrophic effect on fish. Blooms that develop along the coasts of Japan and threaten fish reared in sea net pens include:

Chattonella antiqua

Chattonella marina

*Gymnodinium mikimotoi (*not to be confused with *G.sanguineum* which is similar yet ubiquitous and non-pathogenic)

Cochlodinium polykrikoides Heterosigma akasiwo Gonyaulax polygrama Heterocapsa circularisquama

Hishida et al. (1998) identified that *S.quinqueradiata* have high oxygen requirements and *Seriola* species are highly susceptible to low-oxygen conditions created by *Chattonella* algal blooms. On occasion, the result has been mass mortality of yellowtail in Japan. In addition, and in absolute terms, the oxygen-carrying capacity of water declines as water temperatures increase. This inverse relationship can create low-oxygen rearing conditions in semi-open cage systems during the summer and early autumn. This creates an environmental situation that is problematic for fish, particularly stressed, crowded fish, or fish compromised by sub-clinical infections or debilitated in some other manner.

Moderate risk – Necrotic enteritis (winter syndrome):

Kingfish of Australia, and many other warm- and cold-water fishes in other countries, have succumbed to a complex syndrome related to red necrotic intestines. The likelihood of entry or establishment is already 'extreme' in South Australia in that significant fish mortality has already been experienced during the winter months of 2003 and 2004. The magnitude of impact is assigned a moderate value. The disease has been referred to as 'winter syndrome' (Luzzana et al., 2003). The mechanism and onset of acute death remains largely unknown but suspicious contributing factors are hypothesized to be:

- a) cool water accompanied by slow gut motility, minimal digestion, reduced metabolism and sub-optimal immune protection,
- b) high lipid feeds pellets with energy and oil levels, possibly in excess of the fishes' ability to digest them completely under cold water conditions,
- c) plant proteins pelleted feed made with certain plant proteins not treated for anti-nutritional factors, resulting in bowel irritation, interference with gut absorption and alteration of normal bacterial flora,
- d) opportunistic bacteria these bacteria may find opportunity to colonize and invade the gut, causing more necrosis and shedding exotoxins to the fish's blood stream (Sheppard, 2004).

These factors, when in combination, seem to have the potential to create an inflamed irritated bowel (enteritis), necrosis, ulceration, electrolytic imbalance and death. Other theories suggest the disease is simply a virulent bacterial enteritis whereby potential pathogens such as *Pseudomonas anguilliseptica* infect stressed fish causing mortality rates as high as 30% (i.e. in seabream, *Sparus aurata* (Domenech et al., 1997). Generally speaking, the morbidity rate in kingfish populations is usually low. The mortality rate however can rise sharply in the winter and spring due to the acute onset of enteritis and symptoms of toxaemia.

Luzzana et al. (2003) reports that gilthead seabream, *Sparus aurata*, in Croatia succumb to a seasonal cold-water syndrome that is not associated with a single pathogen. Numerous bacterial opportunists have been isolated from the inflamed intestines of the gilthead and the cause of the syndrome is considered multifactoral. In Croatia, a specific diet was tested to tackle immuno-suppression induced by cold stress and other possible stressors by boosting amino acids. Luzzana states that dietary stress was reduced by including highly digestible raw materials to a balanced feed formulation. Under the conditions of his trial, good results of the winter diet were reported in terms of growth and feed conversion rates. Growth, condition factor, and hepatic energy storage were each improved during cold-water winter season without experiencing the typical and significant mortality rates.

The seasonal nutritional requirements of *Seriola* species continues to be investigated. Shimeno (1992) suggests that *Seriola* species can adapt to low carbohydrate diets although the fish have limited carbohydrate digestion and metabolism. Consequently, some ill-health and poor growth events may arise during cool water conditions when kingfish are consuming diets comprised of substantial amounts of vegetable protein, particularly if opportunistic marine bacteria are present. More research is required on this complex pathological syndrome.

Seedling nutritional deficiencies and syndromes are hazards of moderate risk to kingfish. Nutrition-related inadequacies are commonplace in marine larval hatcheries that rely upon the creation and maintenance of high quality live-feeds and larval rearing environments. Problems of this nature occur in Australia and Japan and it is common to lose up to 80-90% of the larval stock from the time of egg hatch to delivery to net pens (personal communication, 2004; Tachihara et al., 1997; Sorgelous et al., 2001). Some aspects of this mortality were discussed under bacterial hazards (see page 33). More research is required on the complex nutritional and husbandry aspects of *Seriola* culture to improve the efficacy of culturing larvae, broodstock and production fish.

Moderate risk – Deformity and malformations:

Swim-bladder malformations and head/jaw deformities are well known events in the Australian culture of kingfish. The cost and inconvenience of these conditions in *S.lalandi* is high. It can be measured in terms of post-handling mortality, lost growth and performance, and in the elevated labour cost of counting, culling and disposing of dead or compromised fish. The incidence of this syndrome is considered to be declining but estimates of deformities still range from 10-25% (personal communication, 2004). The causes of deformity are complex and numerous.

Toften and Jobling (1996) investigated anatomical malformations, spinal deformation and swim bladder dysfunction in fishes. The paper brings to light the numerous causes of deformation seen amongst intensively reared fish of many types. The major categories of causation identified within Toften and Jobling's review were: hereditary factors, damage during embryonic development, injuries, diseases and damage due to environmental factors, as well as nutrition and chemo-therapeutant toxicities. When kingfish carcasses are counted and categorised by contract divers and farm staff, to date the carcasses are typically identified as 'deformities', 'poorperformers (runts)', and 'post-handling losses' (which include fish suffering from over-inflation of the swim-bladder). Swimbladder malformation of hatchery reared marine fish was documented by Trotter et al. (2001) and may be relevant to the lordosis/kyphosis deformations and over-inflation syndrome experienced in *S.lalandi* of South Australia. More research is required.

Low risk – Haemolytic anaemia (jaundice) syndrome:

Japanese yellowtail are sensitive to this syndrome known as 'odan' or jaundice (yellow discolouration of external and internal tissues), particularly when concurrent infections exist and the water temperature exceeds 23°C. *S.dumerili* do not appear susceptible to the disease whereas *S.quinqueradiata*, *S.lalandi* and the hybrid *Seriola* 'burihira' have been diagnosed with the clinical symptoms; yellowtail more so than the other species (Anon., 1996a, 1998, 2002). The prevalence and significance of this disease in cultured *S.lalandi* is considered relatively low by Japanese farmers. In general, the disease commonly afflicts large fish in net pens during their second or third summer. The fish's red blood cells become fragile and burst resulting in severe acute anaemia, jaundice, necrotic organs and complete organ failure.

Sakai et al. (1998) summarises the occurrence of jaundice in numerous fish species. Iida and Sorimachi (1994) document that since 1980 cultured *S.quinqueradiata* are reported to experience an epizootic disease called 'jaundice', thought to be caused by a filamentous bacterium within the blood (Sorimachi et al., 1993; Maeno et al., 1995a). Sorimachi et al. (1993) applied Koch's postulates to recreate the disease using isolated bacterium. More recently however, researchers discovered that some *S.quinqueradiata* juveniles exhibiting clinical jaundice were not found to be PCR positive for the 'jaundice bacterium'. This brings into question the significance of the bacteraemia (personal communication, Fukuda 2002) versus other etiologies of the

jaundice symptom. Ito et al. (1999) also acknowledges the presence of a systemic bacteraemia and further suggests that haemolysis and anaemia alone do not bring about death and jaundice in yellowtail. Rather, he suggests that lipid peroxidation appears to be the principal cause of jaundice and death.

In summary, there are many causes of jaundice in animals; the cause of the disease in *Seriola* remains questionable. The "chicken and egg" arguments (*in vivo* lipid peroxidation versus bacterial-related haemolytic crises) are debated within the literature. The jaundice syndrome of *Seriola* fish is likely the result of an infectious, pathological, physiological and environmental factor, and the control of the syndrome likely lies in a combination of preventative husbandry, ideal nutrition, early detection of infection, early drug therapy and ideal water quality.

No documentation was found pertaining to **cataract development in Seriola species**, yet producers of kingfish in South Australia and New Zealand have observed the phenomenon and some feel that a specific nutritional deficiency is the most probable cause. In general however, these opacities of the lens usually reflect a complex syndrome influenced by many factors, including: genetics, metabolic disorders, nutrition, trauma, infections, inflammation and toxicity. In this report, cataracts have been assigned a very low overall risk due to their low incidence and minor consequence. Further investigation is warranted if the prevalence increases to include entire groups of fish.

Green liver syndrome is neither a clinical disease, nor does it appear to be a debilitating phenomenon, yet has been observed in both S.lalandi of South Australia and amongst S. quinqueradiata and Pagrus major (red sea bream) of Japan. lts overall risk rating is negligible. Nevertheless, its discovery at harvest processing usually creates some anxiety for the processor and producer so the syndrome is Some literature suggests that green livers arise in S. quinqueradiata noted here. from the excessive use of vegetable protein in fish diets (Watanabe et al., 1998). Watanabe reports that the mechanism of green liver discolouration remains unknown, but the observation of green livers as a necropsy finding amongst the vellowtail group fed non-fish meal diets suggests that diet quality may play a role in this non-clinical syndrome of Seriola fish. Sakai et al. (1990) suggest that severe oxidative stress and haemolysis of the red blood cell may result in jaundice and perhaps discolouration of livers due to the accumulation of bilirubin. Yokoyama and Fukuda (2001) identified microscopic parasites within the gall bladder and biliary tubules of green livers, suggesting tubular inflammation, blockage and biliary stasis as another explanation of liver discolouration of *S. guingueradiata*. More research is required.

Conclusion – risk analysis:

This is a subjective and qualitative risk analysis that reflects information from published literature and expert opinion. It considers farming populations, aquaculture rearing practices in various countries, complex diseases and the dynamic nature of marine ecosystems. The analysis summarises information about plausible fish health hazards and problems that have already, or may soon, confront aquaculturists, researchers and government personnel involved with raising populations of yellowtail kingfish in South Australia. The values assigned to likelihood, consequence and overall risk in this report are based on international experience, veterinary observations, interviews and available literature, yet the risks do invite further interpretation and assessment by each reader according to his or her perspectives and level of comfort.

The 41 hazards to kingfish health discussed in this report may surprise stakeholders of South Australia's developing aquaculture industry, yet only three (3) of these hazards have been assigned an overall risk rating of 'high', and two (2) of those – gill and skin fluke infestations - are well underway to being managed. Any pre-emptive, applied R&D efforts are perhaps most cost-effectively directed to the eleven (11) hazards deemed as 'moderate' risks in Table 2 (page 24); nine (9) of which have already been experienced to some degree in South Australian kingfish culture facilities. However, one can predict that these hazards and diseases may appear with greater frequency or significance in sea net pens unless tools of prevention and control are investigated, developed and made available to veterinarians or Examples of fish health management tools include: diligent and aquaculturists. regular observation of fish, identification of lesions, recognition of changes in fish behaviour and disease patterns, population and environmental monitoring protocols, expanded record keeping, and more efficient access to diagnostic laboratory tests, vaccines and antibiotics.

Currently, the limited surveillance of moribund and dead fish (i.e. for internal lesions, indigenous pathogens, as well as newly emerging pathogens) represents a significant contributing factor to risks facing the kingfish industry of South Australia. Other activities such as standard operating bio-security procedures, and daily phytoplankton and dissolved oxygen monitoring may also exacerbate the risk of losses if unforeseen hazards arrive abruptly. Preventative activities are key to the early identification and/or control of emerging threats to fish health. Without them, the magnitude of impact will increase (should a pathogenic hazard present itself), often leaving the mass mortality of fish as the outcome. Responsive contingency plans arranged in advance of pathogenic and environmental threats are necessary.

Benefits and adoption

This project focuses on the needs and opportunities of the kingfish industry. It highlights specific areas of the industry's development (biology, ecology and socioeconomic perspectives) to facilitate and optimize fish health, productivity and farming efficiencies. If decision makers, enterprises and policy makers apply the guidelines to managing fish health, the kingfish industry will accelerate toward best management practices. An elevated awareness of bio-security and bio-containment procedures, along with fish health training and early recognition skills by divers and farm staff, will help mitigate potential losses before fish diseases become significant in South Australia.

The State agencies that manage aquaculture and environmental regulations will find this report useful to enhance awareness of hazards and risks to yellowtail kingfish, and to facilitate discussions with industry - perhaps leading to amendments of current planning documents and health policies. This report documents opportunities, needs and obstacles that should be considered for new approaches (or at least further negotiations) to fiscal allotments, mandates, and job descriptions within both private enterprises and government agencies. Additional field support and diagnostic services are key topics for discussion.

The research community of Australia will find the list of plausible hazards to kingfish and the literature review relevant when seeking further funding of existing R&D projects, or when developing new project proposals. For example: the comparison of hazards to kingfish relative to other cultured finfish species may be important issues to investigate, or studying viable pathogens carried by raw and frozen ingredients of fish feed (i.e. herring, squid, and pilchard).

National and multinational manufacturers of pharmaceutical and vaccine products will find this risk analysis, and dilemmas facing the YTK industry, useful when investigating aquaculture opportunities and making R&D decisions for products potentially destined for the aquaculture market of South Australia. The dissemination of information within this final report to pharmaceutical and vaccine companies may also help to foster applied research partnerships with stakeholders of the YTK industry. Biotechnology companies are aware of the South Australian finfish market, yet they have not pursued the YTK market, likely due to several factors: the finfish industry's relatively small size (in world terms), the general lack of disease awareness, the minimal disease-related mortality to date, the precautionary approach to industry expansion and the restrictive nature of business development of this primary and sustainable source of seafood raised in highly productive settings.

Fish health personnel of diagnostic laboratories and veterinary services will find the list of hazards and the pathology guidebook useful as tools for training staff and preparing services, or in developing education programmes and expanding the availability of diagnostic tests. By applying the information offered within this report the control measures for each of the hazards listed should now be considered and prepared in advance of problems. In doing so, the YTK industry will remain proactive as opposed to reactive to significant and costly disease outbreaks.

The South Australian Marine Finfish Farmers Association (SAMFFA) has exhibited a responsible and forward-thinking approach to scrutinize their own industry with the intent to plan for the future and prevention problems. The information from this project should be distributed as widely as possible to instill public and consumer

confidence in the producers of finfish aquaculture. The consumers of kingfish will become aware of the integrity of the YTK industry and the government agencies through their combined efforts to maintain pristine marine environments while culturing a high quality, wholesome food-animal that is healthy and safe to eat.

Further Development

Project 2003/216, the detection and management of health issues in kingfish (YTK, *Seriola lalandi)*, will progress somewhat to a related project of preparation and awareness, that of emergency response and preparedness protocols in the event that disease outbreaks do arise in the yellowtail kingfish sector. The related project, Project Number 2003/649 (SAMFFA and Sheppard), will address the immediate emergency protocols required by the farming staff to identify diseases and to deal with mass fish mortality in the first hours and days of a serious fish health emergency. It is important that the *first line of defence* training activities be targeted at farming and diving personnel.

The ongoing distribution and use of the photographic disease guidebook by farming personnel and fish health technicians will help to increase the skills and awareness of aquaculture workers, laboratory diagnosticians and veterinarians. In addition, annual educational training programmes (emphasizing the skills required for diagnostic levels I, II and III) will facilitate more efficient responses to fish illness and mortality and perhaps expand diagnostic services in general, including expanding the repertoire of informative, cost-effective diagnostic tests offered by regional laboratories.

Planned Outcomes

The **output items** from this project are:

- the literature review and bibliography,
- a qualitative risk analysis,
- the guidelines to managing fish health of yellowtail kingfish,
- a photographic pathology guidebook, and
- a fish health training workshop.

Each output stimulates a greater awareness of current and future needs, opportunities and safeguards, mainly for the kingfish aquaculture industry of South Australia (SAMFFA) and PIRSA, but also for the EPA, researchers, diagnostic service personnel and product suppliers to the YTK industry.

The **outcomes** of this project are summarized below. However, some outcomes will become realised as this report achieves broader circulation to stakeholders and other interested groups.

The first outcome includes information transfer, education and training. Hazards and diseases of YTK have been communicated to stakeholders and the kingfish industry now has a clearer vision with which to develop effective disease management plans. The risk analysis, pathology guidebook, and guidelines to fish health will facilitate development of a generic fish health management template that each enterprise can produce and apply to its farm sites, husbandry protocols and personnel job descriptions.

The second outcome is a revitalised discussion between government regulatory agencies and industry members to review existing government policies and planning

related to finfish aquaculture. The qualitative risk analysis offers information to be adopted or debated, and it stimulates information-based revisions of existing policies and practices.

The third outcome of this project affects funding and services. This report offers information and recommendations to allocate funds for:

- future research and development projects,
- more frequent staff training,
- the development of private or government diagnostic services, and
- partnerships of projects of mutual interest to other aquaculture sectors.

The risk analysis and literature review reveal gaps in knowledge where further investigation and research is warranted.

The fourth outcome addresses opportunities regarding public relations, consumer confidence and marketing. The forward-thinking initiative of the YTK industry (SAMFFA) to prioritize a project of this nature is indicative of professionalism and the industry's focus on sustainability. The outputs of this project send a clear message, both nationally and internationally, privately and politically, that the kingfish industry of South Australia is proactive, self-aware, responsible and progressive. This project will enhance industry's productivity and the industry's status in the eyes of suppliers, government officials and consumers.

The fifth outcome facilitates sustainability of the YTK aquaculture industry. The outputs of this project offer tools that can be used to elevate the awareness of hazards and health-related issues, thereby minimizing disease, optimizing survival, and improving feed conversion efficiencies and costs-of-production. The marriage of fish health and productivity is important to the success of management plans. In addition, the project outputs provide information and guidance to the industry and regulatory authorities in their development of policies and programmes related to industry sustainability.

The objectives, milestones, and outputs of Project 2003/216 have been supported by representatives of stakeholder groups, both verbally and in writing, throughout its evolution. Appendices 7A, 7B, 7C and 7D reflect stakeholders' sentiments of this project.

Conclusion

There are four requirements to achieving an effective fish health preventive program. Firstly, one must anticipate the problems one may face. Identifying hazards and assessing the risks of disease development and amplification are important first steps. These have been achieved. Secondly, the accurate identification of clinical signs and pathology by trained, informed farm staff creates a mechanism for early detection and categorization of potential fish health problems. The photographic guidebook of yellowtail diseases facilitates this important activity. Thirdly, an early detection and recording system is required. It includes a mechanism of vigilant observations and documentation that creates an effective form of action-based detection of emerging changes. This commitment to recognize hazards and disease signs must arise in the front-line personnel: the contract divers and enterprise staff. A need for generic guidelines to managing kingfish health and health-related best management practices was identified at the onset of this project so recommendations to minimize problems and achieve disease control objectives are

outlined within Appendix 5 of this report. Appendix 5 can serve as a stand-alone document. It is entitled: *Guidelines to managing fish health of yellowtail*. The fourth requirement to achieving an effective fish health preventive program is that one must be prepared to take action in response to laboratory findings from disease-related or environment-related submissions. Diagnostic tests are conducted to affect and direct one's decision and course of action.

The global expansion of finfish culture is likely to continue to give rise to emerging infectious diseases and there are three main reasons for this:

1) when first discovered, a potential disease-causing organism is generally labelled as 'new' or 'exotic' to the region. However, it is perhaps more appropriate that the microorganism be described as 'previously unidentified' or perhaps 'emerging from the local environment' since opportunistic microorganisms may in fact be indigenous and ubiquitous in the local marine environments in which fish are farmed. Thus, cultured kingfish raised in semi-open net pen systems have the potential to contract infections by indigenous agents;

2) fish reared in enclosures reside in close proximity to one another. Consequently, the horizontal transmission of microorganisms from one fish to the next may be facilitated. If the infections escalate to a disease status, which occurs with more frequency if fish are stressed or overwhelmed by environmental challenges, the pathogens tend to accumulate within the cage environment and amongst the population of affected fish. This is considered an amplifying phenomenon of populations reared within enclosures. It remains unknown to what extent the phenomenon occurs in wild schools of fishes, although commercially caught wild fish populations are frequently found to harbour significant numbers of pathogens, particularly parasites;

3) cultured fish are reared under constant surveillance by farmers such that signs of illness, or mortality, are more likely to be observed and documented. As a result, data is created and diseases are seen to 'emerge' *from within* aquaculture systems, whereas we remain blind to the exact occurrences in finfish swimming freely in the natural marine setting. It is generally accepted that wild fish become ill and die yet that process largely remains undetected. Perhaps we need to accept that observing *farmed* fish is the only viable and affordable means of 'active surveillance' of finfish yet, in the event that a disease emerges in cultured fish, conclusions need to be drawn with care and with a broad perspective. Therein lies the sensitivity and controversy of fish health policies and management of a relatively new YTK aquaculture industry. To date, the YTK industry of South Australia has yet to experience severe mortality or problems related to disease, although the indicators of disease are anticipated to become more evident as awareness and training continues. That is, when we look, we find.

This project offers important viewpoints about allocating government (public) funds for numerous activities: future research, training, development of private or government diagnostic services and infrastructure, and project sharing or expansion to other aquaculture sectors. There is a desire to level the playing field with regard to food-animal production in South Australia. The aquaculture industry wishes to benefit from the same public funds that agriculture is awarded, at least in terms of subsidized diagnostic and field extension programmes to maximize disease prevention. Regulators of fish health and marine environments will use the relative risk analysis and list of hazards to revisit existing disease watch lists and fish health policies. However, the outcomes of this project will evolve most effectively if industry and government bodies work as partners; partners with mutual, progressive intentions for the expansion and sustainable development of this primary resource of South Australia. Once a critical mass (in terms of industry size, economics and market sustainability) is achieved, the YTK industry will then attract the attention of suppliers of vaccines and health products to better ensure industry stability and sustainability. More applied research, and the development of tools to prevent and control diseases, will stimulate movement of the aquaculture enterprises toward best management practices, integrated health management procedures, and disease surveillance programs. The activities may also lead to amendments of the current fish health regulations. This in turn should enhance industry's productivity and the public's acceptance of finfish aquaculture.

The photographic guidebook (Sheppard, 2004) and list of hazards to YTK health have already stimulated fish health specialists to reassess diagnostic plans and prepare other personnel to recognize specific lesions. Personnel of diagnostic laboratories also have new reference material to expand their lists of differential diagnoses or to modify laboratory techniques of pathogen identification. The project outputs should stimulate funding of academic and applied research projects that focus on moderate and high-risk hazards. Consequently, more peer-reviewed literature about *S.lalandi* will emerge and may eventually facilitate the replacement of this qualitative risk analysis with one more quantitative in nature.

Researchers and health professionals have suggested that certain components of this project be published in peer-reviewed journals for wider dissemination of important fish health and YTK information. Continued research and analysis of raw and frozen fish feed for food-borne pathogens may reveal interesting relationships and further risks to finfish aquaculture industries of South Australia, particularly those in close proximity to kingfish net pens such as Southern bluefin tuna, tommies and mulloway. An investigation of various grades of raw fish food comprised of herring, pilchard, sardines, and squid (sometimes used in finfish aquaculture settings) may offer new information for the decision-makers.

In terms of information transfer and dissemination, the training workshop held in July 2004 was well attended by industry stakeholders. It provided hands-on, applicable information for all delegates from contract divers through to veterinarians and enterprise decision-makers. The interactive format of the workshop is an effective approach to training and should form the basis of continuing education programmes for fish culturists and aquaculture service personnel on an annual and repetitive basis.

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Appendix 1: Intellectual Property

Neither the South Australian Marine Finfish Farmers Association (SAMFFA), Sakana Veterinary Services Ltd. (sVs) nor Dr. Mark Sheppard expect to obtain any profit from the intellectual property (IP) arising from Project No. 2003/216 per se, but an IP and Copyright agreement has been reached with regard to the concurrent and future sale and distribution of the photographic pathology guidebook (ISBN 0-920225-14-4) published as an adjunct enhancement of this YTK project. FRDC also encourages Principal Investigators to publish specific components of Animal Health Subprograms to help disseminate the information as widely as possible and by various means.

Appendix 2: Staff

Mark Sheppard, President of Sakana Veterinary Services Ltd. (sVs). Martin Hernen, Executive Officer of South Australian Marine Finfish Farmers Association Pty. Ltd. (SAMFFA).

Appendix 3: Australia Questionnaire (09/2003) – YTK Health

Our goal is to identify the needs, gaps and opportunities related to maintaining YTK health and productivity, as well as to identify issues confronting YTK health in South Australia. To assist us in the development of disease recognition and in managing priorities affecting Yellowtail kingfish **(Seriola lalandi)** please answer the following questions. When applicable, please circle your response. To indicate your greatest to least concern or priority, please use 1, 2 or 3 where #1 indicates the greatest concern. If this questionnaire does not address specific issues, or if you wish to further clarify your answer(s), please comment freely. All information will be helpful and remain confidential to the contractor. Thank you.

Does the identification of potential infectious diseases of YTK, and guidance for managing the health, require immediate attention? Yes, a priority. No, it can wait.

List the top 3 causes of YTK MORTALITY that you feel are most important, to date:

Have your YTK experienced any of these yet?	Yes	No	All	
List the top 3 causes of POOR-PERFORMANCE (i.e. of gro significant:	wth or	quality	/) that	are most
Has your YTK's performance been affected by any of these?	Yes	No	All	
What cumulative mortality rate do you feel is respectable in ponding to cage entry (i.e. cage entry to hark				
Overall, would you describe your cumulative YTK mortality rate higher than planned, as planned, or low?	to be:			
Since 1999, have you noticed any change in the type or sev symptoms (i.e. emerging signs) from the YTK populations?			tious (San	
Have these infections been formally diagnosed? Or, simply obs	erved/re	ecorde	ed at t	he farm?
Is adequate laboratory and diagnostic support in SA important t	o your ı	needs	? Yes	No
Is there adequate diagnostic support available to you now?Yes Any suggestions?	No			
Do you rely upon services and facilities within SA, or in Ta	asmania	a?		
Have diagnostic labs or services answered your questions and No	met you	ur nee	ds, to	date? Yes
Is the diagnostic facility easy to submit samples to? Yes No Any suggestions?				
Is the diagnostic facility close enough for timely submissions?	Yes	No		
Is the diagnostic service affordable and cost-beneficial to you?	Yes	No		
Has the lab personnel given you helpful guidance in how be	st to si	ıbmit -	fish s:	amples for

Has the lab personnel given you helpful guidance in how best to submit fish samples for quality diagnoses? Yes No

Appendix 3, continued

Sheppard, M.E., May 2005

Is there an adequate number of fish health diagnosticians available to address your questions/problems about YTK and bio-security? Yes No Are they easily available to you? Yes No

Is there sufficient support & service with regard to parasite problems in YTK? Yes No

Is there sufficient support & service with regard to bacterial and viral problems? Yes No

Is there sufficient support & service with regard to harmful marine algae? Yes No Any suggestions?

Is there sufficient support & service with regard to YTK hatchery health and efficiencies? Yes No

In general, is the current level of support from the MUNICIPAL government satisfactory to the needs of YTK farming, health and development? Yes No

In general, is the current level of support from the STATE government satisfactory to the needs of YTK farming, health and development? Yes No

In general, is the current level of support from the FEDERAL government satisfactory to the needs of YTK farming, health and development? Yes No

Is the current legislation and policy related to YTK: Consistent? Inconsistent? Supportive? Unrealistic? Focused on enforcement?

In general, do you find the current YTK-related support projects useful and applicable? Examples please: _____

Or, are the YTK projects too academic and potentially enforcement-orientated? Examples please:

In general, has the public's perception of YTK farming been: positive negative or indifferent?

Do you feel the general public is accurately informed about YTK aquaculture? Yes No

Which group should be responsible to educate the public about YTK farming?

Do you feel that YTK farming is environmentally sustainable under its current practices? Yes No

If yes, will it remain environmentally sustainable if the annual production doubles in each farm location? Yes No

Do you feel the "environmental footprint" of YTK farming is, as it's practiced now: severe (deep), moderate, or minor (shallow)?

Are the environmental impacts of most YTK farms: permanent, temporary, beneficial?

Is the current environmental legislation/policy: too restrictive? too loose? workable?

Appendix 3, continued

Which do you anticipate is most likely to occur?:

1) YTK will contract and express infections of indigenous disease agents already naturally present in the marine environment? or

2) in the near future, YTK will contract exotic, imported disease agents? Or 3) both?

What time frame do you anticipate these events may arise?within monthswithin yearsdecades away

Do you think there are undiscovered "exotic" disease agents in the waters of SA now? Yes No

Is your staff adequately trained and prepared for a YTK outbreak of disease? Yes No

Do you routinely examine your YTK carcasses and record categories of lesions/death? Yes No

Do you have a recording system that monitors changes or trends in cage mortality rates? Yes No

Does your system signal you when a significant change has occurred over a pre-determined period of time? Yes No

Does your record system monitor changes in cause-of-death categories? Yes No

Do you have access to "tools" to control or prevent diseases if they arise? Yes No Consulting services? Anti-microbial drugs? Vaccines?

Do you already implement sanitizing and bio-security protocols as part of your farming activities? Yes No

If so, which best describes your protocols?:

1) constant and stringent bio-security measures.

2) casual and ready to implement, if/when necessary.

Who do you feel should be the first person (or department) to contact in the event of the discovery of an unidentified lesion in a several fish?

Who do you feel should be the first person (or department) to contact in the event of a massmortality of YTK? _____

Overall, which best describes your YTK operation?:

1) your husbandry activity is ready to control a disease and mortality problem once it occurs.

2) your husbandry activity applies a preventative approach already.

3) both.

4) neither.

Below, please circle the diseases that you have already had diagnosed, or you have had experience with, in YTK, if any:

Comments and additional information:

Pasteurellosis Lactococcus

Appendix 3, continued

Sheppard, M.E., May 2005

Nocardiosis Mycobacteriosis Yellowtail Ascites Virus (dropsy) Iridovirus Vibriosis Necrotizing enteritis Dermatitis, erosive or ulcerative Flexibacter dermatitis Pale gills, anaemia Curved spine (scoliosis) Bent tails Meat microsporidiosis Branchial epitheliocystis Yellow jaundice/visceral necrosis Skin flukes Gill "flukes" Green liver syndrome Other parasites

Appendix 4: Japan Questionnaire - HIRAMASA Fish Health (2003)

Would you please be so kind to answer the following questions to help me learn more about *Seriola lalandi* compared to other *Seriola* species by using numbers 1, 2, 3 to indicate the highest to lowest problem? (i.e. #1 indicates the biggest problem).

	Hiramasa	YT	Kampachi
Which fish have the least problems and mortality due to infections/disease? (please indicate only one):			
Rensa is a significant infection/disease in: Hiramasa get Rensa infections: Yes No	(i.e. 1)	(3)	(2)
Ruiketsu is a significant infection/disease in: Hiramasa get Ruiketsu infections: Yes No			
Nocardia is a significant infection/disease in: Hiramasa get Nocardia infections: Yes No			
Mycobacterium is a significant infection/disease in: Hiramasa get Myco infections: Yes No			
Fukusui is a significant infection/disease in: Hiramasa get Fukusui infections: Yes No			
Iridovirus is a significant infection/disease in: Hiramasa get Iridovirus infections: Yes No			
Vibrio is a significant infection/disease in: Hiramasa get Vibrio infections: Yes No			
Odan is a significant infection/disease in: Hiramasa get Odan infections: Yes No			
Curved spine (brain infection) is most common in: Hiramasa get curved spines: Yes No			
Beko is a significant infection/disease in: Hiramasa get Beko infections: Yes No			
Edwardsiella is a significant infection/disease in: Hiramasa get Edwardsiella infections: Yes No Others: BRANCHIAL EPITHELIOCYSTIS			
FLEXIBACTER KASSO-SAIKIN "HISAYOSHI DZ-SYNDROME"			

Results of Japanese survey (below):

Appendix 4, continued

HIRAMASA kingfish questionnaire/survey. Japan, May-Dec 2003

n-7 Jananese farr	mers (4 Saiki, 2 W.Kyushu, 1 Kochi)							Asked,
					% response	% response	% response	but no
n=2 Japanese drug reps (Saiki)				Kingfish	Yellowtail	Amberjack	response, o	
n=2 Japanese DzCntre Microbiologists					S.lalandi	S.quinquer.	S.dumerili	unknown
Which fish have the fewest problems and least mortality due to disease/infection?					73%	9%	0.ddineini	18%
	•	hat responded:	Yes	No		070		1070
Kingfish contract	Lactococcus infections/mortality:		88%	13%	-			
Lactococcus	creates significant mortality	in which fish?				91%		
Lactococcus	creates moderate mortality	in which fish?			64%		27%	
Lactococcus	is not a problem, not known, insignif.	in which fish?			<u>36%</u>	<u>9%</u>	<u>73%</u>	
Kingfish contract	Photobacterium infections/mortality:		70%	30%				13%
Photobacterium	creates significant mortality	in which fish?			27%	64%	9%	
Photobacterium	creates moderate mortality	in which fish?			36%	<u>36%</u>	45%	
Photobacterium	is not a problem, not known, insignif.	in which fish?			<u>36%</u>		<u>45%</u>	
Kingfish contract	Nocardia infections/mortality:		78%	22%	_			25%
Nocardia	creates significant mortality	in which fish?			22%	44%	44%	
Nocardia	creates moderate mortality	in which fish?				44%	44%	
Nocardia	is not a problem, not known, insignif.	in which fish?			<u>78%</u>	<u>11%</u>	<u>11%</u>	
Kingfish contract	Mycobacterium infections/mortality:		33%	67%	_			50%
Mycobacterium	creates significant mortality	in which fish?				78%	22%	
Mycobacterium	creates moderate mortality	in which fish?			11%	<u>22%</u>	33%	
Mycobacterium	is not a problem, not known, insignif.	in which fish?			<u>89%</u>		<u>44%</u>	
Kingfish contract	YAVirus infections/mortality:		71%	29%	_			38%
YAVirus	creates significant mortality	in which fish?			11%	89%	11%	
YAVirus	creates moderate mortality	in which fish?			22%		33%	
YAVirus	is not a problem, not known, insignif.	in which fish?			<u>67%</u>	<u>11%</u>	<u>56%</u>	
Kingfish contract	Iridovirus infections/mortality:		89%	11%				25%
Iridovirus	creates significant mortality	in which fish?			10%	67%	30%	
Iridovirus	creates moderate mortality	in which fish?			30%	17%	30%	
Iridovirus	is not a problem, not known, insignif.	in which fish?			<u>60%</u>	<u>17%</u>	<u>40%</u>	
Kingfish contract	Vibrio infections/mortality:		82%	18%				
Vibrio	creates significant mortality	in which fish?			10%	60%	20%	
Vibrio	creates moderate mortality	in which fish?			30%	20%	60%	
Vibrio	is not a problem, not known, insignif.	in which fish?			<u>60%</u>	20%	20%	

/ ...continued

.../table continued

Appendix 4, continued

					% response Kingfish	% response Yellowtail	% response Amberjack	Asked but no response, or
	Of those t	hat responded:	Yes	No	S.lalandi	S.quinquer.	S.dumerili	unknown
Kingfish contract	Jaundice infections/mortality:		43%	57%		- 1. 1.		38%
Jaundice	creates significant mortality	in which fish?			10%	<u>100%</u>		
Jaundice	creates moderate mortality	in which fish?			20%		43%	
Jaundice	is not a problem, not known, insignif.	in which fish?			<u>70%</u>		<u>57%</u>	
Kingfish contract	Curved spine infections/mortality:		78%	22%	_			25%
Curved spine	creates significant mortality	in which fish?				67%	30%	
Curved spine	creates moderate mortality	in which fish?			22%	22%	40%	
Curved spine	is not a problem, not known, insignif.	in which fish?			<u>78%</u>	<u>11%</u>	<u>30%</u>	
Kingfish contract	Microsporidium infections/mortality:		33%	67%]			38%
Microsporidium	creates significant mortality	in which fish?				44%	11%	
Microsporidium	creates moderate mortality	in which fish?			22%	22%	22%	
Microsporidium	is not a problem, not known, insignif.	in which fish?			<u>78%</u>	<u>33%</u>	<u>67%</u>	
Kingfish contract	Edwardsiella infections/mortality:		0%	100%	_			38%
Edwardsiella	creates significant mortality	in which fish?						
Edwardsiella	creates moderate mortality	in which fish?						
Edwardsiella	is not a problem, not known, insignif.	in which fish?			<u>100%</u>	<u>100%</u>	<u>100%</u>	
Kingfish contract	Epitheliocystis infections/mortality:		25%	75%	_			63%
Epitheliocystis	creates significant mortality	in which fish?				67%	33%	
Epitheliocystis	creates moderate mortality	in which fish?			50%			
Epitheliocystis	is not a problem, not known, insignif.	in which fish?			<u>50%</u>	<u>33%</u>	<u>67%</u>	
Kingfish contract	Enteritis infections/mortality:		17%	83%	_			60%
Enteritis	creates significant mortality	in which fish?				11%	11%	
Enteritis	creates moderate mortality	in which fish?			22%	11%	22%	
Enteritis	is not a problem, not known, insignif.	in which fish?			<u>78%</u>	<u>78%</u>	<u>67%</u>	
Kingfish contract	Dermatitis infections/mortality:		0%	100%				100%
Dermatitis	creates significant mortality	in which fish?				<u>100%</u>		
Dermatitis	creates moderate mortality	in which fish?						
Dermatitis	is not a problem, not known, insignif.	in which fish?			<u>100%</u>		<u>100%</u>	
Kingfish contract	Flexibacter infections/mortality:		0%	100%	_			100%
Flexibacter	creates significant mortality	in which fish?				<u>100%</u>		
Flexibacter	creates moderate mortality	in which fish?						
Flexibacter	is not a problem, not known, insignif.	in which fish?			<u>100%</u>		<u>100%</u>	

Appendix 5: Guidelines to managing fish health of YTK

Guidelines to Managing Fish Health of Yellowtail Kingfishthe foundation for a health program for finfish aquaculture of Australia.

(an output of FRDC Project No. 2003/216)

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TABLE OF CONTENTS (GUIDELINES TO MANAGING FISH HEALTH OF YTK)

i. Definitions:	76
ii. Intent of the Guidelines	79
iii. Objectives of the Guidelines	80
iv. Principal Components of Managing Fish Health	80
v. General Duties of Fish Culturists	81
Five Main Principles:	82
Principle #1. Characterizing, diagnosing and recording disease.	
Part A. Fish health records Part B. Monitoring infection & disease	83
Principle #2. Identifying and managing risks to fish health.	84
Part A. Water Quality	
Part B: Vaccination	
Part C: Segregation & fallowing	85
Part D: Stress & handling	85
Part E: Broodstock	86
Principle #3. Reducing exposure to and spread of pathogens.	88
Part A: Outbreak investigation	
Part B: Isolation "sick" units	
Part C: Managing dead fish	
Part D: Contract divers	
Part E: Disinfection, equipment & visitors	90
Principle #4. Adequate nutrition and feed management.	92
Part A: Clean cooked feed	92
Part B: Meat quality	
Part C: Transport & storage of feed	
Part D: Seasonal physiology & digestibility	
Principle #5. Judicious use of drugs in cultured fish.	
Part A: Diagnostic support	
Part B: Prescription records & food safety	
Part C: Tools of treatment & prevention of disease	
Part D: Feed rates & conditioning hatchery live-food	
Part E: Fish welfare	97
Appendix A-Gfh: Australian National List of Reportable Diseases of Aquatic A (Finfish), 2003	Animals 99

Appendix B-Gfh: Legislation	related to fish health	100
Typenan D Offic Degistation	<i>ciaica io jisti ticaitti</i>	100

I. DEFINITIONS:

Diagnosis: The use of skillful methods to establish the cause, nature and name of a disease. Three levels of diagnostic skill are generally required to make a definitive diagnosis.

Disease: Changes in the structure or function of a fish that presents a particular set of signs and symptoms that are distinctly different from what is considered a normal state.

Disease screening: Testing for evidence of early signs of disease or for factors that could predispose a fish or population to disease, such as infection.

Disinfection: Action undertaken to destroy infectious and parasitic disease-causing microorganisms. Often used synonymously with chemical sanitation.

Drug: A chemical reagent or chemo-therapeutant (including antibiotics, antimicrobials and topical pesticides) that has minimal effect on the fish host yet a specific toxic effect upon disease-causing microorganisms.

Endemic disease: A continuously recurring clinical disease in a relatively small number of fish within a geographically defined area, yet the disease-causing agent is present within the fish community at all times. Endemic is used in contrast to sporadic or outbreak, and it should not to be confused with indigenous disease.

Epidemiology: Investigating the cause of disease by identifying and explaining the interrelationships of host, disease-causing agent, environment and ecology.

Epidemiologically-linked: When groups of animals share risk factors for the disease of concern.

Emerging disease: A disease that becomes increasingly evident or it reflects an increasing incidence within a population or region. The disease may be significant or not, exotic or not, or perhaps previously recognized in an individual, yet initially the disease-causing microorganism was not considered to be problematic.

Exotic disease: A disease not previously recognized or diagnosed within a geographically defined area, such as: the nation or state. It represents the new introduction of a disease-causing microorganism to an area.

Fish culture facility: A fish culture facility is defined here as a location or facility that alters the normal movements, feeding and ecology of fish in order to affect their productivity in terms of reproductive success, growth or marketable fish products for commercial. A fish culture facility holds and handles fish for a period of time.

Fish health management plan: A written document that outlines the '*what and why*' principles a culture facility will abide by to meet the objectives and basic requirements of fish health (for more, see Standard Operating Procedures).

Fish health staff: People assigned the responsibility for daily fish health monitoring and management at a fish culture facility.

Health: The extent to which a fish, or group of fish, is able to satisfy biological and social needs and cope with changes in the environment, and not merely the absence of disease.

Holding unit: The basic physical structure containing fish, such as: net pens, tanks, troughs and raceways.

Husbandry: Any human activity related to the safe and effective rearing of fish housed in a ranching or fish culture facility. It includes containment, feeding, welfare, handling, movement, treatment, manipulation of rearing environments, etc.

Indigenous disease: Refers to a disease with a long history of occurring naturally and commonly as part of the ecology of an area. Not to be confused with endemic disease.

Infection: Infection is not to be confused with disease but it has the potential to give rise to disease. Rather it is the condition in which a fish, or part of it, is invaded by a microorganism which, under favourable physiological or environmental conditions, has the potential to multiply and produce injurious effects or symptoms of disease.

Operators: A collective term used to describe people responsible for the management, health and welfare of fish at fish culture facilities.

Outbreak: As an epidemic or epizootic (animals), an unexpected occurrence and rate of death or disease that affects a group of fish at the same time. This term applies to: (1) disease occurring outside of the typical geographic or host range, (2) a previously unrecognized problem, (3) an indigenous disease occurring at a rate higher than expected.

Pathogen: An infectious microscopic organism, including viruses, bacteria, fungus and parasites, capable of causing disease. A disease-causing microorganism.

Qualified fish health professional: A phrase used to describe people with adequate post-secondary training and experience in the recognition of diseases in fish to qualify them for certification by a recognized body.

Standard operating procedures: A written document and set of actions that describe in detail the *'how, who and when'* a culture facility will meet the objectives and basic requirements of fish health.

Treatment: Any husbandry actions, drugs, chemicals or biological agents given or applied to fish, to prevent or mitigate the impacts of disease on a fish, or group of fish.

Vaccine: A biological product comprised of specific proteins or protein-creating nucleic acids that produces a protective immune response in fish to a specific disease-causing microorganism. Vaccines are a preventative control measure and they are often more cost-effective than alternative methods of disease control. If the specific disease does arise subsequent to vaccination, the incidence and severity of that disease is usually significantly reduced.

Wild fish: Fish that have spent no part of their life cycle in a private or public fish culture facility.

II. INTENT OF THE GUIDELINES

The intent of these guidelines is to suggest a standard of health care and welfare for yellowtail kingfish cultured in South Australia. The concepts within this document originated from a Centre for Coastal Health project (Stephen, Sifton and Sheppard, 2001) for the British Columbia Ministry of Agriculture, Food and Fisheries (BCMAFF, Canada). The subsequent Manual of Accepted Practices (www.agf.gov.bc.ca/fisheries/health/, 2003) has now been adopted by the provincial government and aquaculture industry of B.C. Of course similar concepts, codes and plans appear in other published works, including: Aquavetplan (AFFA, 2001), FAO/NACA Technical paper 402 (2000), and various emergency response manuals.

The primary purpose of these guidelines is to offer a set of objectives and basic principles of fish health management to maintain the health of cultured yellowtail kingfish by means of prevention and reduction of disease. Equally important secondary objectives include:

- Animal (fish) welfare: the provision of a culture environment that serves to meet the physiological and behavioural needs of cultured fish,
- Food safety: the avoidance of fish health management actions that would adversely affect public health, and
- Environmental sustainability: the practice of fish health management in a manner that minimizes environment impact and disease risks to wild aquatic species.

Fish culturists are encouraged to continue fish health practices, reiterated in these guidelines, within their respective corporate plans wherever possible. However, it is acknowledged that these guidelines are just that. They must remain flexible and they may not accommodate all environments, infrastructure and goals of each yellowtail kingfish culture facility in the State. Guidelines suggested here are not necessarily 'best practices' nor do they represent the only way to achieve health and welfare. If culturists opt to use additional or different practices in raising their fish, it is hoped that the operators' actions provide at least an equivalent level of protection as recommended within these guidelines. It is further acknowledged that not all sectors of fish culture will be able to implement all practices suggested here due to shortfalls of financial and human resources and limitations of the culture facility itself, yet companies are encouraged to continually make commitments toward improving fish health and welfare activities.

Aspects of these guidelines undoubtedly touch on regulatory issues enforced by more than one government agency (Appendix B-Gfh of this document). As such, those agencies responsible for fisheries and aquaculture must examine any guideline manual to ensure the impacts of actions are in accordance with existing regulations. It is expected that fish health approaches will be regularly reviewed, negotiated and updated by government agencies and fish culturists to address future advances in fish culture techniques and any changes in disease risks facing aquaculture in the State. It is assumed by these guidelines that primary benefits to wild fish will arise from adequate disease management in private fish culture facilities.

III. OBJECTIVES OF THE GUIDELINES

- **Objective #1:** Fish culture methods and facilities must promote the health, welfare and productivity of fish kept at the facility.
- **Objective #2:** Prevent the introduction of exotic disease or disease-causing microorganisms.
- **Objective #3:** Reduce the number of cases of disease and/or the spread of diseasecausing microorganisms.
- **Objective #4:** Maintain an environment that does not increase the susceptibility of fish to disease or minimizes their exposure to disease-causing microorganisms.
- **Objective #5:** Fish culture methods and facilities must be managed to safeguard public health through practices that promote food and water safety.
- **Objective #6:** Protect public health through rational and responsible use of prescribed drugs and chemicals.
- **Objective #7:** Fish culturists and regulators must have appropriate information upon which to make rational, *evidence-based* health management decisions and to allow adequate evaluation by appropriate regulatory agencies.

IV. PRINCIPAL COMPONENTS OF MANAGING FISH HEALTH

The following elements are considered the main preoccupations of fish health management at fish culture facilities and this summary is intended to address the objective of these guidelines:

1. Characterizing, diagnosing and recording disease:

- Fish health management requires a careful system of records capable of describing patterns of death and disease at a facility. These guidelines reflect the attributes of a health record system intended to help operators detect trends in disease in time to react to minimize health impacts on nearby fish groups. Accurate records and historical accounts can also facilitate the timely approval of special permissions and permits from regulatory agencies in emergency situations.
- Sound observations provide value to records. These guidelines present the features of a monitoring programme intended to identify *changes* in disease status of fish at a facility.

2. Identifying and managing risks to fish health:

- By monitoring all factors that can affect fish health, operators may be able to take actions before significant negative health effects arise. Water quality management, vaccination plans, segregation and broodstock management are offered in this section of the guidelines.
- A cornerstone of fish health management is the reduction of stressors that could make fish more susceptible to disease. Recommendations in these guidelines address handling, crowding and rearing, anaesthetic protocols, and predator / prey interactions.

3. Reducing exposure to and spread of disease-causing organisms:

• Disease impacts can be confined or eliminated by minimizing opportunities for disease-causing microorganisms to spread within and between groups of fish. Management of disease outbreaks, care in the handling of dead fish, hygiene and disinfection practices, and fish movements are each considered.

4. Adequate nutrition and feed management:

• Feeding fish to maintain health and welfare is a major component of finfish culture. Feed management also bridges health with fish performance, production, and corporate sustainability. These guidelines offer basic nutritional considerations to reduce susceptibility to the onset of disease.

5. Judicious use of drugs in cultured fish:

• The consumer, environment and fish are more protected by proper application of drugs and chemicals to control disease. Practices concerning the need for adequate diagnostic support, and drug / chemical storage, drug use and handling are addressed in this section of the guidelines.

V. GENERAL DUTIES OF FISH CULTURISTS

- Everyone involved in fish culture must manage fish in a manner that respects welfare and does not increase adverse health effects.
- A fish culturist must not contribute to the increased prevalence or distribution of infectious disease-causing agents by moving fish known to be infected off site until the threat to other fishes and aquatic animals is assessed by a qualified fish health professional, and recommendations are acted upon.
- When there is reason to believe that a disease problem will spread from its site of origin to other locations or aquatic animals, fish culturists must notify the parties with the ability and authority to act in time to restrict or prevent the spread of the disease-causing microorganisms.
- For diseases appearing on the Australian National List of Reportable Diseases of Aquatic Animals (Appendix A-Gfh of this document), facility operators must provide information and access to locations, activities and samples required to prevent or control the disease.
- It is the responsibility of fish culturists to ensure their operations are in full compliance with applicable regulations (Appendix B-Gfh).

FIVE MAIN PRINCIPLES:

Principle #1. Characterizing, diagnosing and recording disease. Part A: Fish health records

Part B: Monitoring infection & disease

Part A. Fish health records

Culture facilities should have a record keeping system that allows the early recognition of changes in fish health. The records should be capable of generating information on the health status of the fish group at any time during the production cycle. Records should be kept in a manner that allows the identification and recreation of the disease and management history of fish in a particular holding unit.

Fish health records should include information on:

- consistent code identifiers for every group of fish and allows for tracking the movement, splitting and harvest of fish groups,
- the causes and rates of death (% over time) in each fish group,
- information about actions taken to treat, prevent or control disease,
- other health risk factors relevant to a specific site.

A.1 Basic attributes of health records:

- i. **Identifying fish groups:** Establish and assign a consistent code identifier to each group of fish that depicts its origin, date of arrival, year-class, relocation and mixing history.
- ii. **Regular and prompt data entry:** Record data before information is lost or forgotten. Data entry should be a routine event.
- iii. **Ease of data access, and frequent analysis:** To identify trends or emerging issues affecting health. Identify things that may require immediate action.
- iv. **Consistent set of measurements:** Fish inventory at start and end of period, fish weight at start and end of period, biomass, daily feed rates, weekly mortality rates.
- v. **Chronological events:** To link, correlate and recreate events and trends.
- vi. **Incidental data to support evidence-based decisions:** Both at the individual holding-unit level (i.e. pen) and at the culture facility level (site or region).

A.2 Basic information within health records:

- i. **Patterns and causes of death:** Regularly examine all carcasses and assign them obvious reasons for death. Describe mortality rates (number dead per number living per time period).
- ii. **Diagnostic information:** Samples collected, fish group, when, how and where sent. The diagnostic tests applied and results.
- iii. **Patterns and causes of disease:** Observations, slow-swimmer numbers, culled, external lesions, daily feed consumed by group, diagnostic test and result.
- iv. **Treatment or control events:** Group ID, dates, rationale, nature of control event (i.e. net change), drug or chemical if used, approval and prescription copies, dose, duration, residue withdrawal time.
- v. **Fish movements:** Original groups and inventories, original locations, final locations and inventories, adjusted yet linked ID codes for fish groups.

vi. **Health risks:** Water quality parameters, dates of handling, density and stress, predator attacks, equipment movement, feed management, weather and environmental events.

Principle #1. Characterizing, diagnosing and recording disease, continued:

Part B. Monitoring infection & disease

Operators and fish health staff should have access to disease information, access to diagnostic support, and support staff sufficiently trained to recognize fish behaviour and external lesions in order to detect early changes in the health status of a fish population. This may help to prevent the spread of disease-causing microorganisms and to minimize the impact of disease on other fish groups.

A monitoring programme for both routine and emergency mortality situations will indicate changes in population health and health risks rather than simply managing numbers of dead fish. A culture facility may find it beneficial to budget for routine collections of representative fresh dead and moribund fish for in-house Level 1 diagnostics training (basic visual) as well as Level 2 (laboratory) readiness. The aim is to increase in-house databases and awareness of local indigenous diseases.

Useful basic equipment includes: a compound microscope, a few select stains, a sink and dissection counter, and a refrigerator to facilitate some primary investigations and marine algal monitoring.

B.1 Basic attributes of a monitoring programme:

- i. **Awareness of potential diseases and risks to health:** Training and reference materials are helpful to first learn what potential troubles may arise.
- ii. **Diagnostic support:** Training, resource personnel (biologists, veterinarians) and lab tests are required for prompt action.
- iii. **Routine observation of carcasses, slow-swimmers and living behaviour:** Fish need to be watched routinely in order to detect subtle variations in activity, appearances, feed responses, and minor lesions. Each dive and feeding event is an ideal opportunity for collection and assessment of fish by both divers and farm staff trained in basic fish health.
- iv. **Sampling when mortality rates increase:** Monitoring and laboratory efforts should increase if staff observations reveal higher than usual mortality or morbidity. Basic, visual diagnoses should perhaps advance toward a greater level of confirmation by trained lab personnel.
- v. **Sampling during an outbreak:** All fish on site, visibly affected or not, now require intensified observation and dive sampling perhaps daily or everyother-day, or by deploying carcass collection rings within each net pen to facilitate daily carcass removal and minimizing pathogen concentrations.

Principle #2. Identifying and managing risks to fish health.

- Part A: Water Quality
- Part B: Vaccination
- Part C: Segregation & fallowing
- Part D: Stress & handling
- Part E: Broodstock

Part A. Water Quality

Operators must maintain water quality and quantity to support the needs of the fish. The water should minimize the fish's exposure to disease-causing organisms and not increase the fish's stress or susceptibility to disease. Water quality assurance obviously requires different approaches in sea pens versus land tanks supported by mechanical pumps and filters. Regardless, operators should have a routine procedure of monitoring water quality and measuring facility-specific quality thresholds. The operators should also have a tangible and local response plan that can be enacted when incidents of poor water quality are detected.

A.1 Basic considerations:

- i. **Stocking densities:** This will vary depending on age, size, species, health and water source but, in general, 10-20 kg biomass per cubic meter is quite typical within fish culture units.
- ii. **Water source:** Water exchange, removal of metabolic products, recirculation, filtration versus flow-through volumes, water sources free of human and industrial pollutants, and low pathogen contamination, temperature and oxygen capacity are each important factors to consider with regard to risks to fish health.
- iii. **Measurements and equipment:** Basic electronic equipment and chemical test kits are required to regularly monitor parameters of water quality.
- iv. Action plans: If adverse conditions arise, plans must be in place to supplement water, change water sources, alter flow, or move fish to safe areas.
- v. **Factors during transport of fish:** Water conditioning (i.e. supplemental oxygen) in a closed system is required and care must be taken to minimize stress, time, metabolic accumulation and the transfer of pathogens from the origin.

Principle #2. Identifying and managing risks to fish health, continued:

Part B: Vaccination

Immersion and injectable vaccines are common measures of prevention for specific diseases within many countries where finfish industries are of substantial size, yet face significant fish mortality due to disease. In the absence of disease losses, vaccination is sometimes viewed as an unnecessary cost-of-production, but operators are encouraged to remember that preventative approaches are the key to continued fish health.

Unlike antibiotics, vaccines are designed to prevent (or minimize) the onset of specific diseases by stimulating a fish to create its own protective immunity. Neither vaccines nor antibiotics should be relied upon to substitute other important aspects of fish health management. Unfortunately, vaccine suppliers (and regulators) may not be enthused to facilitate the research and development of these biological products for South Australia's aquaculture industry until the needs and economics of

the aquaculture industry become more apparent. Various bath, injection and in-feed vaccines for warm-water fish are commercially available now in other countries.

Principle #2. Identifying and managing risks to fish health, continued:

Part C: Segregation & fallowing

Whenever possible it is best to create a physical or at least a functional separation of year-classes of fish, especially newly arrived fish from existing fish groups. All-in and all-out production models are ideal to minimize the transfer of microorganisms but this schedule is difficult to achieve in aquaculture because fish growth cycles extend past 12 months. At sea sites, new fry from the hatchery should be afforded fresh net pens located away from all other fish, and brood pens should also be segregated from normal production fish. Designating specific staff and equipment to juvenile cages or sites, and restricting visitation to those cages may also help to minimize the flow of pathogens from production populations to the immunologically naïve juvenile fish.

Perhaps one of the most significant and effective plans to minimize the transfer of disease-causing organisms from one out-going group of fish to the next incoming generation of fish is to leave the growing area completely empty (and dry if possible). This is known as fallowing, or a state of idle activity. Fallowing is applicable to land-based culture facilities, individual holding units, and net pen sites. At semi-closed facilities, fallowing helps to disrupt pathogen life cycles and allows the natural local flora and fauna of the environment to return to its historical natural state. Each production unit should remain devoid of fish for as long as possible between production cycles; four to six months, as a minimum, is quite typical. Of course, additional approved empty marine leases are required to best facilitate long-term fallowing, yet continued fish production. Thorough scrubbing, scraping, tidying, sanitizing and drying of all residual organic material is recommended in attempt to break life cycles of pathogens and disease-harbouring hosts.

Principle #2. Identifying and managing risks to fish health, continued:

Part D: Stress & handling

It is well accepted that stress has a profound and measurable effect in terms of suppressing the immune system of animals, including fish. The transition from a quiescent, non-clinical infection to overt disease and mortality very frequently occurs 3 to 10 days following a stressful event and prolonged stress can significantly reduce growth and productivity. Stressors appear in many forms, so providing fish with suitable rearing environments, adequate nutrition, minimal crowding, gentle handling, smooth equipment surfaces, protection from predators, etc. all help to minimize physiological changes (stress) and reduce fish's predisposition to infection and disease. Largely, fish culturists address animal welfare topics here, and farmers are well aware of the health (and marketing) advantages of raising animals under optimal environments and practices.

D.1 Basic approaches to minimizing stress:

i. **Housing, rearing and feeding:** Behavioural and physiological needs (space, food and protection) to minimize stress is the goal. Considerations such as: water quality, moderate density, adequate lighting, waste removal, are requirements within a safe, secure and non-damaging holding unit. Each factor should be measured, monitored and assessed regularly.

- ii. **Observations and handling guidelines:** Watch fish behaviour as often as possible, especially before and after handling procedures. All equipment designed to contact fish should have smooth, rounded, wet surfaces. Avoid holding fish by the tail or gills; rather provide support to the body. Maximize oxygen, minimize crowding and crushing, and locate containers adjacent to one another to avoid "walking" fish to new destinations. When transporting fish from one facility to another, plan for road or vehicle delays (flat tires, mechanical break downs, road detours). A contingency plan should be established and include things, such as: communication, additional ice, on-call back-up vehicles, etc.
- iii. **Anaesthesia:** Physiological stress is greatly reduced by sedating and anaesthetising fish for measurement or invasive procedures. Use licensed, labelled, prescription anaesthetic products and create a checklist of necessary equipment for the handling procedure. Use clean, smooth containers, clean water as well as supplemental oxygen whenever possible. Balance the depth of anaesthesia with the risk of overdose, drug residues and the optimum level of stress relief.
- iv. **Scavengers and predators:** It is extremely difficult to stop predator interest and attacks on groups of fish. The predator is acting naturally, yet it is incumbent upon operators to inspect units, detect predator-fish interactions, to reduce predator opportunities and interests as much as possible, and to constantly improve protection of livestock using scare tactics and physical barriers. Remove and dispose of dead fish as frequently as possible to avoid attracting scavengers to the rearing area.

Principle #2. Identifying and managing risks to fish health, continued:

Part E: Broodstock

Unless one is "ranching" wild fish (capturing wild fish for containment and growth), the propagation of fish generations arises from captured or cultured maturing adult fish. The adults are held in safe, secure rearing tanks to eventually release and fertilize gametes once the broodfish experiences favourable, specific environmental and physiological conditions.

E.1 General considerations:

- i. **Selection of brood:** Although South Australia permits may facilitate the intermittent capture of wild yellowtail kingfish (to enhance genetic variability, etc.), the capture of wild fish is difficult and traumatic. This technique is likely to be replaced by the selection of cultured broodfish expressing desirable genetic traits. Handling should be as gentle and infrequent as possible, and sedation or anaesthesia techniques should be implemented and are most beneficial.
- ii. **Containment and transport of brood:** Water conditioning (i.e. oxygen and anaesthesia) in a closed system is required and care must be taken to minimize stress, trauma, time, and accumulation of metabolic wastes.
- iii. **Identification:** A safe and innocuous labelling or branding programme to identify individuals or family groups of broodfish is important for breeding advances. Many safe, effective and hygienic options are known. Anaesthesia is essential for all handling activities.
- iv. **Housing and feeding:** Clean water, daily use of clean cooked feed, variable lighting and temperature regimes must be planned to manipulate breeding activity yet still provide a high level of animal welfare.

- v. **Hygiene and bio-security:** The creation of a stable biological environment is helpful. Limit attending personnel, quarantine and condition any new fish before adding to groups, develop protocols of disinfection and visitation, and try to limit (or replace) raw food diets. Uncooked food may contain pathogens.
- vi. **Pathogen screening of gametes:** Laboratory tests to detect diseasecausing microorganisms (especially vertically transmissible agents) should be developed. A plan should also be enacted if pathogens are ever detected. To emphasize prevention and due diligence it may be helpful to schedule a complete diagnostic screening of a rejected or dying brood fish. PIRSA and/or the diagnostic laboratory should be consulted first to determine which samples they recommend, and which tests can be coordinated or contracted (i.e. virology, histology of all tissues, PCR primer availability for certain pathogens). In the future perhaps a single blood sample from broodfish will be sufficient to screen for the main infections of concern.
- vii. **Action plans:** If adverse conditions (water quality or disease) arise within the brood holding unit, action must occur to supplement water, change/filter the water supply, alter flow rates, or segregate fish. Spare tanks, pens and equipment should be available for health emergencies.

Principle #3. Reducing exposure to and spread of pathogens.

- Part A: Outbreak investigation
- Part B: Isolation "sick" units
- Part C: Managing dead fish
- Part D: Contract divers
- Part E: Disinfection, equipment & visitors

Part A: Outbreak investigation

Operators should have a plan to detect and manage outbreaks (epizootics) of infectious and non-infectious disease, and they need access to the resources, equipment and skills required to contain problems in a timely fashion. Immediate diagnostic support from labs, State fish health professionals and trained personnel is essential.

A.1 Basic attributes of investigations:

- i. **Personnel:** Fish health staff and/or a consulting veterinarian should coordinate the activities and bio-security/containment of other farm staff. Once an outbreak is suspected, farming activities must accentuate and emphasize hygiene, isolation and quarantine (more than the daily routine) until a diagnosis is reached. Divers, operators, fish health staff, and veterinarians should all be contacted immediately. The veterinarian is responsible for obtaining a diagnosis and helping to develop interim and final outbreak management plans.
- ii. **Records and assessment:** Past and present mortality rates, patterns of diagnoses, stress events and water quality must be easily accessible and assessed from existing and up-to-date data. A relationship with a veterinarian and/or fish health professionals with knowledge of disease patterns in aquaculture is helpful. An "outbreak case history" should be generated with information reflecting: management history, fish age, entry dates, source, vaccination, movements, treatments, past samples and diagnostics results (from labs or in-house), water quality parameters, feed history, previous month mortality rates, fish behaviour, and clinical symptoms.
- iii. **Diagnosis and notification:** Health coordinators within PIRSA should be informed of farm and lab investigation activity, and be invited to make further recommendations. Neighbouring farms will also appreciate advanced direct communication of this nature. Representative numbers of moribund and dead fish should be examined by a trained fish health professional, and samples should be prepared and delivered to a diagnostic lab. Collect, store and label samples of current food and water used by affected (vs. unaffected) fish.
- iv. **Surveillance of disease distribution:** While enacting strict disinfection and bio-security diligence (see Principle #3E), epidemiologically-linked groups of fish should also undergo surveillance and examination for similar behavioural and disease problems. Designated fish health staff should perform surveillance activity. Other staff movements should be restricted.
- v. **Restrictions and action:** Once an outbreak is suspected, operators should halt movement of live affected fish and epidemiologically-linked fish until a diagnosis is achieved. The moribund and dead fish however need to be carefully removed (see Principle #3C) in order to reduce the environmental load of disease-causing microorganisms, decomposing organic material, and fish carcasses that will attract predators.

Principle #3. Reducing exposure to and spread of pathogens, continued:

Part B: Isolation "sick" units

Unlike land-based agricultural farms, aquaculture sometimes faces difficulty in achieving physical and functional isolation of cultured and wild populations; particularly with respect to semi-open net pen facilities where water and organic material is shared amongst wild and cultured fish alike. So the concept of aquaculture "sick pens" is not quite equivalent to cattle feedlot "sick pens". Decisions to move or "isolate" a group of sick fish should reflect the diagnosis, the biology and significance of the pathogen, the stage of infection, state of affected fish, and the potential effect (if any) on wild fish in the newly proposed holding area. The pros and cons of moving fish need to be weighed in each case. For non-infectious insults (i.e. blooms, toxins, cycling parasites) fish movement is often most appropriate.

It is reasonable to have additional land-based fish holding units and perhaps marine leases that, with special permission, allow an operator the flexibility to deal with unique groups of brood or production fish. Additional holding units and empty leases must have a water supply that is different than that of other fish and must define both a physical and functional isolation of fish placed there. The goal would be to minimise the risk of shedding and transfer of disease-causing microorganisms from sick populations to surrounding unaffected groups of fish, but whether this is timely and feasible depends on many environmental, management and regulatory factors.

Principle #3. Reducing exposure to and spread of pathogen, continued:

Part C: Managing dead fish

Containment and hygiene are key components of the high-risk activity of carcass collection and disposal. Moving dead fish from holding units in a manner that will not spread infectious disease-causing microorganisms is difficult and this activity requires extreme diligence. The frequency of removal of carcasses should depend on the current degree of normal background mortality, yet not be so infrequent as to miss the early recognition of elevating mortality rates or outbreaks. In general the removal of carcasses at least once per week is considered a minimum, and a sufficient number of leak-proof containers with lids is essential to accommodate both normal and also excessive numbers of carcasses should diseases arise.

Carcass buckets and transport vehicles (i.e. diver boats) can be the most highly contaminated source of organic material and pathogens. Raingear, hands and the boat deck should also be sprayed down due to the extensive splashing by sampled fish. Vehicles should be spray-disinfected and scrubbed after carcass collections, and containers should be soaked in bleach totes and scrubbed with bleach at the dumping or storage station. In the event of elevated mortality rates (for example, if thousands of fish die overnight due to algal blooms) there should be sufficient lifting equipment (cranes) and additional transport capacity to accommodate the removal of carcasses. A contingency plan for special, massive dumping will likely involve the regulatory approval dumping permits and this approval process should be discussed and developed well in advance of the future event.

Principle #3. Reducing exposure to and spread of pathogens, continued:

Part D: Contract divers

By nature of the job, contract dive teams and their equipment inherently represent one of the highest risks with respect to bio-containment of disease-causing microorganisms and the bio-security of fish populations. Diligent hygiene and disinfection is extremely important. Below are some safeguards to consider emphasizing during carcass retrieval routines of dive teams:

D.1 General considerations:

- i. **Establish a routine dive sequence** that begins with the youngest and healthiest fish groups, then move to the yearling and then broodstock. The concept is a "clean-first to dirty-last" flow of diving; from the fish most healthy to those fish more likely to be silent carriers of pathogens.
- ii. **Sick pens last:** the highest priority is to dive the problem or "sick" pens LAST (regardless of age-class).
- iii. **Disinfection:** find a diver- and gear-friendly disinfectant to sanitize boat decks, dive equipment, hands, feet, raingear, etc. after carcasses collections from each pen.
- iv. **Bleach totes:** use a 500ppm bleach container that is of sufficient size to soak two (2) carcass-collecting sacks or rings that are used in alternating fashion from pen to pen.
- v. **Carcass containers:** locate watertight carcass containers at the rear of the dive boat or away from foot traffic areas. The goal is to transfer dead fish from the pen, directly into containers (with minimal drips), then place collection sacks/rings directly into an adjacent bleach container. This area of the boat would be considered the "dirty zone", whereas the diver would re-enter the boat at a distant "clean zone" of the boat deck.
- vi. **Excess carcasses:** Under no circumstances, including outbreak scenarios, should an operator or diver allow dead fish to over-flow from buckets onto the boat deck or ground. A plan needs to be in place to contain and accommodate all dead fish, even if the diving must be terminated to coordinate and collect more containers.
- vii. **Educate and train divers** (and all staff) about identifying diseases, fish symptoms, transference of microorganisms, and the importance of bio-security.

It is helpful if the dive team is adequately trained in disease recognition to categorize carcasses as fish are counted. Useful basic carcass descriptions (external lesions) may include: old, normal silver, predation, deformed head/jaw, deformed spine, post-handling damage, non-performer, skin lesions, other external lesions of concern, carcass sampled, etc.

Principle #3. Reducing exposure to and spread of pathogens, continued:

Part E: Disinfection, equipment & visitors

Staff moving between live fish-holding units should ensure they are not transporting potentially infectious material on their footwear, clothes, hands, dip nets, equipment or vessels. Hygiene, disinfection and bio-security standard operating procedures (SOPs) are helpful to develop and implement BEFORE disease problems arise.

Rubber foot and hand baths, and/or garden sprayers containing disinfectant, should be considered in numerous locations, including boats. Dipping boots and spraying raingear should be the first and last thing that a staff member does at boats, docks and thresholds. Fluke sampling totes, hooks, hand-lines, and anaesthetic water should also be bleached and rinsed well before the staff and gear leave the sampled pen.

FRDC Project 2003/216

Within land-based hatchery facilities, fish larvae are quite susceptible to infections prior to the development of their immune systems. Live-food sources at hatcheries create additional complexities in terms of hygiene and inadvertent contamination of fish populations. The accumulation, multiplication and transmission of potential pathogens within rearing tanks is commonplace, so sanitization and bio-security SOPs may be most relevant BEFORE problems arise.

E.1 General considerations:

- i. numerous foot baths (i.e. at each "critical control location" or passage way),
- ii. numerous hand wash stations,
- iii. numerous disinfection sprayers (i.e. one per room or per boat, per dock),
- iv. restricted (or designated) staffing work areas (i.e. functional quarantine zones
 no movement from brood areas to larvae areas, etc), or the use of colourcoded boots, or one-way traffic flow between designated zones, etc.,
- v. finding cleaner food sources for larvae and/or monitoring bacteria loads twice daily.

Principle #4. Adequate nutrition and feed management.

- Part A: Clean cooked feed
- Part B: Meat Quality
- Part C: Transport & storage of feed
- Part D: Seasonal physiology & digestibility

Part A: Clean cooked feed

Nutritional, pathogen-free, cooked food from a reputable supplier that meets good manufacturing practice requirements must be transported and stored in a manner to avoid ingredient tampering and degradation. Feed of this nature is usually nutrient dense, highly palatable and digestible. In addition, cooked feed helps to maintain good quality rearing environments by introducing minimal particulate matter, limited organic loading, and low environmental oxygen demands.

It is generally accepted that raw, uncooked, frozen or freeze-dried feeds will contain numerous microorganisms, a number of which may create challenges for the immune systems of the consuming fish. Introducing uncooked feed to cultured fish (and to the rearing environment) is a considerable fish health risk that is not necessary.

A routine protocol to monitor the bacterial identification and counts of larvae and rearing pools should be implemented as an in-house and an external lab event, particularly when culturing and applying live food (algae, artemia, rotifers) within larval hatcheries. It may be important to document the normal flora of food cultures in order to best investigate and develop probiotic applications to the feed and/or pools. Chemical conditioning of live feed sources to minimize or control bacterial overgrowth is a management technique applied in some countries to prevent or control disease outbreaks. Safe, "organic" sanitizers are commercially available in addition to prescription antibacterial products.

Principle #4. Adequate nutrition and feed management, continued:

Part B: Meat quality

As with all farmed animals, fish are completely dependent upon the human operators to provide good nutritious food in adequate amounts and with regular frequency in order to sustain health and minimize stress. The main source of widespread global chemicals, such as PCBs and dioxins, appears to be from basic feed ingredients (i.e. fish meal, fish oils, vegetable oils, etc.). The farmer must interact with the supplier of feeds to ensure that fish will remain wholesome and safe for consumers of seafood.

For pharmaceutical issues, farmers and veterinarians take great pride in ensuring that any medicinal products used to control fish disease are adequately metabolized to meet all regulatory and food safety standards.

Gall bladder staining has been a common harvest-related problem in salmonid aquaculture, and may very well arise as an issue for yellowtail kingfish farmers as well. As food is withheld before harvest, food stops flowing through the gut of fish, the gall bladder stops releasing its stored biliary fluids so the salts concentrate in the gall bladder. The bladder becomes dark green and the adjacent belly wall may become discoloured, thereby reducing the quality and price of the processed fish. Optimum food clearing periods immediately prior to harvest should perhaps be investigated (using yearling fish, and at various water temperatures) to test the hypothesis of "gall bladder staining" amongst yellowtail kingfish.

Principle #4. Adequate nutrition and feed management, continued:

Part C: Transport & storage of feed

The transportation and storage of a perishable oily product has its challenges, especially in hot climates. Fatty acids can oxidize and become rancid, and antioxidant ingredients within the feed (selenium, vitamin E, vitamin C) may become depleted before fish have opportunity to eat the feed. The inevitable exposure of fish feeds to heat, sunshine and humidity suggests a need (and benefit) in freezing and labelling small aliquots of each feed order, in the event that subsequent rancidity and vitamin analyses are required. Frozen fish tissues can also be informative in terms of diagnosing long-term nutritional deficiencies and oxidative reactions. Alternatively, the use of temperature-regulated transport containers (or refrigeration units) for dry feed shipments would certainly address the maintenance of quality of the perishable feed product.

If anti-oxidant insufficiencies do arise in fish feeds, a large top-coating (on-site) mixing drum may become a useful piece of standard equipment to fish culturists. If properly designed the drum may be an efficient way to add supplemental vitamin premixes or medications, oils, probiotics, etc. to the surface of feed pellets. Top-coating is a common practice at feed mills of other countries, particularly since some antibiotics and vitamin premixes cannot withstand heat and steam extrusion or hot, humid storage.

Principle #4. Adequate nutrition and feed management, continued:

Part D: Seasonal physiology & digestibility

The food should be regularly and adequately available to all fish to elicit normal behaviour and physiological function. In most cases growth and optimal feed conversion into flesh are the goals. Both excessive feeding and inadequate feeding are unacceptable farming practices. A fish's digestive and metabolic rate is highly dependent upon water temperatures, so as seasons change often feed rates and feed ingredients also need adjustment to address such thing as: physiological needs, feed competition, fish stress, body weight and fat distribution, even bacterial reproduction rates in rearing environments.

Physiological syndromes, such as Green Liver Syndrome and Winter Syndrome, are likely examples of conditions that may very well be avoided by fine-tuning feed types and feed management at specific times of the year. Both vegetable and fish protein source are desirable ingredients of fish feed yet each has its benefit and detriment.

Juvenile fish should be afforded all possible nutritional benefits by offering them the best quality diet formulation in amounts adequate to maximize their health, immunity and early growth. Broodstock also have specific nutritional needs. To the cultured brood, extruded brood diets (fortified with various vitamins and nutrients) should likely be offered for the 8-12 months prior to planned spawning periods. The wild brood should also be fed a fortified diet. Semi-moist feed mixture is likely useful however, perhaps a nutrient dense extruded pellet could be added to the mixture to help supplement the brood fish with concentrated vitamins and essential fatty acids. Egg and sperm development, fecundity, and fertilization are all likely to be enhanced through optimal nutrition and low stress. The frozen/raw components of the current

FRDC Project 2003/216

brood diets (pilchard and squid, etc.), although "feed grade" quality, should perhaps be analysed routinely for viable fish pathogens, and specifically pathogens known to cause disease in yellowtail kingfish. High-temperature cooking (i.e. extruded pellets) generally kills most fish pathogens whereas freezing and thawing raw natural seafood may not kill all disease-causing microorganisms. Principle #5. Judicious use of drugs in cultured fish.

- Part A: Diagnostic support
- Part B: Prescription, records & food safety
- Part C: Tools of treatment & prevention of disease
- Part D: Feed rates & conditioning hatchery live-food
- Part E: Fish welfare

Part A: Diagnostic support

Disease treatments using drugs or chemicals must be based on a reasonable and professional diagnosis. That is, it is imperative that the cause of disease is first determined in order to initiate an adequate control measure, specifically a measure involving a drug treatment. The type of pathogen found, its biology and epidemiology will guide treatment options, if indeed the microorganism is treatable by chemicals at all.

For problematic bacteria, the minimal diagnostic requirements are confirmation of visual lesions, bacterial culture and isolation, followed by an antibiotic sensitivity test. These tests are all quite immediate approaches and possible at in-house or on-site facilities if fish health staff is sufficiently trained. Other disease-causing microorganisms may require more extensive diagnostic activity at Level II or III lab facilities. A veterinarian familiar with the fish population should always be consulted to interpret diagnostic finding and before drugs, chemicals or biological agents are considered for control.

Principle #5. Judicious use of drugs, continued:

Part B: Prescription records & food safety

Veterinarians are well trained in pharmacology and the pharmacokinetics of drugs used in animals. Upon prescribing a chemotherapeutic product, the veterinarian assumes the responsibility for drug use and safety within fish, provided the aquaculture operator complies with all details of the prescription and drug application. By law, both the prescribing veterinarian and the aquaculture operator are required to keep accurate documentation and treatment records that can be retrieved (if need be) for a period of years after a treatment. Records of this type comply with regulations and standards related to HACCP (Hazard Analysis and Critical Control Points) as well as issues surrounding product traceability.

The use of drugs must conform to the drug manufacturer's recommendations and existing regulations concerning occupational safety, public health, environmental impacts and consumer food safety. All food animals, including fish, must be free of measurable drug or chemical residues prior to harvest to ensure product wholesomeness and consumer safety. Prescriptions assign the required residue withdrawal time for the drug to clear the fish's tissues and aquaculture operators must confer with food processors to double-check all harvest, withdrawal dates and quality assurance standards.

Principle #5. Judicious use of drugs, continued:

Part C: Tools of treatment & prevention of disease

Specific antibiotics should be considered, investigated, pre-approved and secured for emergency drug application in South Australia over the next few years. The risk analysis of diseases likely to afflict yellowtail kingfish (FRDC project 2003/216) anticipates a number of bacterial diseases to arise in the future, so it is advisable to make administrative preparations sooner than later. The antibiotic premixes of choice (and those likely to be HACCP-friendly globally) are: 40-60% oxytetracycline, 40-60% erythromycin, 30-40% potentiated sulfonamide(s) like Romet 30 or Tribrissen 40, amoxicillin, and florfenicol. An Australian list of licensed animal drugs is published for medical practitioners, pharmacists and veterinarians and these drugs can become available under special permission and prescription if emergencies arise. Other anti-parasitic agents, such as praziquantil and hydrogen peroxide will also be useful products for application under integrated pest management approaches.

It may also be powerful and efficient to lobby the need for vaccine research and development in yellowtail kingfish. A number of useful vaccines already exist in other countries and attention to vaccines will emphasize aquaculture's focus on *preventative medicine and continued health,* as opposed to awaiting the control of disease by *mass medication*.

C.1 General considerations:

- i. **Regulatory agencies** must be consulted to discuss any concern regarding potential transient environmental impacts of chemicals and drugs.
- ii. **Shipment, handling and storage:** the shipment, storage, labelling and topcoating application of all drugs and medicated feeds must comply with all regulatory requirements. These products should be stored in a location that is protected from sunshine, excess heat and humidity, and stored in a place prevents tampering or contamination by other chemicals or pests.
- iii. **Measures:** accurate weigh scales (one measuring grams, one for kilograms) plus volumetric equipment (for powders, liquids and feeds) are essential at farm sites in order to measure, dispense and top-coat medicated products. Designated staff should be trained for these specific measuring and mixing events. Other essential equipment includes protective health and safety clothing (goggles, mask, gloves) as well as designated mixing utensils and clean-up facilities.
- iv. **Bathing and dipping:** bathing fish with chemicals or drugs (i.e. to remove external parasites or to sanitize semi-closed environments (i.e. pools) due to heavy burdens of bacteria), can be both traumatic and stressful to fish due to physical manipulation of holding units and crowding abrasions. Great care and compassion is required from staff to conduct this type of fish medication, and regulatory guidelines needs to be strictly adhered to given that local environments may be transiently affected.

Principle #5. Judicious use of drugs, continued:

Part D: Feed rates & conditioning hatchery live-food

Medicated feeds likely taste different than non-medicated feeds, so in one's decision to orally medicate a group of fish it is important to anticipate that the normal daily feed rate will probably decline. This aspect of treating fish (particularly sick fish whose feed rates may have declined from normal already) must be addressed in order to assure the following:

- the complete consumption of a therapeutic target dose each day,
- an appropriate treatment duration, and
- minimal loss of medicated pellets to the environment.

In other words, a sufficient maximum number of medicated pellets must be presented to a sick population of fish to ensure non-competitive consumption of the feed by all fish, yet not so much feed as to satiate the fish and risk the loss of expensive, uneaten medicated pellets dropping to the bottom of the fish holding unit. In addition, if the number of pellets is insufficient we run the risk of over-dosing the most aggressive fish while under-dosing the subordinate; those that are likely in most need of the medication. Veterinarians are trained to assist fish health staff and feed manufacturers in calculating accurate and appropriate target doses.

With regard to hatchery pools and larval rearing situations, routine feed bacteriology should be an in-house or external lab event when culturing live food such as algae, artemia, and rotifers. It will be important to document the normal flora of food cultures in order to best investigate and develop probiotic applications to the feed and/or pools. Conditioning of live feed sources using drugs and/or safe sanitizers, to minimize or control bacterial overgrowth, is sometimes an efficient and effective way to prevent bacterial overgrowth of larvae and pools environments. This prophylactic conditioning of larval and live-food tank may be necessary at critical times of larvae (and food) development. Again, it must be emphasized that routine bacteriology and monitoring of the target fish and rearing water, both before and after bath treatments, will be useful in making evidence-based decisions.

Principle #5. Judicious use of drugs, continued:

Part E: Fish welfare

The majority of the guidelines written above address and emphasize key aspects of animal welfare. Various perspectives of fish welfare continue to develop and the entire topic remains widely investigated and debated (Chandroo et al., 2004a, 2004b). The recently adopted OIE guiding principles for animal welfare (May 2004, Final minutes of the International Committee meeting) are likely to be reflected within finalized OIE Codes (OIE, 2004). Regardless, one largely accepted perspective suggests that cultured fish should be raised in a manner to ensure their freedom from:

- hunger and thirst,
- discomfort,
- fear and distress, yet also enable
- freedom of expression (i.e. conduct natural behaviours), and
- freedom from pain, injury or disease.

With specific regard to freedom from disease, humans rearing animals face a dilemma related to the controversial topic of therapeutic mass medications, particularly in the event of higher-than-normal morbidity and mortality rates amongst

FRDC Project 2003/216

the cultured animals. It is incumbent upon the fish culturist to attempt to alleviate discomfort and disease by some means of management and husbandry, and it is at this point that the application of drugs must become a serious and viable consideration, even if the animals are being raised according to an "organic production" framework.

Operators are encouraged to use alternative methods to reduce the need for drugs or chemicals. These methods include: vaccination, use of pathogen-free water in hatcheries, broodstock screening, egg disinfection, low stress and limited stocking densities. It is understood and accepted by most that the use of drugs and chemicals to prevent disease in healthy populations should be avoided whenever possible. That said, certain disease-causing microorganisms and population infections are quite seasonal, historic and predictable in which case the prophylactic or early use of drugs (to minimize hidden infections and discomfort) can be an effective, preventative, humane approach; an action highly supportive of animal welfare.

SUB-APPENDIX A-GFH: AUSTRALIAN NATIONAL LIST OF REPORTABLE DISEASES OF AQUATIC ANIMALS (FINFISH), 2003

(www.affa.gov.au and www.oie.int)

Listed in the OIE Aquatic An. H. Code	Listed regionally (OIE/NACA)	Exotic to Australia
Yes	Yes	No
Yes	Yes	Yes
Yes	Yes	No
Yes	Yes	Yes
Yes	Yes	Yes
Yes	Yes	No
Yes	Yes	Yes
No	No	Yes
No	No	No
No	No	Yes
No	No	Yes
No	Yes	Yes
	OIE Aquatic An. H. Code Yes Yes Yes Yes Yes Yes Yes Yes Yes Ye	OIE Aquatic An. H. Coderegionally (OIE/NACA)YesNoNoNoNoNoNoNoNoNoNoNoNo

SUB-APPENDIX B-GFH: LEGISLATION RELATED TO FISH HEALTH

(www.austlii.edu.au/form/search/)

Commonwealth

Agriculture and Veterinary Chemicals Act, 1994 and Regulations, 1995 Environment Protection and Biodiversity Conservation Act, 1999 Freedom of Information Act, 1982 Network of Aquaculture Centres in Asia and the Pacific Regulations, 1998 Privacy Act, 1988 and Privacy (Private Sector) Regulations, 2001 Protection of the Sea (Powers of Intervention) Act, 1981 Quarantine Act, 1903

State

Agricultural and Veterinary Products Act, 2002 Aquaculture Act, 2001 Controlled Substances (Pesticide) Regulations, 1988 Development Regulations, 1993 Environment Protection Act, 1993 and Regulations, 1994 and Water Quality Policy, 2003 Fisheries Act, 1982 Fisheries (Exotic Fish, Fish Farming and Fish Disease) Regulations, 2000 Freedom of Information Act, 1991 Land Not Within a Council Area (Coastal Waters) Development Plan, Livestock Act, 1997 Pharmacists Act, 1991 Veterinary Surgeons Act, 1985

Policy & Papers

Aquaculture Chemical Use Policy Paper, PIRSA 2001 Aquatic Animal Health Paper, PIRSA 2001 Draft Code of Best Management Practices, SA Marine Finfish Farming Association 2004 State Planning Strategy,

International publications

ICES, Code of Practice for Introduction and Transfer of Marine Organisms CITES (for culture and sale of protected listed species) OIE, International Aquatic Animal Health Code for Finfish, Molluscs & Crustacea, 2004

Appendi	x 6: Parasites id	ientinea in	Seriora	iaiaiiui		
Parasite	Parasite Genus	Parasite	Host	Host	Locality	Noted
Group		Species	Genus	Species	-	Pathogen
Copepoda	Caligus	aesopus	Seriola	lalandi	New Zealand	Unknown
	C.	lalandi	Seriola	lalandi	New Zealand	Unknown
	C.	spinosus	Seriola	lalandi	Japan	Yes
	Lernanthropus	sp.	Seriola	lalandi	New Zealand	Unknown
	Neobrachiella	sp.	Seriola	lalandi	New Zealand	Unknown
Digenea	Bucephalopsis	elongates	Seriola	lalandi	Japan	No
Digenea	B.	elongates	Seriola	lalandi	Japan	No
	Bucephalus	ololigatoo	Conola	lalandi	USA	No
	B.	introversus		lalandi	Columbia & Mexico	No
	Dinurus	coryphaenae	Seriola	lalandi	USA	No
	Distomum	hispidum	Seriola	lalandi	USA	No
	D.	monticellii	Seriola	lalandi	USA	No
	D.	sp.		lalandi	USA	No
	Ectenurus	trachuri	Seriola	lalandi	Qld-GBR Heron Is.	No
	Erilepturus	hamati	Seriola	lalandi	Japan	No
	Gasterostomum	sp.	Seriola	lalandi	USA	No
	Lecithaster	stellatus	Seriola	lalandi	Qld-GBR Heron Is.	No
	Lecithochirium	magnaporum	Seriola	lalandi	China -Fujian Province	No
		magnaporum	Seriola	lalandi	Galapagos Islands	No
	Nannoenterum	gorgon	Seriola	lalandi	USA	No
	Paradeontacylix	sanguinico- loides		lalandi	Atlantic Ocean - Miami, Florida	No
	Prosorhynchus	facilis		lalandi	Japan	No
	Stephanostomum	filiforme	Seriola	lalandi	UŚA	No
	S.	hispidum	Seriola	lalandi	British W. Indies, Mexico & Panama	No
	Tormopsolus	orientalis	Seriola	lalandi	British W. Indies & Japan	No
Monogenea	Benedenia	seriolae	Seriola	lalandi	New Zealand	Yes
	Neobenedenia	girellae		lalandi	Japan	Yes
	Paramicrocotyloides	reticularis	Seriola	lalandi	Australia	No
	Zeuxapta	seriolae	Seriola	lalandi	Australia, Japan & New Zealand	Yes

Appendix 6: Parasites identified in Seriola lalandi *

* This list of Copepod and Monogenean parasites is courtesy of Drs. C.Chambers and I.Ernst of the University of Adelaide, Australia (2002). The Digenean list is courtesy of Dr. Tom Cribb of University of Queensland, Australia (2002).

Appendix 7A: Stakeholder feedback Responses from workshop delegates (20 of 35 forms returned):

On July 16, 2004 thirty-five (35) workshop delegates were asked to complete workshop evaluation forms, of which 20 individuals returned the sheet voluntarily. Overall, 70% of the 20 respondents felt all aspects of this workshop were excellent and the other 30% answered that all aspects were good. The overall sentiment (both written and verbal) was that the workshop was very practical, it built knowledge and hands-on skill for use in the aquaculture work environment. Numerous attendees also mentioned that "this type of ongoing training and flow of information was very necessary for our industry".

The questions of the evaluation form are summarised below:

"Were the presentations clear and easy to understand?" 60% answered excellent, 40% good, 0% fair.

"Were the presentations and discussions organised and focused?" 60% answered excellent, 40% good.

"Did the speakers offer challenging new information and insight about fish health?"

75% answered excellent, 25% good.

"Did you find the workshop information interesting and worthwhile?" 90% answered excellent, 10% good.

"Did you find the information relevant and useable for your work and planning?"

60% answered excellent, 40% good, 0% fair.

When asked about speaker knowledge and effectiveness of communication, 58% of the delegates felt the speakers were excellent, 34% felt they were good and 8% fair (but this latter score reflects presenters who were poorly audible as heavy rain fell onto the tin roof of the meeting room). A consistent recommendation was that a microphone and PA speaker system would have been helpful for selected presenters. The Clean Seas facility was effective however because it is centrally located for the industry members and many delegates had not had previous opportunity to visit the Clean Seas aquaculture site. This location and gathering helped delegates to network, break down communication barriers and galvanize cooperative efforts for the future.

Appendix 7B: Stakeholder feedback News release from industry (July 2004, post-workshop)



South Australian Marine Finfish Farmers Association Pty. Ltd. ABN 55 797 303 657

SA Marine Finfish Farmers' Commitment to Fish Health

The SA Marine Finfish Farmer's Association ran an extremely successful Fish Health training workshop for its member companies at Arno Bay on 15 and 16 July 2004.

The training program was delivered by Dr Mark Sheppard, a Canadian vet, who is an internationally recognized authority on the health of Yellowtail (Seriola) Kingfish.

Attendees picked up extensive knowledge and hands on skill from Dr Sheppard and other presenters, and had the chance to network face to face with others from fish farms, vets, lab staff, PIRSA, the Environment Protection Agency and the Universities of Adelaide and Tasmania regarding related projects.

Lyndon Giles, CEO of Southern Star Aquaculture Pty Ltd attended the workshop. He said that "it's a fantastic achievement and puts us ahead of every one else in the industry by establishing a proactive, eco-friendly, risk management approach to farming fish".

Dr. Sheppard commented that the Industry is now coming of age and setting an example of how it should be done.

The Chairman of the Association, Dr Simon Stone said that "This industry has a bright future. Our Strategic Development Plan is based upon marketing the strengths of our product, our industry and our environment and that they are all safe, healthy and sustainable. Fish Health is one of several programs, which will underpin our Plan and provide the proof that customers require. This will then allow us to grow domestic and export markets in a sustainable fashion, matching supply with demand".

In addition, Martin Hernen, the Association's Executive Officer thanked the Australian Government for its financial support in establishing the training workshop through the Fisheries Research and Development Corporation (Project 2003/216) and for assisting the industry to develop its Strategic Research and Development Plan.

(Written and circulated by Executive Officer, Martin Hernen, July 17th, 2004)

Appendix 7C: Stakeholder feedback From the research community



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4th September 2004

Mark Sheppard Sakana Veterinary Services 173 Engles Road, Campbell River British Columbia V9H 1J4 Canada

Dear Mark,

As a member of the Yellowtail Kingfish Research and Development Steering Committee I have been invited to comment on your milestone 4 for FRDC project 2003/216: "Risk analysis of disease hazards – detection and management of yellowtail kingfish (*Seriola lalandi*, YTK) health issues".

I found the report to be well constructed and a pleasure to read. I am sure it will be a useful study for the YTK industry in SA. In particular I am impressed with the practicality of the document. In my opinion it avoids being bogged down in technicalities and it strikes a useful balance between the needs of industry and those of professionals. For professionals it provides an excellent summary of YTK related health literature. I believe this report and other outputs from 2003/216 to be excellent achievements for the SA kingfish industry.

Best regards,

Ingo Ernst

Appendix 7D: Stakeholder feedback – comments during interviews

- keep the project outcomes simple, tangible and user-friendly for the farmers and lab personnel; "what diseases are most likely to arise?", "what are the relative risks?", "what shall we do to reduce those disease risks and/or control the disease, should they arise?", and "what tools do we need to plan for that?".
- reconsider estimates of overall risk for each disease hazard by comparing the Qualitative Risk Analysis Matrix presented in Appendix E, Table E of the Risk Management publication of Standards Australia (Anon., 1999) against the matrix referenced in Aquavetplan (AFFA, 2001; AFFA 2003a). Principal Investigator considered but used the latter.
- consider expanding the preliminary disease hazard list to include all possible and documented parasitic infections of kingfish (including those of incidental academic discovery) perhaps as an appendix format. See Appendix 6.
- diagnostics (lab and vet services) and lack of chemo-therapeutant tools/options remain the key concerns of farmers and support personnel.
- lab personnel appear willing to develop fish diagnostic expertise, at least to Level II diagnostic capability, once the infectious hazard list and diagnostic demand from this project is identified. The lab companies have also expressed an interest in making presentations and assisting at the 2004 training workshop.
- local veterinarians, despite their limited experience with aquaculture, appear willing to learn more and offer as-needed services to the industry when asked to do so.
- the project's disease hazard list and risk analysis will be useful to many groups: kingfish aquaculturists, lab microbiologists, pathologists, new veterinary practitioners, researchers, biological and pharmaceutical companies and regulators.
- plan a 1.5 day kingfish training workshop mid-July in Whyalla that reviews and summarises this project and addresses: kingfish disease risks, control measures, dissection/collection techniques, and presents thorough case scenarios of five diseases of high risk or likelihood.