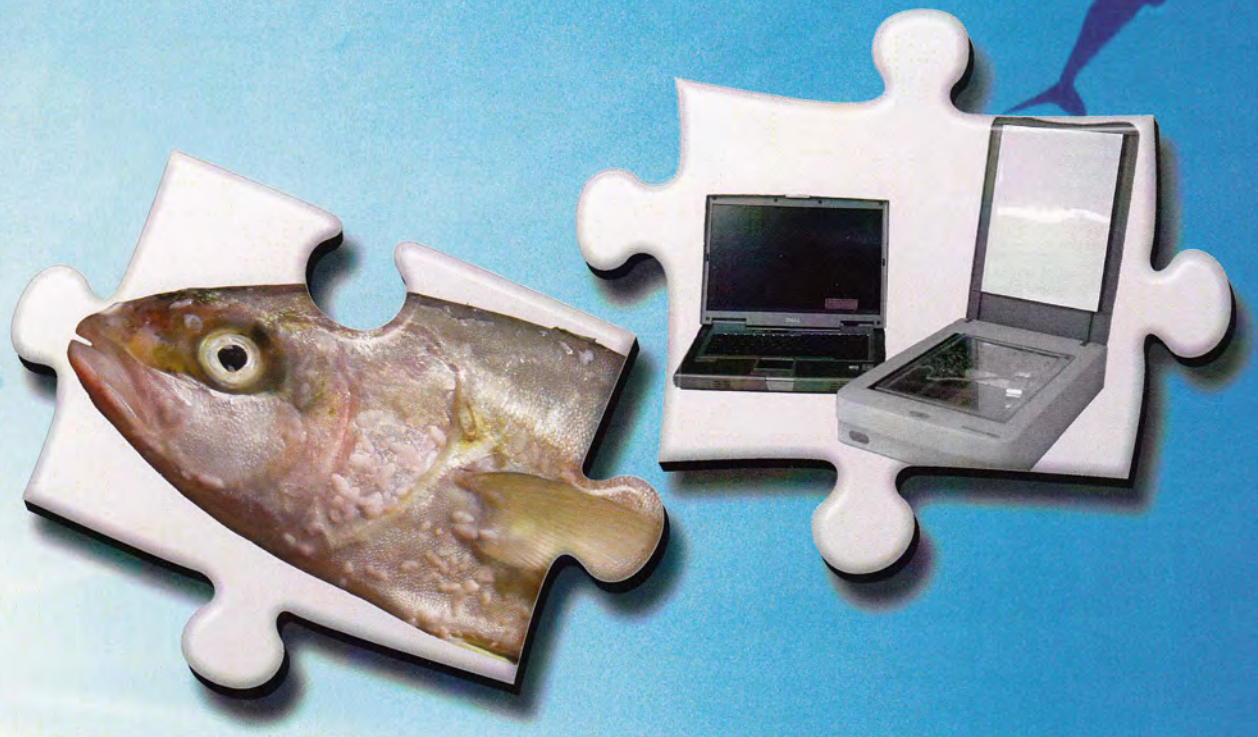
Innovative Solutions for Aquaculture: Assessment of *in situ* monitoring techniques and life history parameters for monogenean skin and gill parasites



Final Report

I.D. Whittington, A.P. Shinn, J.E. Bron & M.R. Deveney

FRDC Project No. 2003/221

July 2011

UNIVERSITY OF STIRLING

THE UNIVERSITY of ADELAIDE

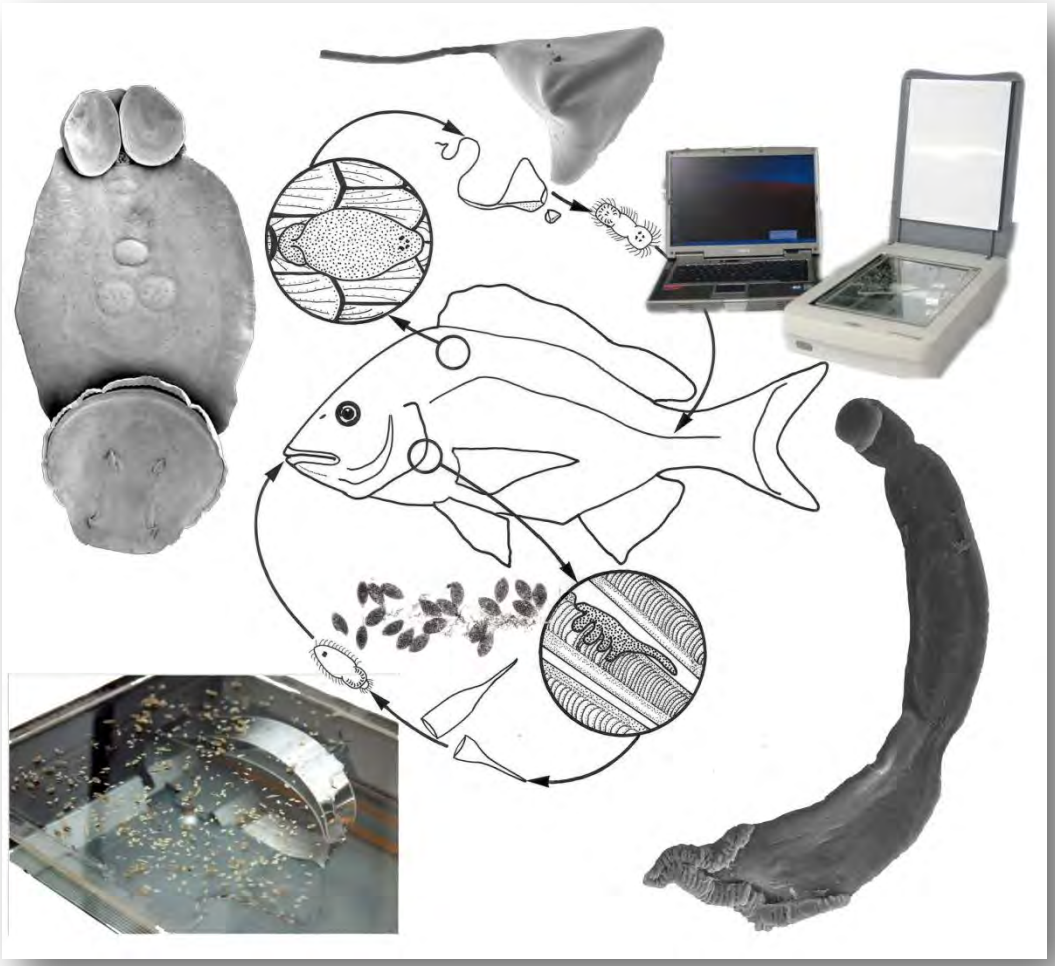
Australian Government
Fisheries Research and Development Corporation

Government of South Australia

SARDI
South Australian Research and Development Institute

South Australian Museum

Innovative Solutions for Aquaculture: Assessment of *in situ* monitoring techniques and life history parameters for monogenean skin and gill parasites



Final Report

I.D. Whittington, A.P. Shinn,
J.E. Bron & M.R. Deveney

FRDC Project No. 2003/221

30 July 2011

Innovative Solutions for Aquaculture: Assessment of *in situ* monitoring techniques and life history parameters for monogenean skin and gill parasites

I.D. Whittington[1,2,3], A.P. Shinn[4], J.E. Bron[4] & M.R. Deveney[5]

[1]*Marine Parasitology Laboratory, School of Earth & Environmental Sciences (DX 650 418), The University of Adelaide, North Terrace, Adelaide, South Australia 5005, Australia*

[2]*Monogenean Research Laboratory, Parasitology Section, The South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia*

[3]*Australian Centre for Evolutionary Biology & Biodiversity, The University of Adelaide, North Terrace, Adelaide, South Australia 5005, Australia*

[4]*Institute of Aquaculture, The University of Stirling, Stirling FK9 4LA, Scotland*

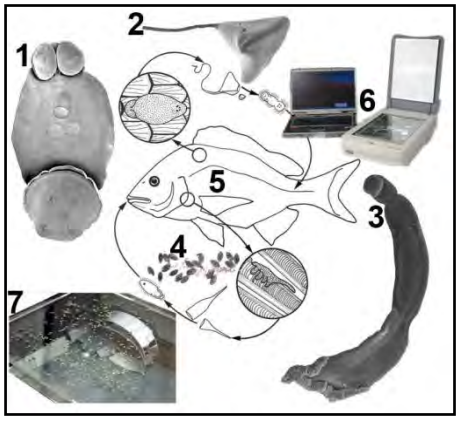
[5]*SARDI Aquatic Sciences, South Australian Research & Development Institute, PO Box 120, Henley Beach, South Australia 5022, Australia*

Final Report for the Fisheries Research & Development Corporation

FRDC Project No. 2003/221

30 July 2011

Cover images: left jigsaw piece, experimental tank infection by *Benedenia seriolae* on skin of *Seriola lalandi* (photograph by R.E. Williams); right jigsaw piece, see **6** below.



Frontispiece images: **1**, scanning electron micrograph (SEM) of the skin fluke, *Benedenia seriolae* from *Seriola lalandi* (photograph by B.W. Cribb [BWC] & I.D. Whittington [IDW]); **2**, SEM of *B. seriolae* egg (photograph by IDW); **3**, SEM of the gill fluke, *Zeuxapta seriolae* from *S. lalandi* (photograph by BWC & IDW); **4**, light micrograph of *Z. seriolae* eggs (photograph by IDW); **5**, generalised lifecycle of Monogenea (drawing by IDW); **6** & **7**, the deliverable from this project is „BEAST" (=Benedeniine Enumeration And Segmentation Techniques), a computerised system to recognise, count and measure skin & gill flukes in mixed samples collected from yellowtail kingfish, *S. lalandi*, in Spencer Gulf, South Australia, using a PC or laptop & flatbed scanner (photographs by A.P. Shinn).

This publication may be cited as:

I.D. Whittington, A.P. Shinn, J.E. Bron & M.R. Deveney. 2011. Innovative Solutions for Aquaculture: Assessment of *in situ* monitoring techniques and life history parameters for monogenean skin and gill parasites. Final Report to FRDC (Project No. 2003/221). The University of Adelaide, Adelaide, South Australia. 41 pp.

The University of Adelaide
Marine Parasitology Laboratory
Discipline of Ecology, Evolution & Landscape Science
School of Earth & Environmental Sciences
The University of Adelaide
Darling Building (DX 650 418)
North Terrace, Adelaide, South Australia 5005
Tel: +61 8 8207 7463
Fax: +61 8 8207 7222

ISBN 978-0-646-55907-0

Authors: Ian D. Whittington, Andrew P. Shinn, James E. Bron & Marty R. Deveney

Review Status: This report was reviewed by Primary Industries & Research South Australia Fisheries & Aquaculture Division.

Distribution: FRDC, FRAB representatives, scientific contributors, beneficiaries, National Library of Australia, CSIRO Library, institutional libraries, investigators

Circulation: Public Domain

Table of Contents

List of Figures

2003/221 **Innovative Solutions for Aquaculture: Assessment of *in situ* monitoring techniques and life history parameters for monogenean skin and gill parasites**

Ian D. Whittington, Andrew P. Shinn, James E. Bron & Marty R. Deveney

PRINCIPAL INVESTIGATOR: Associate Professor Ian D. Whittington
ADDRESS: Marine Parasitology Laboratory
Discipline of Ecology, Evolution & Landscape Science
School of Earth & Environmental Sciences
The University of Adelaide
Darling Building (DX 650 418)
North Terrace, Adelaide, South Australia 5005
Telephone: 08 8207 7463 Fax: 08 8207 7222

OBJECTIVES:

1. Develop a prototype automated (computerised) image analysis system for counting mixed samples of skin and gill fluke parasites that infect yellowtail kingfish, *Seriola lalandi*, in South Australia.
2. Test the accuracy of the system through manual counts.
3. Compile a User"s Manual for the system.
4. Provide training to potential users.
5. Consider policy implications and outline a draft implementation plan.

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

A prototype automated (computerised) image analysis system to count mixed samples of skin and gill flukes infecting farmed yellowtail kingfish in Spencer Gulf, South Australia, has been developed. Using a standard PC or laptop operating Windows, a generic flatbed scanner and the appropriate software loaded and encrypted on a software lock, mixed samples of fish flukes can be counted in less than 3 minutes. Parasite size data, that can be used to assess the population structure and reproductive status of the sampled flukes, is automatically measured and may be exported in an Excel format. At the time of completion of this report, the counting system has not been released and is not in general use by the kingfish farm community. On demonstration to industry representatives at a workshop in February 2011, there was general interest in the technology and recognition that it could become a valuable on-farm tool to help manage the fluke problem on kingfish. The counting system will also benefit future research projects that require rapid and accurate enumeration of flukes when investigating alternative chemotherapeutants, infection pressures among farm leases and other elements of parasite biology from kingfish. A standardised counting system is a first step towards an integrated pest management system for skin and gill flukes, including an industry management system that could decrease reliance on chemical treatments for control, and a governance structure to limit the environmental release of parasite eggs. The accuracy and efficiency of the counting system will be useful in studies examining efficacy of treatments and contribute to the development of Minor Use Permits. This is the first time image analysis and recognition technology has been applied to flukes of kingfish.

External parasitic flukes that infect the skin and gills of yellowtail kingfish are among the most serious health issues for the culture of this species. Fingerlings grown in land-based hatcheries are

free of parasites when transferred to sea-cages for grow out. The skin and gill parasites occur naturally and infect wild yellowtail kingfish stocks. Fluke populations proliferate on captive, sea-caged stocks due to the direct lifecycle of the two parasite species. Fluke infections require regular monitoring by farm staff throughout the production cycle of yellowtail kingfish. Infections contribute to reduced growth, morbidity and if fluke populations reach sufficient intensity, the parasites can cause kingfish mortality on farms.

The current control method for flukes is by bathing in hydrogen peroxide, but bath timing is critical to attain maximum efficacy at killing flukes and therefore reducing the speed of re-infection after treatment. Optimal timing for bathing is determined by regular sampling and monitoring of fluke numbers on cultured fish. Data collected are used to maximise treatment efficacy and minimise the impacts of fluke infections on fish health and production costs. Currently, fluke monitoring is achieved by removing parasites from a sample of fish, fixing them and manually counting the numbers of skin and gill flukes using a stereomicroscope. During counting, the developmental stages of the two fluke species are assessed. Skin flukes are divided into juvenile and adult stages and counted separately. Gill flukes are commonly divided into six stages and counted separately: hatched juveniles, small, medium and large juveniles, mature and egg-laying adults. This reproductive assessment informs farm health managers of the optimal period between strategic bath treatments to achieve the most effective reductions in fluke population sizes and re-infection pressure. Manual counts of the population structure of skin and gill flukes require skilled staff, are time-consuming and depending on competence level, may suffer from variable results between operatives.

This project has developed a prototype automated, computerised software system based on image analysis to recognise shape characteristics for skin and gill flukes that infect yellowtail kingfish in Spencer Gulf, South Australia. This is the first application of this technology to flukes of kingfish anywhere in the world. The original premise for the project when conceived in 2003 was for an automated system to provide separate counts for mixed samples of skin and gill flukes. Farm procedures in 2003 dictated that the parasite species were removed collectively from sampled fish after freshwater bathing (to remove skin flukes) followed by a bath in praziquantel (to remove gill flukes). The system as conceived originally, therefore, counted skin and gill flukes in mixed samples and was developed based on parasites collected from the research activities of several PhD students working on an Australian Research Council (ARC) Linkage project and associated projects from 2003 to 2007. Accuracy of the automated system was tested and assessed and achieved the minimum 95% congruence required between carefully manually counted samples and results from the automated system, providing separate counts for mixed samples of skin and gill flukes. To ensure consistency, repeatability and accuracy of the parasite counts, it is critical that the collection and preservation of specimens is standardised. A rigid protocol for the collection and fixation of specimens, therefore, has been included in the User's Manual.

The software runs on a standard PC or laptop operating Windows XP and Windows 7 (using Windows XP mode) and requires a generic flatbed scanner capable of scanning to 600 dots per inch (dpi), a Perspex plate on which to pour the parasite sample and the software program that runs on an external software lock (a dongle) plugged into the USB port. Tests demonstrate that ~300 flukes can be counted accurately in approximately 3 minutes. The software also identifies and measures each individual parasite, allocates it to skin or gill fluke species and can export the length dimensions of each individual fluke to a text file or Excel file to enable a size distribution of the sampled parasites to be analysed by farm health staff. It is estimated that our automated system may save staff up to 15 hours per week on one large farm (based on 1 hour per sample for a total of 15 samples per week) but the software additionally generates accurate and useful data about the size, and therefore the frequency distribution, of reproductive stages in fluke samples.

A detailed, comprehensive User‟s Manual has been compiled to allow an untrained user to load and initiate the software and provides firm guidelines about the standardised collection and preservation protocols that must be used to ensure consistency, repeatability and accuracy.

The deliverable output from this project is a software macro called BEAST, an acronym for Benedeniine Enumeration And Segmentation Techniques. The purpose and aim of the product is its use by kingfish farmers in Spencer Gulf, South Australia, who may experience problems due to infection of their fish stocks by skin and gill flukes. The system was developed using specimens of skin and gill fluke species from wild and farmed kingfish in this region. It specifically uses shape and size characteristics of these two fluke species to identify, discriminate, count and measure large numbers of individuals rapidly. The principle beneficiaries of this product will be kingfish farmers because BEAST will count large numbers of parasites and also provide data about the frequency distribution of fluke sizes for each species, which will assist farms to manage infections by monogeneans. This will reduce the person hours required to obtain these data manually. The system also improves the quality of data required for monitoring. Depending on its adoption, government departments such as PIRSA Fisheries & Aquaculture may choose to recommend it as part of a fluke management plan / integrated pest management strategy. The BEAST system will also prove to be a powerful tool for appropriate research projects that may investigate flukes of kingfish that require rapid counts of parasite populations. Potential beneficiaries of this technology, therefore, lie in three areas: the kingfish aquaculture industry; related research projects needing fluke counts; regulatory authorities.

The prototype system was demonstrated to industry stakeholders on February 15th 2011 at a workshop in Port Lincoln, South Australia. We learned that parasite monitoring now entails that skin and gill flukes are collected and enumerated separately by farm staff. More importance is now placed by farm managers and staff to assess the reproductive stages of the parasites. The use of shape descriptors for the flukes permits the separation of parasite species and although the maturity status of each worm is not specifically determined from body features, in the updated version of the software, reproductive status is inferred on the premise of size and width of each specimen. Future work, however, subject to available funding, could explore the potential of being able to categorise specimens based on their maturity status.

If and when the software system is used by the South Australian yellowtail kingfish farming industry, we welcome their input and suggestions as to how the prototype developed here can be further enhanced, with additional financial resources, to address the changing needs of farms and their staff.

KEYWORDS: Yellowtail kingfish, aquaculture, skin and gill flukes, monogenean parasites, Spencer Gulf, South Australia, automated computerised counting system, parasite monitoring, management of fish health

Acknowledgements

The Fisheries Research & Development Corporation (FRDC) plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries & Forestry, jointly funded by the Australian Government and the fishing industry. The authors gratefully acknowledge the FRDC who funded this investigation (project no. 2003/221) through the „Innovative Solutions for Aquaculture" initiative of Primary Industries & Resources South Australia (PIRSA) Fisheries in 2003. This research has also formed part of the work of the Marine Innovation South Australia Biosecurity Node.

The original concept for this project sprang from discussions between Dr Ingo Ernst (now Executive Officer, Aquatic Animal Health Committee, Department of Agriculture, Fisheries & Forestry, Canberra), Dr Clinton Chambers (now of Worley Parsons, Perth) and co-investigator Dr Andrew Shinn during the *6th International Symposium on Fish Parasites* in Bloemfontein, South Africa in September 2003.

We thank PIRSA Fisheries & Aquaculture for supporting the Innovative Solutions program. Dr Jane Rathjen and Ms Rosanne Rositano of Adelaide Research & Innovations, The University of Adelaide, provided administrative, legal and contractual support. At the University of Stirling, Mr Eric Gibb assisted in executing the sub-contractual agreement between The University of Adelaide and The University of Stirling.

We are indebted to Professor Mehdi Doroudi, Executive Director, PIRSA Fisheries & Aquaculture, for setting the project on track in 2009/10 and to Peter Lauer (Manager, Environment & Biosecurity Programs, PIRSA Fisheries & Aquaculture) and Luke Fraser (Environmental Assessment Officer, PIRSA Fisheries & Aquaculture) for their administrative help in steering the project to conclusion. Chelsea DuBois (Research Grants Officer, Research Branch, The University of Adelaide) provided important administrative and financial advice and timely reminders about due dates, in a friendly and efficient manner, to the investigators throughout the project.

Pre-counted specimens of the skin fluke, *Benedenia seriolae*, and of the gill fluke, *Zeuxapta seriolae*, were provided by two former PhD students from the Marine Parasitology Laboratory, The University of Adelaide: Dr Kate Hutson (now of the Parasitology Laboratory, Discipline of Aquaculture, School of Marine & Tropical Biology, James Cook University, Townsville) and Dr Rissa Williams (now of Biosecurity New Zealand, Wellington). We thank Kate and Rissa for access to, and use of, this material and for their manual counts because these specimens and data proved invaluable in initial development of the software.

Damian Critchley (Cleanseas Aquaculture, Port Lincoln) provided us with significant and useful assistance and information about farm management procedures, routines and sampling during our visit to Port Lincoln. He also supplied samples of juvenile flukes according to developmental criteria used by Cleanseas Aquaculture in their kingfish fluke monitoring protocols that greatly assisted the development and completion of the project to meet the changing requirements of industry.

Background

Monogenean flatworms are ectoparasites of fish (also known as fish flukes) with a direct lifecycle. Two species, *Benedenia seriolae* and *Zeuxapta seriolae*, infect the skin and gills respectively of yellowtail kingfish, *Seriola lalandi* in Japan, the Mediterranean, New Zealand and Australia (Ernst *et al*. 2002). These monogenean species occur naturally on wild *S. lalandi* in Australia (Rohde 1978; Whittington 1996; Hutson *et al*. 2007*a*). In sea-cage culture, each parasite species can present serious health concerns to farmed Australian stocks (Ernst *et al*. 2002; Hutson *et al*. 2007*a*, *b*; Lackenby *et al*. 2007). Fingerlings reared in land-based hatcheries are parasite-free but when transferred to sea-cages for grow out, fluke populations can proliferate due to their direct lifecycle and the reservoir infections on wild *S. lalandi*. Fluke infections require regular surveillance by farm staff throughout the *S. lalandi* production cycle (Mansell *et al*. 2005; Lackenby *et al*. 2007). *Benedenia seriolae* feeds on host epithelial cells and *Z. seriolae* consumes blood (Ernst *et al*. 2002; Whittington & Chisholm 2008). Burdens of skin flukes (Figure 1a) can cause epithelial lesions, the fish may succumb to secondary infections and suffer osmoregulatory problems. Gill fluke burdens (Figure 1b) can cause anaemia. Both parasite species may affect growth and morbidity and should fluke populations reach sufficient intensity, they can cause kingfish mortality on farms (Ernst *et al*. 2002; Whittington 2005; Whittington & Chisholm 2008).

Figure 1. (a) Experimental infection of *Benedenia seriolae* on the skin of *Seriola lalandi*. (Photograph by R.E. Williams). (b) Experimental infection of *Zeuxapta seriolae* on the gills of *S. lalandi*. (Photograph by A.J. Mooney).

The current control method for flukes on farmed kingfish in South Australia is to bathe entire fish cages in hydrogen peroxide (Chambers & Ernst 2005; Mansell *et al*. 2005; Williams *et al*. 2007). Bath timing is critical to attain maximum efficacy at killing flukes and therefore reduce the speed of re-infection by larvae in the local environment (Lackenby *et al*. 2007). The most advantageous time for bathing is determined by regular sampling and monitoring of fluke numbers on cultured fish by farm staff. Data collected are used to maximise treatment efficacy and minimise the impacts of fluke infections on fish health and production costs. The present method to monitor flukes is to remove parasites from a sample of caged fish and manually count the numbers of skin and gill flukes using a stereomicroscope. During the counting procedure, the developmental stage of the two fluke species is assessed. Staff at Cleanseas Aquaculture (CSA) currently use the following procedure: skin flukes are divided into juvenile and adult stages and counted separately whereas gill flukes are commonly divided into six stages and counted separately as hatched juveniles, small, medium and large juveniles, mature adults and egg-laying adults (Damian Critchley, personal communication). Assessment of the reproductive stage of the parasites allows farm health managers to determine the optimal time required between strategic bath treatments to achieve the greatest reduction of mature, egg-laying stages and therefore of re-infection pressure (*e.g.* Lackenby *et al*. 2007). The manual assessment of the population structure of skin and gill flukes requires

skilled staff, is time-consuming and depending on levels of expertise, may suffer from variable results between personnel.

The principal aim of this project was to develop a prototype fully or semi-automated software system based on image analysis and recognition characteristics for the skin and gill flukes that infect farmed *S. lalandi* in Spencer Gulf, South Australia. The automation concept is based on technology developed by Shinn and colleagues for image analysis and recognition of European *Gyrodactylus* species, which employs shape characteristics to classify unique morphometric features for rapid discrimination and identification (Kay *et al*. 1999; Shinn *et al*. 2000, 2001, 2004). At conception in 2003, the original premise for the project was an automated system to provide separate counts for mixed samples of skin and gill flukes because farm sampling procedures then removed the two parasite species together after freshwater bathing (to remove skin flukes) followed by a bath in praziquantel (to remove gill flukes). The system was initially developed and perfected based on skin and gill flukes in mixed samples collected during the research activities of several PhD students working on an Australian Research Council (ARC) Linkage project and associated grants from 2003 to 2007. Implicit in the development of an automated counting system was to ensure a standardised collection and preservation protocol to guarantee repeatability, comparison and accuracy. To achieve this, the time and resources available to farm staff were discussed to implement procedures that are familiar and standard to most farm operations.

The prototype system was demonstrated to industry stakeholders on February 15th 2011 at a workshop in Port Lincoln, South Australia. At this workshop, however, it was determined that parasite monitoring by CSA now entails that skin and gill flukes are collected and counted separately and more importance is placed by farm managers and staff on assessing the reproductive stages of the parasites (Damian Critchley, personal communication). Following the industry workshop, further modifications to the program were made and although the maturity status of each worm can be inferred from its size, the current version of the software does not specifically extract key morphological features from each fluke specimen to categorically determine its maturity. This, however, could be explored in a future study.

Need

Monogenean (flatworm) parasites have threatened *Seriola* spp. culture elsewhere (*e.g.* Japan, see Egusa 1983; Whittington *et al*. 2001; Whittington 2005; the Mediterranean, see Grau *et al*. 2003; and New Zealand, see Tubbs *et al*. 2005). Yellowtail kingfish aquaculture in Spencer Gulf, South Australia began in the late 1990s. Two monogenean species, *Benedenia seriolae* and *Zeuxapta seriolae*, have proven to be a serious problem for the emerging Australian kingfish industry (*e.g.* Chambers & Ernst 2005; Whittington & Chisholm 2008). For effective fluke management, accurate monitoring is essential but this can consume considerable human resources. Flukes can be collected readily by bathing fish and filtering treatment bath water. This process is rapid and non-destructive to sampled hosts but can generate numerous samples and parasites must be counted manually using a stereomicroscope. Single samples may take from 15 minutes to several hours to process depending on the number of flukes recovered and the extent of detritus such as scales, mucus, faeces and other material. Counting flukes in the farm laboratory therefore places significant demands on staff time. For a large farm at periods in the production cycle when sea water temperatures are high and the fluke lifecycle proceeds rapidly, it is critical for samples to be processed in a timely fashion so that parasite data can be interpreted and acted on appropriately.

The origin of this project emerged from discussions concerning the development of an automated, computerised system based on the skills and expertise of Andrew Shinn to discriminate species of *Gyrodactylus* from Europe based on the shapes of their sclerotised attachment apparatus (Kay *et al*. 1999; McHugh *et al.* 2000; Shinn *et al*. 2000, 2001, 2004). The bodies of adult specimens of *B. seriolae* and *Z. seriolae* from the skin and gills, respectively, of kingfish have different morphology that can be used to allow rapid and accurate counting and measuring of mixed fluke samples based on shape descriptors and image recognition. This is the first application of this technology to flukes of kingfish anywhere in the world.

The automated system that we developed aims to improve significantly the quality of monitoring data collected on farms and may allow considerable cost savings by reducing the time invested in counting parasites manually. For a large farm with multiple sea-cages, it may take one staff member up to 1 hour to count flukes in a single sample from 1 cage. Based on this, we make a conservative estimate that current manual counting procedures may take up to 15 hours / week). Personnel can then spend more time on other critical farm tasks such as feeding, assessment of growth, grading and other management issues. The system developed will also be a useful research tool for future projects assessing how nutrition, chemotherapeutants, immunostimulants and vaccines may affect parasite populations, because these studies need to assess fluke numbers rapidly. In previous research investigations (*e.g.* Hutson 2007; Lackenby *et al*. 2007; Williams 2009), considerable person hours have necessarily been devoted to accurate manual counts of flukes from wild *S. lalandi* or from fish subject to control and test treatments in experiments undertaken by students, research assistants and research associates. Use of the automated system described in this report would save considerable amounts of laboratory labour that could be reallocated to benefit other elements of the research programs.

Changes in how the largest kingfish grower in South Australia, CSA, collect and classify the different reproductive stages of skin and gill flukes from Spencer Gulf, however, required further modifications to the program following its demonstration to industry stakeholders at a workshop in Port Lincoln in February 2011.

Objectives

1. Develop a prototype automated (computerised) image analysis system for counting mixed samples of skin and gill fluke parasites that infect yellowtail kingfish, *Seriola lalandi*, in South Australia.

   Objective achieved.

2. Test the accuracy of the system against manual counts.

   Objective achieved.

3. Compile a User‟s Manual for the system.

   Objective achieved.

4. Provide industry training to potential users.

   Objective achieved.

5. Consider policy implications and outline a draft implementation plan.

   Objective achieved.

Methods

The project set out to develop an image analysis system for enumerating and sizing skin (*Benedenia seriolae*) and gill (*Zeuxapta seriolae*) monogeneans from yellowtail kingfish, *Seriola lalandi*, based on parasite populations from wild and farmed fish in Spencer Gulf. Within the first phase of our work, we developed image processing and analysis software to allow for semi-automated quantification of these parasites. At the onset of this project, the skin fluke species appeared to be more prevalent on, and problematic for, cultured kingfish so we named the system "BEAST", an acronym for Benedeniine Enumeration And Segmentation Techniques.

The concept for the software design was to allow users to perform a range of operations on a single image field comprising a sample or sub-sample of flukes removed from single or multiple hosts. The software runs on a standard personal computer (desktop or laptop) running the Windows XP or Windows 7 (using Windows XP mode; see page 24) operating systems. Images are captured using a low-cost flatbed scanner and then analysed using the software, which allows several levels of interaction. This permits the enumeration and measurement of parasites using a range of morphological descriptors which can then be used to inform farm management about the composition of the fluke population and help with the implementation of control strategies.

The Zeiss KS300 system (ver. 3.0 Carl Zeiss Vision GmbH, Germany, 1997) is a widely recognised and employed image processing and analysis platform. For the purposes of the current work, Dr Andy Shinn has employed this platform to develop protocols and algorithms allowing image processing and analysis to be carried out on images of fluke assemblages collected from *S. lalandi*. The developed solutions have been incorporated into bespoke macros which are run within the stand-alone Zeiss KSRun platform, which may be used to run, but not to program, scripts / macros. This software platform employs an external (USB) hardware lock / key that allows users to run the software through which the BEAST macro runs.

Phases of the project

1. Establish standard preparation of samples
2. Develop prototype image analysis system to count and size the supplied fluke samples (achieved by 31 July 2007)
3. Further development and validation including improved segmentation of flukes and separation of the two species in mixed samples.

i) Sample preparation

The first component of the research conducted involved development of a prototype image processing and analysis system for the enumeration of the two key monogenean species. Typically, a sample for evaluation is collected following the immersion of fish in freshwater, which causes the monogeneans to detach. The detached parasites are allowed to settle, the water is poured off and 10% neutral buffered formalin (NBF) is added to the concentrated sample of parasites to allow long-term preservation. The collected sample, however, contains considerable potential "noise" in terms of fish mucus, detached scales and various detritus that are present in addition to the parasites of interest that comprise the signal to be analysed (Figure 2a).

The accurate determination of parasite numbers and shape requires that the sample is principally free of contaminating material. To remove most of the extraneous material, therefore, the sample was passed twice through a 700 µm mesh filter (Figure 2b). This retained the smallest parasite

stages present in the field samples sent to the University of Stirling from The University of Adelaide from activities of PhD students (2003–2007).

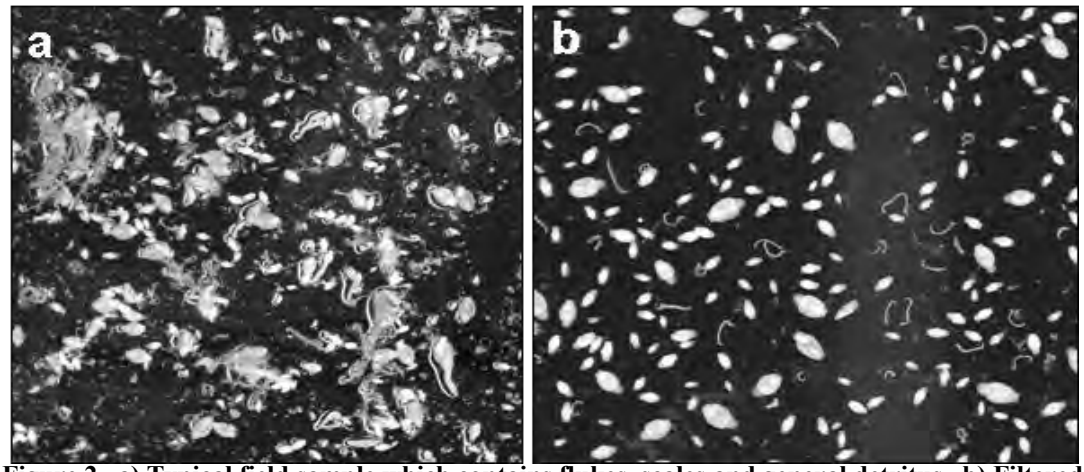


Figure 2. a) Typical field sample which contains flukes, scales and general detritus. b) Filtered sample which retains the smallest flukes but removes most of the contaminating material.

ii) Image capture for analysis

Having obtained clean samples, the next step in the process was to capture representative images for processing. To ensure that a large number of specimens could be analysed quickly and accurately, it was important to get the right balance between the size and resolution of the image field (*i.e.* how many parasites could be analysed simultaneously), the size of the image to be processed and the cost and practicality of the equipment employed for capturing the image. If images are too large then this significantly reduces the subsequent speed of processing. The best solution incorporated a standard scanner bed with an additional shallow tray made from Perspex that fitted onto the scanner plate (Figure 3).

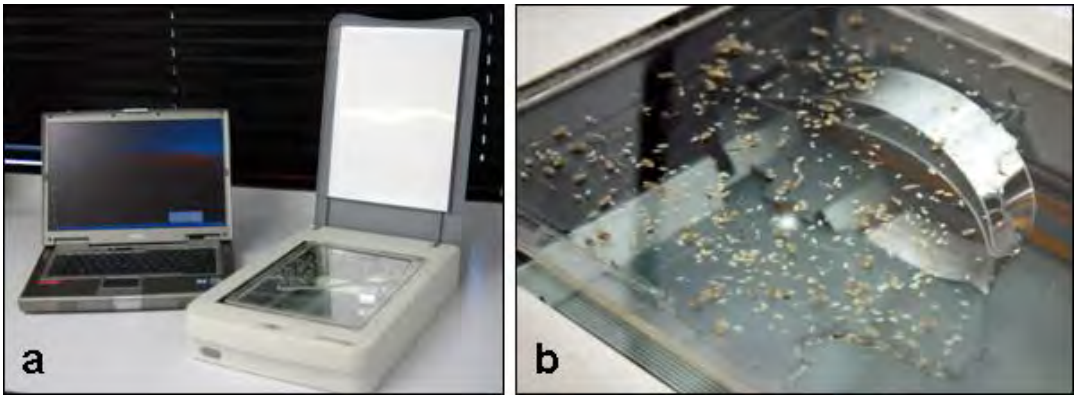


Figure 3. Large sample assemblages of flukes can be imaged by (a) placing samples within a Perspex tray and laying the tray on a conventional scanner bed (b), then capturing an image of the flukes lying within a specified area.

By using an image field measuring 18.5 (w) × 13.7 (l) cm, parasite samples at low resolution (2118 × 1605 pixels at 300 dpi; 4327 × 3209 pixels at 600 dpi) could be rapidly obtained without affecting the ability of the image analysis system to subsequently process the images. Using low resolution images negates the need for expensive image capture equipment making this technology readily accessible and affordable as a farm-based tool.

iii) Calibration of the image field

A calibration sub-routine within the main image analysis macro, used in conjunction with a suitable calibration slide placed on the scanner bed, allows for any make or model of scanner to be used and calibrated (Figure 4).

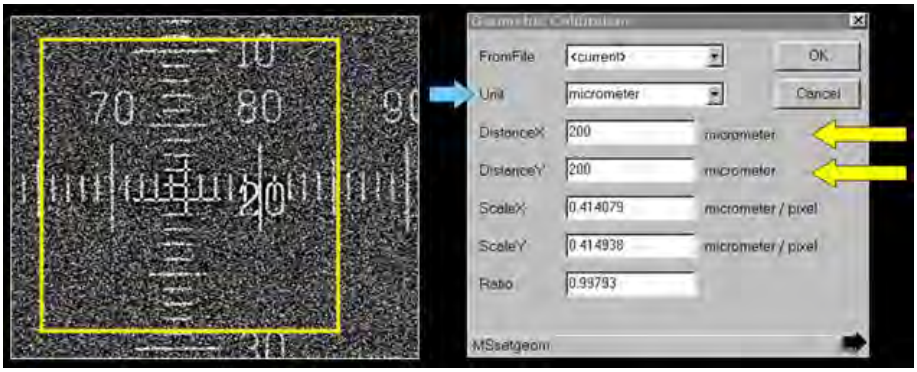


Figure 4. The inclusion of a calibration sub-routine within the BEAST program allows the system to be rapidly set up and adapted for any make or model of scanner.

iv) The BEAST macro

The BEAST macro has been written within an image analysis program which permits features of the main program to be incorporated into bespoke macros, the software being unlocked with a hardware key or dongle (Figure 5). Using a hardware key circumvents the need to purchase the parent program, the cost of which is prohibitive and also ensures that pirating of the software is difficult.

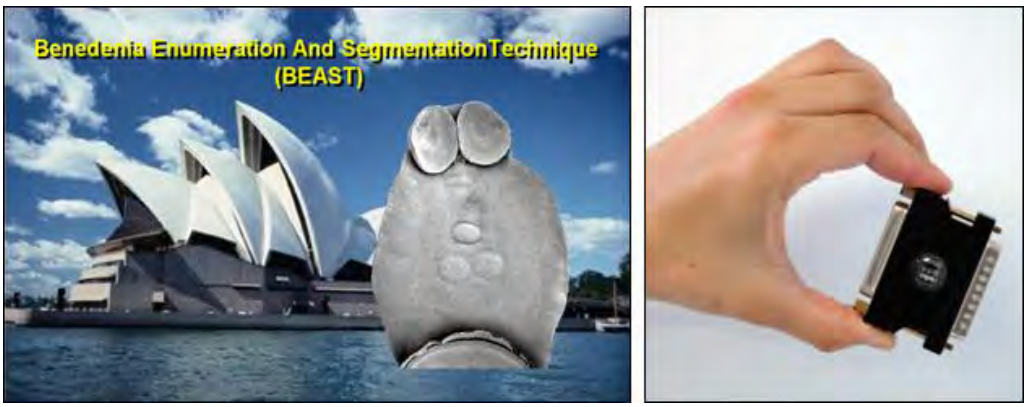


Figure 5. The BEAST program splash page (left). The BEAST macro is accessible through the use of a hardware key or dongle (right) - a plug-in, now via a USB port, which allows the user to access the parent software through which BEAST runs.

The BEAST macro allows for several levels of interaction. The first level can be used by the farms to obtain a rapid assessment of parasite samples while the second level allows for more interaction and the specific selection of parasites within the image field and may be employed for more detailed analysis or research applications. Once the sample has been filtered and placed on the tray on the scanner bed, the accuracy of the subsequent processing can be maximised if the operator takes a few seconds to spread out the parasites using a fine paintbrush so that flukes are minimally in contact (this is discussed in detail later). Once the image has been captured, the operator uses an interactive thresholding segmentation procedure to manually choose a level at which all the flukes are selected (Figure 6). Here, the parasites turn to green via the software as they are manually selected (see Figure 6).

Once features have been segmented, the image is converted to a black and white image (Figure 7a). Objects measuring below 70µm in size are then removed (Figure 7b). The macro does, however, allow the user to select and change the size of objects to be removed.

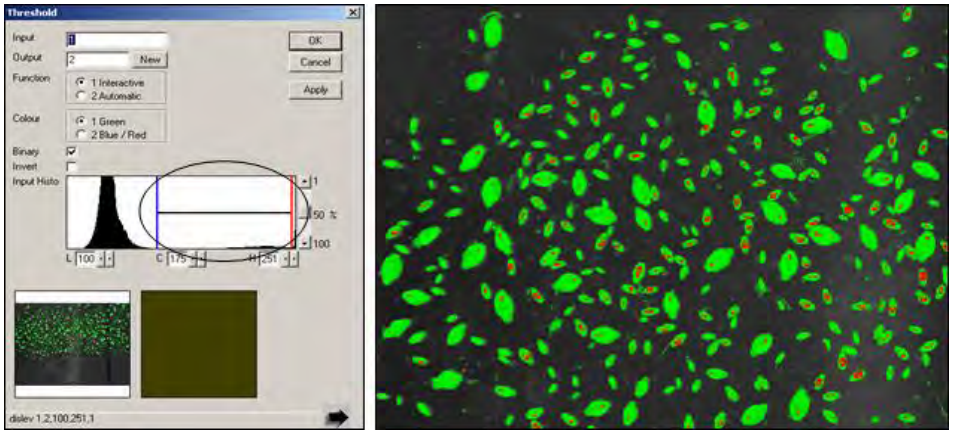


Figure 6. The BEAST macro has a step allowing the user to conduct an interactive thresholding, selecting features on which the macro will subsequently perform a segmentation. By adjusting the bars in the "threshold" window (circled, left), features that are selected turn green (right).

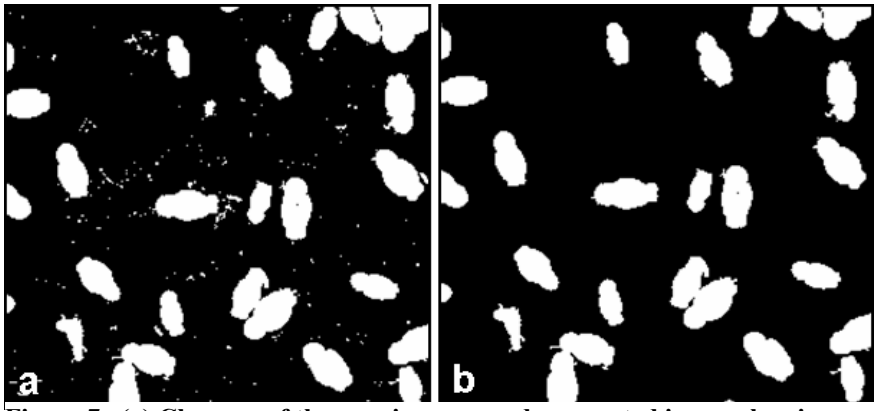


Figure 7. (a) Close-up of the pre size-screened segmented image showing lots of 'noise' within the image. (b) Image following removal of the smallest features using the macro.

When the image has been cleaned up, an automated parasite separation algorithm detects individual flukes and the software gives each one a unique colour code (Figure 8).

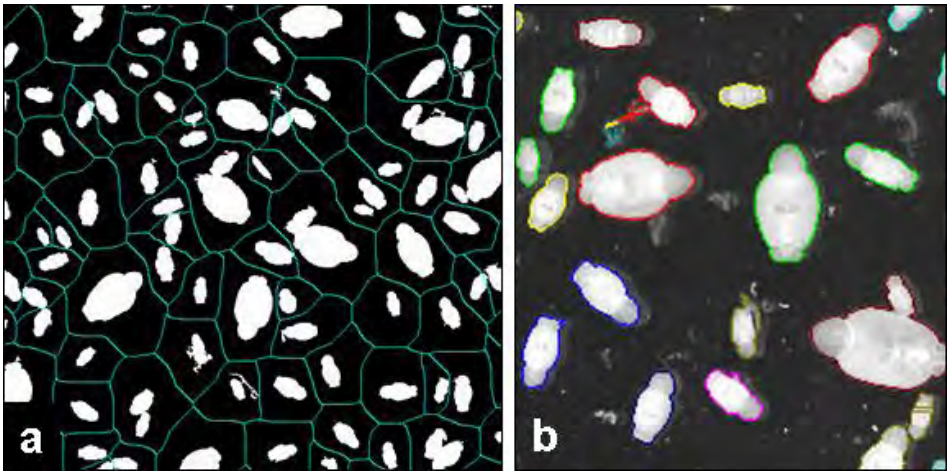


Figure 8. (a) Parasite separation algorithm. (b) A unique colour value is then assigned to each fluke to identify it.

Once the flukes have been identified, they are subjected to the shape description step within the macro. The current version of BEAST uses seven key shape descriptors to characterise each fluke (Figures 9, 15). By way of an example, once the image has been captured, the macro enables the user to characterise 260+ individual flukes in detail within 90 seconds. The macro enables the generated data to be exported as a text or Excel file. These data can then be appropriately manipulated and analysed.

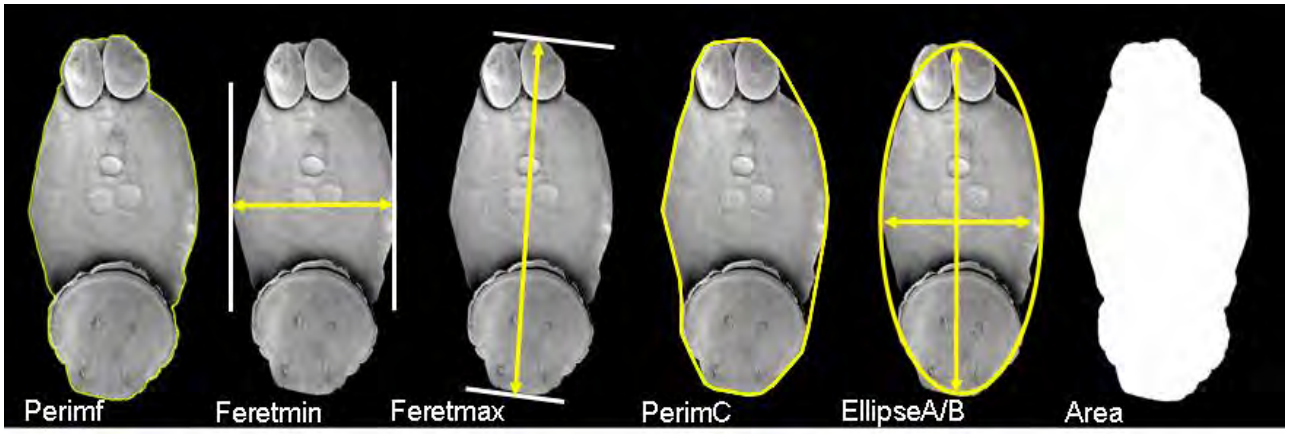


Figure 9. Examples of some of the shape descriptors used to characterise each individual of *Benedenia seriolae* (skin fluke) identified by the BEAST macro.

Results & Discussion

Production and validation of the BEAST macro

The BEAST macro now permits the user to run either a fast (<90 second) analysis which requires minimal user interaction ("automatic") or an advanced, more interactive level to allow the user to select certain flukes for measurement ("semi-automatic"). The output from the analysis, which also permits the separation of *Benedenia* from *Zeuxapta*, allows for farm managers to consider each population of flukes separately.

Figure 10, for example, uses the „feretmax" distance (see Figure 9) determined for each fluke in the sample to show the approximate state of development of individuals within the population. If a farm manager knows, therefore, that the skin fluke *B. seriolae* begins to lay eggs when the parasites reach ~4mm long (*e.g.* based on Lackenby *et al*. 2007), then the farm needs to monitor the maturity status of the population carefully and then implement treatments to ensure that the majority of skin flukes do not reach egg-laying size and that infections can be managed. Figure 10 also shows that there is a strong relationship between fluke maximum width („feretmin", see Figure 9) and fluke maximum length („feretmax") and that these parameters, therefore, are good indicators of size and therefore maturity status.

One notable benefit of using shape descriptors was that these could then be used to separate mixed infections of *Benedenia* and *Zeuxapta* which could then be analysed in separate databases.

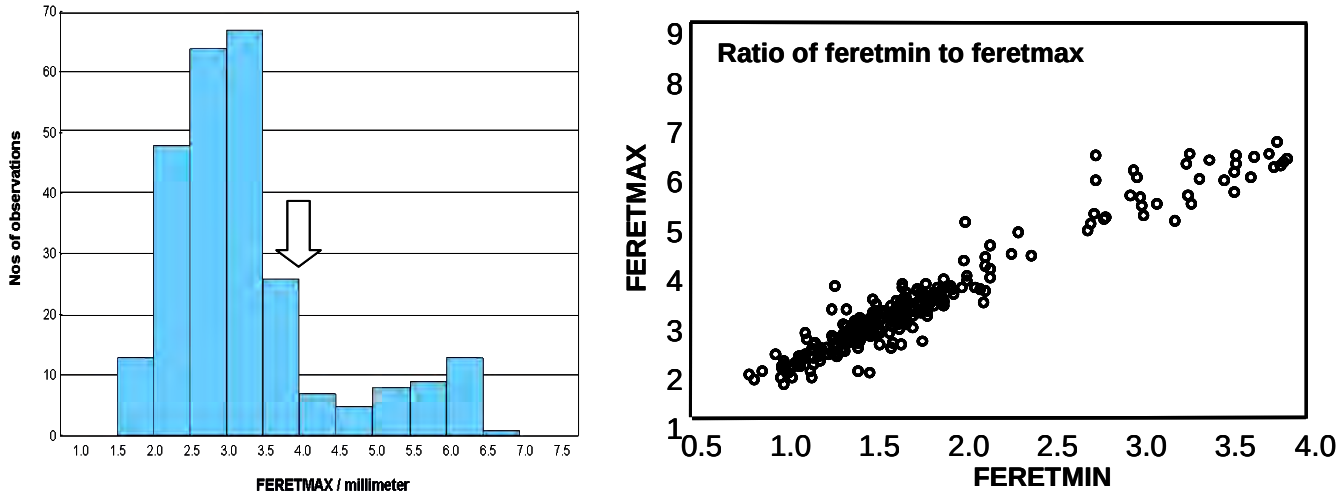


Figure 10. Sizing parasites allows farm managers to determine what proportion of the skin fluke population is near egg-laying size (arrow, left, around 4 mm maximum length) when using, for example, a histogram of 'feretmax' data against the number of observations (left). The plot on the right shows the linear relationship between maximum width ('feretmin') and maximum length ('feretmax') and their suitability as predictors of maturity within the skin fluke population.

Preliminary validation of the BEAST macro

Preliminary comparisons were conducted between point-to-point length measurements taken by a human operator and maximum lengths obtained through image analysis (5 replicates per specimen, 10 specimens). The results indicate that the manual length measurements are predictably related to the maximum length measurements ($R^2 = 0.9945$, $p < 0.00001$) (Figures 11, 12). Further validation and development of the system using large field samples is outlined below.

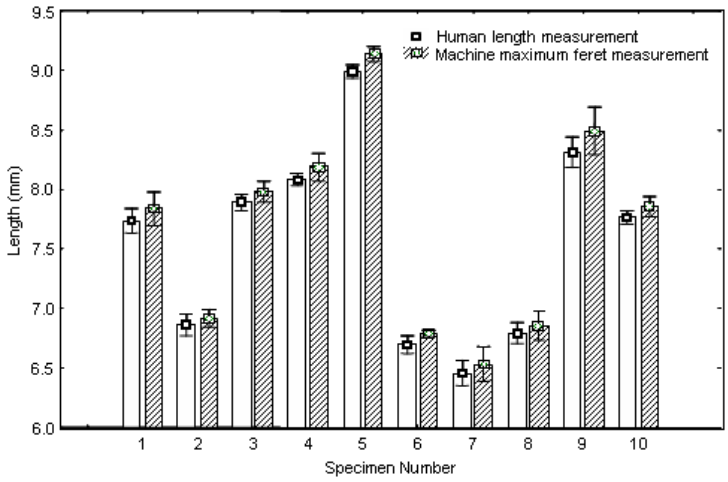


Figure 11. Comparison between parasite lengths measured manually by human operator and maximum lengths measured by the software (n = 5, mean ± 95% C.I.).

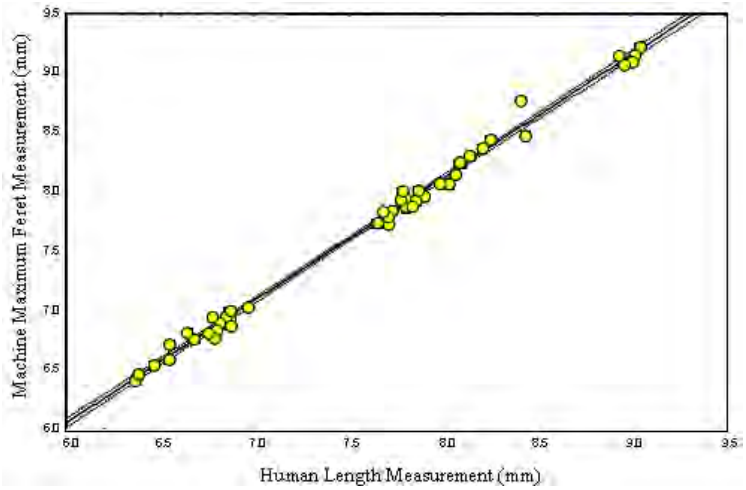


Figure 12. Linear regression of relationship between human and software measurements of parasite length. Regression ± 95% C.I.

User interaction within the BEAST macro

Further, more advanced developments were made so the user may run either the fast analysis (<90 second) requiring minimal user interaction (an "automatic" mode) or the advanced, more interactive level that allows the user to select certain parasite individuals for measurement (a "semi-automatic" mode). In Figure 13, the fluke specimens were quickly scattered onto the scanner tray *without* taking the extra few seconds to ensure that they were not touching each other. Regardless, BEAST was able, in the majority of cases, to identify touching specimens, separate them using the programmed algorithm and measure them (Figure 13a). In other situations, this was not possible (Figure 13b), or pieces of detritus were processed as parasites (Figure 13c), or large / damaged parasites were cut in half and regarded as two separate parasites by the software (Figure 13d). This latter situation was a notable problem when analysing samples containing large numbers of *Z. seriolae* (Figure 14) – a much more elongate fluke compared with *B. seriolae* (*e.g.* Figure 15). To address this, an additional step allowing the user to select specific parasites using an "object select / reject" feature, means that accurate analyses, free from potential semi-automated errors, can be conducted (Figure 14).

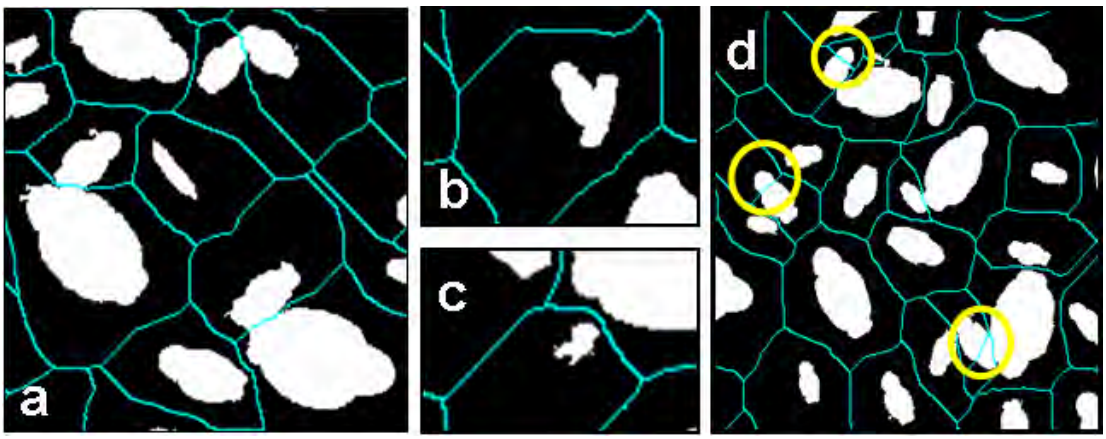


Figure 13. Features of the "automatic" level of image analysis within the BEAST macro. (a) Touching parasites can be separated, in certain cases, by the parasite separation algorithm; (b) touching parasites not separated; (c) large pieces of detritus are regarded as objects for analysis unless removed; d) parasites are sliced in two, in certain cases, by the parasite separation algorithm.

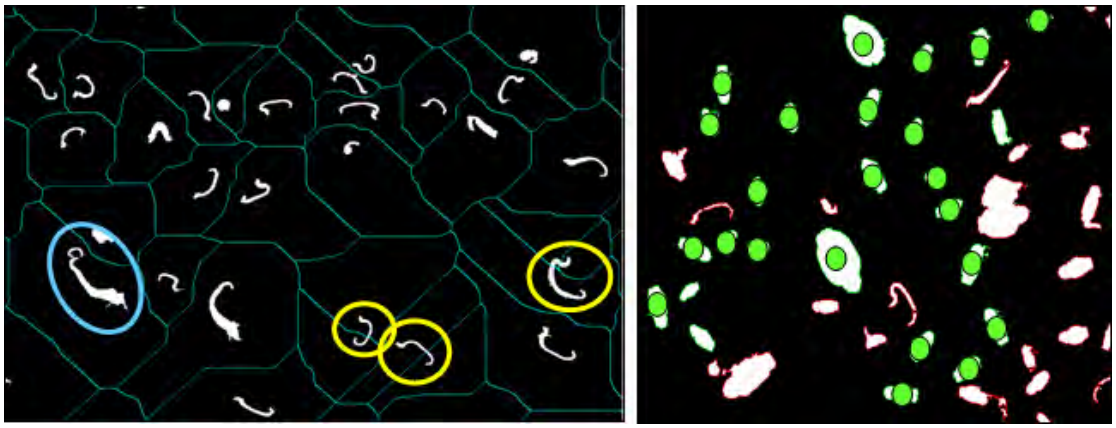


Figure 14. Options within the advanced "semi-automatic" level of BEAST. Above left: The elongate gill fluke, *Zeuxapta seriolae*, with attached detritus (blue circle) or parasites that ordinarily would be sliced in two by the "automatic" level of BEAST (yellow circles) can be rejected from analysis by the selective sampling of representative samples (green dots, above right).

Further development and validation phase

By July 2007, we had established that BEAST functioned with clean samples (*i.e.* minimal detritus), providing accurate automated counts, measurements and identification of *B. seriolae* and *Z. seriolae* from mixed samples. The next project phase was to further refine the software to work with dirtier samples more representative of the condition on farms (*e.g.* containing sediment and other particulate matter, mucus, faeces, scales etc) and validate the automated measurement function of the system on a larger sample of parasites that had been manually counted and measured. The process to refine and validate the software can often be more arduous than the initial development of the system.

The performance of the system was assessed on a sub-set of samples collected and pre-counted manually during the course of two PhD projects on monogenean parasites of wild and farmed kingfish (Hutson 2007; Williams 2009). A total of 29 archived, 10% NBF preserved samples of *B. seriolae* and *Z. seriolae* collected either from sea-caged cultured stocks or from wild *S. lalandi* following freshwater treatment some dating back to 2003, were analysed. The non-parasite material

(*i.e.* fish mucus, detached scales, various detritus such as fish faeces) commonly associated with each field sample was removed by passing the filtrate, twice, through a 400 µm mesh filter and then by transferring the flukes retained on the mesh into a Perspex tray containing distilled water. Any persistent, large captured pieces of non-parasite material were removed and the flukes distributed using a fine paintbrush such that the number of specimens touching each other was minimised. Image fields of the parasites (18.5 cm wide × 13.7 cm long) were scanned using a conventional flatbed scanner bed (HP ScanJet 6200C) at low resolution (300 dots per inch (dpi); 2118 × 1605 pixels).

To circumvent potential measurement errors induced through the use of different fixatives, we agreed on a standard 10% NBF–based protocol for the preparation and fixation of specimens prior to assessment using the BEAST program. A copy of the standard protocol is included in the User"s Manual.

Ongoing refinements to the image analysis macro now permit the operator, once an image has been scanned and loaded into the software, to interactively set the threshold level at which parasites are segmented (*i.e.* selected within a digitised image) or to use automatic preset levels. Pixel noise within the segmented, binary image is removed using an interactive size filter where objects below a certain pixel size can be removed; alternatively a default setting can be used. A Gaussian filter is then applied to the image and a subsequent binary grain reconstruction algorithm detects individual parasites and labels each object with a unique colour code (*e.g.* Figure 8b). This latter object contour image is then overlaid on the original scanned image to allow an assessment of the accuracy of edge detection prior to feature extraction. A series of shape features (*e.g.* perimeter length [„Perimf"], maximum breadth [„Feretmin"], maximum length [„Feretmax"] and area [„Area"]; Figure 9) are then extracted automatically for each object, and are stored within an exportable database (as a text file or in Excel format).

Validation of the BEAST macro based on evaluation of the archive samples

To ensure concordance between the shape features extracted by the BEAST macro and a human operator, repeated point-to-point length measurements on a random sub-sample of *B. seriolae* were taken using a point-to-point morphometrics macro running within the Zeiss KS300 image analysis platform. „Feretmax" measurements generated by the BEAST macro were compared against the total length measurements determined manually by a human operator. The results indicated that the manual length measurements were predictably related to the „Feretmax" measurements (R^2 = 0.9945, $p < 0.00001$). Further validation using the 29 formalin fixed, field samples, however, provided lower initial performances necessitating several iterative improvements to the BEAST macro followed by re-assessment of the field samples.

The initial lower performances were the result of: 1) the inclusion of smaller sized specimens than those provided in the training sets used to develop and assess the performance of the prototype system; 2) minor deterioration in soft bodies of the archived specimens (some were 8 years old!), notably in the thinner, sometimes thread-like, *Zeuxapta* specimens (*e.g.* Figures 14, 15).

Subsequent improvements to the image analysis software have been achieved through: 1) the inclusion of a sub-routine to permit mixed samples of *Benedenia* and *Zeuxapta* to be separated using the shape descriptor „FCircle" which is $[4 \times \pi \times \text{area}]/\text{perimeter}^2$ (an estimate of sphericity; *e.g.* Figure 15) as part of the basis for discrimination to provide separate summary statistics for both parasite species; 2) a feature to permit the user to select specific parasites using an "object select / reject" feature means that accurate analyses, free of potential semi-automated errors, can be conducted; 3) further optimisation of the BEAST macro specifically to address the enumeration

performance of the smaller-sized *Zeuxapta* specimens including scanning conditions (background colour, dpi at which images are scanned); and 4) making further rigid recommendations regarding the collection, fixation and storage of specimens.

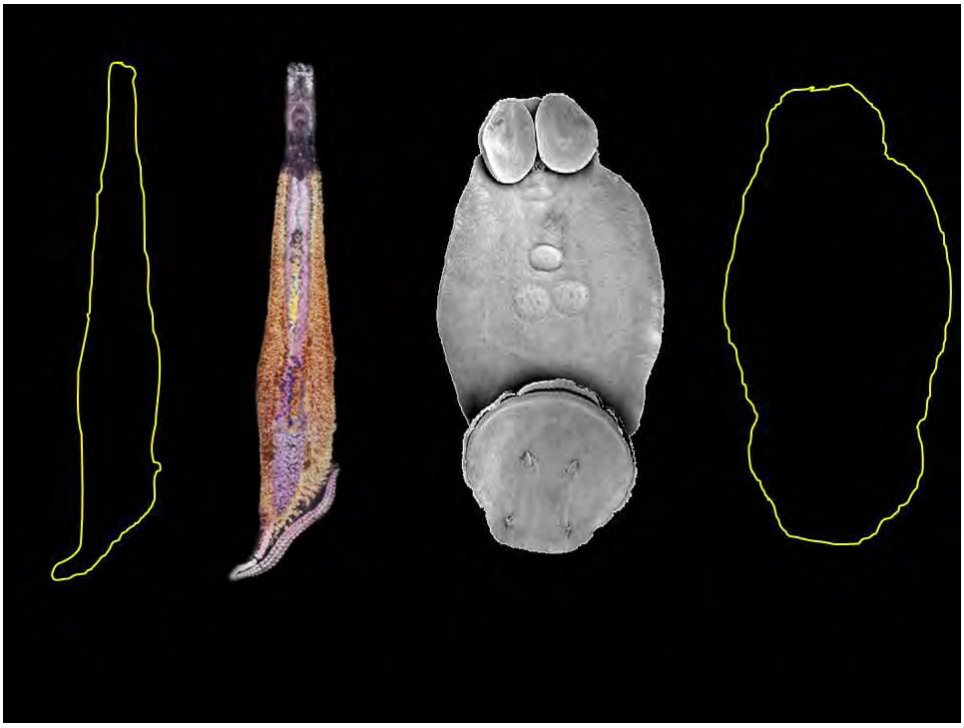


Figure 15. Images to show the distinctly different shapes of *Zeuxapta seriolae* (left) and *Benedenia seriolae* (right). In a revised version of BEAST, the shape descriptor 'FCircle' (= [4 × π × area]/perimeter^2), which is an estimate of the sphericity of an object, was used in combination with other shape descriptors in a sub-routine to better separate mixed samples and provide separate summary statistics for each fluke species.

For the anticipated use of BEAST, it is not expected that old archived samples are likely to be used. Nevertheless, the archived samples and provision of small specimens of *Z. seriolae* in late February 2011 by Damian Critchley (CST) provided some significant challenges and permitted important improvements and advances to the macro that have enhanced its applications.

In readiness for a workshop for industry scheduled for mid-February 2011 in Port Lincoln, South Australia and coordinated through PIRSA Fisheries & Aquaculture, the BEAST software was demonstrated and discussed. A User"s Manual was compiled and completed.

Modifications made to the macro post-industry workshop

The new version of BEAST (progeny of BEAST?) is more powerful because instead of the analysis working directly on a sample of worms *in situ*, the software now breaks down the parasite image that is scanned into „skin fluke (*Benedenia*)"-like and „gill fluke (*Zeuxapta*)"-like populations and then runs separate sub-routines for each „group". The sub-routines then mark flukes with „*particular characteristics*" according to each „group" and analyses each „group" separately. The resultant analysis is therefore more powerful. It is further enhanced because the „*particular characteristics*" can be specified by the operator allowing a focus on parasites of each species. Based on the shape analyses during the course of this study, a set of parameters that best „describe" each parasite species have been generated (*i.e.* descriptors for skin flukes and descriptors for gill flukes). These values can be adjusted by the operator (a "semi-automatic" mode) or can be run as a default setting (*i.e.* an "automatic mode").

Improvements have also been made to the output data generated by BEAST after a fluke sample has been scanned and analysed. The output graphs now provide a full complement of population statistics for the samples (e.g. mean values, ranges and standard deviations).

When this project was conceived in 2003, the standard Microsoft operating platform for most PCs was Windows 2000 and Windows XP. The intervening years have seen the release of Windows Vista and most recently Windows 7. BEAST has been written and developed using Windows XP as the standard PC operating system, but we know that our macro will run in the Windows XP Mode on the Windows Virtual PC which is an optional component of Windows 7 that is available for download from Microsoft. Some versions of Windows 7 such as Professional and Ultimate already have the XP mode loaded and enabled. For more information, explanation and access to download Windows XP Mode and Windows Virtual PC for Windows 7, see: http://www.microsoft.com/windows/virtual-pc/

The User's Manual

This manual provides step-by-step instruction in installing, operating and using the BEAST macro and interpreting the results generated. The manual also includes a firm protocol for the preparation and fixation of all specimens destined for analysis using BEAST.

Policy implications

This system is an initial step towards development of an integrated pest management (IPM) strategy for skin and gill flukes of kingfish. IPM is a system of management that comprises:

- Maintaining acceptable pest levels
- Preventive cultural systems
- Monitoring
- Non-chemical controls
- Responsible treatment use

The BEAST system we have developed is integral for an IPM strategy because flukes need to be counted to obtain data for the development of all other components of the management system. Development of a threshold at which flukes have an unacceptable impact on the host needs to be balanced with parasite egg release into the environment, but can be achieved by better understanding fluke feeding and pathogenicity. This could be achieved using experiments that examine parasite feeding, and host growth and condition index. Although some preliminary data exists for Australian conditions (*e.g.* Chambers & Ernst, 2005), separation of cages, their arrangement in the current and the distances required to establish independent management units (IMUs) need to be investigated further. Data used to establish IMUs could also be used to develop guidelines for egg release and environmental egg load. The effects of single year class stocking, fallowing, use of antifoulants and different net materials should be established, facilitating implementation strategies that minimise fluke numbers. A monitoring plan that satisfies industry and governance needs should be developed and referred to in license conditions to provide a consistent, stable operating environment with good industry practices contributing to environmental sustainability and fish welfare. Controls that do not rely on direct chemical treatment of adult flukes including ways to reduce egg load in the environment or treat egg concentrations, larval traps or decoys, devices to allow fish to mechanically remove skin flukes by rubbing and investigating other ways to manage flukes *without* chemicals should be examined. Dose and treatment time for existing medicines should be optimised, alternatives to hydrogen peroxide and praziquantel should be identified and their efficacy assessed. Environmental effects of treatments should be considered

further and strategies to minimise the broader release of chemical products should be identified. These data could be combined to obtain Minor Use Permits for those products.

This IPM strategy should be developed as a component of a broader finfish biosecurity plan, analogous to those developed for terrestrial livestock industries under Ausvetplan. This plan could then be gazetted as an approved code of practice and used to maintain minimum standards in industry.

Draft implementation plan

2011-2012 – Development of a finfish industry biosecurity plan
2011-2012 – Development of a plan for an IPM strategy
2012-2015 – IPM research module 1 – assessment of acceptable loads and environmental effects of egg release, optimisation of current treatments and assessment of two new treatments
2015-2018 – IPM research module 2 – Development of two non-chemical treatments and assessment of a further two treatments

Benefits and Adoption

The deliverable from this research is computer software known as "BEAST (Benedeniine Enumeration And Segmentation Techniques)" which is a macro written within Zeiss KS300 ver. 3.0 image analysis software (Carl Zeiss Vision GmbH, Germany, 1997). Features of the complex main image analysis and processing program are incorporated into tailored macros which are operated using Zeiss KSRun. The parent software is unlocked with a hardware key (dongle) that plugs into the USB port of a standard PC or laptop and allows the user to access the software through which the BEAST macro runs. The parent KS300 software costs AUS$23,000 to AUS$31,000 whereas the KSRun costs approximately AUS$3,875 and avoids the purchase of the parent program.

A benefit of the system developed and a major stimulus when the project was conceived was for the counting software to run on a basic low-cost computer and a cheap, standard flatbed scanner capable of scanning images at 300 dpi (Appendix 7). We assume that all fish farms and research institutions likely to use the system will either already possess this equipment or it can be purchased at relatively modest expense.

At the time of the publication of this report, we estimate that purchase of a BEAST package comprising hardware key dongle complete with the BEAST macro, Perspex tray onto which samples are poured and the User"s Manual will cost approximately AUS$4,500 subject to currency exchange rates and current costs for KSRun licence and dongle.

Prior to the industry workshop held in February 2011 (Appendix 3), the software was not in use at any of the kingfish farms. At this workshop there was keen interest in the counting system and recognition that it could save personnel significant amounts of time if used to replace manual counts by stereomicroscopy. It is understood that the time taken to count flukes varies throughout the year, often related to seasonal changes in sea water temperature which affects the rate at which the lifecycles of the parasites are completed. Depending on time of year and numbers of cultured fish, a job that could take several hours a week could be completed in one to two hours. Additional data generated in terms of frequency distributions of fluke lengths for each parasite species in text or Excel file format will provide an accurate snapshot of the current parasite infection. These data can be used strategically to plan and coordinate treatment intervention to remove flukes. All electronic data such as scanned sample images and spreadsheet information can be archived on hard disks, USB drives, CD or DVD so that farms can establish weekly, monthly and annual records. Over time, these data can help inform farms about potentially „difficult" times of year when heightened surveillance of flukes and additional treatments may be necessary. These data can be reviewed to continually improve farm husbandry and management procedures.

We anticipate that each company with kingfish farms in Spencer Gulf (*i.e.* CSA and Southern Star) will likely benefit from BEAST. As the operations of CSA are spread between three sites (*i.e.* from north to south: Whyalla, Arno Bay; Port Lincoln), it is likely that each site may choose to operate and use individual installations of BEAST. Broad adoption of BEAST in South Australia will depend on the perception of its utility by industry.

BEAST will also have enormous benefits for research projects that require the numbers and sizes of flukes from kingfish to be assessed. In recent years, companies supplying feed to the aquaculture industry such as Ridley, Skretting and Nutreco have expressed interest in developing in-feed chemotherapeutants against flukes. Independent research projects funded variously by the Australian Research Council, CRC Aquaculture, SARDI Aquatic Sciences and CSA have also investigated the efficacy of chemicals delivered orally to farmed fish. Furthermore, there is a need to investigate alternative and safer bathing practices to control fluke infections on kingfish in

Spencer Gulf. Current use of dilute hydrogen peroxide baths is stressful to kingfish, duration and toxicity to fish is dependent on ambient water temperature and the procedure requires aeration of caged stocks. Human error has been attributed to stock losses during bathing using hydrogen peroxide (McIlwraith, 2010). A significant component of any research budget in such investigations is personnel required to count flukes manually. We anticipate that BEAST will be a valuable research tool for any future studies that require large numbers of flukes to be counted and measured, quickly and accurately.

PIRSA Fisheries & Aquaculture and other regulatory authorities may choose to require the use of BEAST or another system proven to be equivalently accurate as part of a fluke management plan.

BEAST was developed using fluke samples sourced only from Spencer Gulf, South Australia. We cannot guarantee its effectiveness or accuracy on fluke samples from *Seriola* species elsewhere, but our expectation is that it will work on the same parasite species (*i.e. Benedenia seriolae* and *Zeuxapta seriolae*) from kingfish (*Seriola lalandi*).

Further Development

Further field testing and refinements to the system

The BEAST system (parent software module and our software macro encrypted onto a USB dongle software lock, Perspex tray and User"s Manual) costs approximately AUS$4,500 (note: this price is subject to change due to currency exchange rates and current costs for KSRun licence and dongle. We will make a copy available to CSA for them to use and to assess its performance over a period of 60 days. During this period of use, we will ask that users provide feedback on its utility and comment on whether further refinements to the system are required for its operation for the purposes for which it was originally developed. Specifically, we recommend that CSA have their employees use the system at their three main sites (Whyalla, Arno Bay and Port Lincoln) to assess its applicability and ease of use by different operatives and different operations. We will also ask for feedback on the User"s Manual to ensure that the step-by-step instructions are logical and easy to follow and that the style of language is clear.

Any reasonable and necessary modifications to the program and User"s Manual will be made within 60 days of the end of the stakeholder trial period. The further modifications, however, will not address the evaluation of the smallest fluke stages (hatched larvae and the youngest settled post-larval stages) or the maturity status of the flukes as this is a larger and significant challenge and should be addressed in future funded work.

Press release

Dependent on the success of the stakeholder trial and modification period, we may organise in consultation with FRDC and PIRSA Fisheries & Aquaculture an official „release" with media in attendance. It is hoped that the system may also serve as a useful analysis and management tool for *Seriola* growers in other parts of the world *e.g.* Chile, Japan, México, New Zealand and some countries around the Mediterranean Sea. Since the current software was developed using fluke samples only from Spencer Gulf, South Australia, we cannot guarantee its effectiveness or accuracy on fluke samples from elsewhere. We anticipate, however, that BEAST will work on the same parasite species (*i.e. Benedenia seriolae* and *Zeuxapta seriolae*) from kingfish (*Seriola lalandi*).

Publication of the results in scientific literature

A manuscript for submission to the international peer-reviewed journal *Aquaculture* (Excellence in Research for Australia [ERA] ranking, A) which is published by Elsevier, is in preparation and it is hoped will be submitted to the refereeing process later this year, after its release is approved by PIRSA. The publication will provide a detailed overview of the project, highlighting the role of FRDC and PIRSA in funding and guiding the work and stating the person at PIRSA Fisheries & Aquaculture to contact to acquire the system or to obtain more details (*e.g.* Appendix 7).

Supplementary development

In addition to the further developments that could be made to the BEAST macro that have already been discussed above (*i.e.* enumeration of the smallest flukes and the assessment of parasite maturity), it is hoped that use of BEAST will play a critical role in an effective program of Integrated Pest Management (IPM) to build on and extend the biological research on the skin and

gill flukes of *Seriola* species in Australia and Japan by Whittington and colleagues (*e.g*. Whittington 1996, Whittington *et al*. 2001, Ernst *et al*. 2002, 2005, Chambers & Ernst 2005, Mansell *et al*. 2005, Mooney et al., 2005, 2008, Hutson *et al*. 2007*a*, ,; Lackenby et al. 2007, Williams *et al*. 2007, Whittington & Chisholm 2008). There are growing concerns regarding chemotherapeutant practices within the aquaculture industry, many of which are still heavily reliant on the use of synthetic chemical products for the treatment of a broad range of disease agents. These concerns are not only about the chemicals themselves, but also the volumes being used, their doses and efficacy, the frequency of necessary treatments, impacts on the environment and non-target species, the withdrawal period required, residues in fish flesh and the development of resistance by the pathogens. If BEAST is used effectively, it is hoped that farmers will be able to optimise their treatment programs, strategically targeting the pre-adult parasite population theoretically resulting in the need for fewer treatments. This is a first step in developing an IPM strategy for farms in Spencer Gulf, South Australia.

Planned Outcomes

The deliverable output from this project is the software macro called BEAST. The purpose and aim of this product is its use by kingfish farmers in Spencer Gulf, South Australia, who may experience problems due to infection of their fish stocks by monogenean parasites. This image analysis program was developed using specimens from wild and farmed kingfish in this region infected by the skin and gill fluke species, *Benedenia seriolae* and *Zeuxapta seriolae*, respectively. It specifically uses shape and size characteristics of these two fluke species to identify, discriminate, count and measure large numbers of individuals rapidly. The principle beneficiaries of this product will be kingfish farmers because BEAST will count large numbers of parasites and also provide data about the frequency distribution of fluke sizes for each species which will assist farms to manage infections by monogeneans. This will reduce the person hours required to obtain these data manually. The system also improves the quality of data required for monitoring. Depending on its adoption, government departments such as PIRSA Fisheries & Aquaculture may choose to recommend its use as part of a fluke management plan / IPM strategy. The BEAST system will also prove to be a powerful tool for appropriate research projects that may investigate flukes of kingfish that require rapid counts of parasite populations. Potential beneficiaries of this technology, therefore, fall in three areas: the kingfish aquaculture industry; related research projects relying on fluke counts; regulatory authorities.

To date, the outcome of this project is the development of BEAST for use by farms, researchers and regulatory authorities to count monogenean skin and gill flukes from kingfish and make use of the data generated.

Conclusions

Infections of *Benedenia seriolae* and *Zeuxapta seriolae* on sea-cage cultured stocks of yellowtail kingfish *Seriola lalandi* are a major concern for fish health and welfare. Heavy infections, if left untreated, can cause epithelial hyperplasia and haemorrhaging which may lead to secondary infections and osmotic stress resulting in host mortality. To manage these pathogens effectively and to allow strategic deployment of chemotherapeutic controls, frequent monitoring of parasite prevalences, incidences and life-stages is required. Current monitoring programs conducted by stakeholders in the kingfish industry involve high manpower and costs. This project which set out to develop a prototype automated (computerised) image analysis system for counting mixed samples of skin and gill fluke parasites that infect yellowtail kingfish has been successful. The BEAST (Benedeniine Enumeration And Segmentation Techniques) software co-developed by the University of Stirling, UK and The University of Adelaide, permits rapid semi-automatic quantification and shape-analysis of skin and gill flukes (300+ in ~180 seconds), using low cost imaging hardware and a standard PC or laptop operating Windows XP or Windows 7 (using Windows XP Mode). Images of a sample or sub-sample of monogeneans removed from single or multiple hosts are captured using a standard flatbed scanner and then analysed using the software which allows several levels of interaction. This time saving exercise (*e.g.* perhaps ~15 hours per week on a large farm) permits the enumeration and measurement of parasites using a range of morphological descriptors which can then be used to inform farm management when to apply control strategies. This is the first time image analysis and recognition technology has been applied to flukes of kingfish. The system was demonstrated and trialled at a workshop in Port Lincoln in February 2011 after which a few further modifications were made to the system. Future work could develop the system more to evaluate the smallest stages of fluke parasitising kingfish and / or categorise specimens based on their level of maturity.

References

Chambers C.B. & Ernst I. 2005. Dispersal of the skin fluke *Benedenia seriolae* (Monogenea: Capsalidae) by tidal currents and implications for sea-cage farming of *Seriola* spp. *Aquaculture* **250**: 60–69.

Egusa S. 1983. Disease problems in Japanese yellowtail, *Seriola quinqueradiata*, culture: a review. *Rapports et proces-verbaux des réunions Conseil International pour l'exploration de la Mer* **182**: 10–18.

Ernst I., Whittington I., Corneillie S. & Talbot C. 2002. Monogenean parasites in sea-cage aquaculture. *Austasia Aquaculture* **Feb./Mar. 2002**: 46–48.

Ernst I., Whittington I.D., Corneillie S. & Talbot C. 2005. Effects of temperature, salinity, desiccation and chemical treatments on egg embryonation and hatching success of *Benedenia seriolae* (Monogenea: Capsalidae), a parasite of farmed *Seriola* spp. *Journal of Fish Diseases* **28**: 157–164.

Grau A., Crespo S., Pastor E., Gonzalez P. & Carbonell E. 2003. High infection by *Zeuxapta seriolae* (Monogenea: Heteraxinidae) associated with mass mortalities of amberjack *Seriola dumerili* Risso reared in sea cages in the Balearic Islands (western Mediterranean). *Bulletin of the European Association of Fish Pathologists* **23**: 139–142.

Hutson K.S. 2007. *Parasite interactions between wild and farmed yellowtail kingfish (*Seriola lalandi*) in southern Australia*. PhD thesis, School of Earth & Environmental Sciences, The University of Adelaide, xv + 195 pp.

Hutson K.S., Ernst I., Mooney A.J. & Whittington I.D. 2007*a*. Metazoan parasite assemblages of wild *Seriola lalandi* (Carangidae) from eastern and southern Australia. *Parasitology International* **56**: 95–105.

Hutson K.S., Ernst I. & Whittington I.D. 2007*b*. Risk assessment for metazoan parasites of yellowtail kingfish *Seriola lalandi* (Perciformes: Carangidae) in South Australian sea-cage aquaculture. *Aquaculture* **271**: 85–99.

Kay J.W., Shinn A.P. & Sommerville C. 1999. Towards an automated system for the identification of notifiable pathogens: using *Gyrodactylus salaris* as an example. *Parasitology Today* **15**: 201–206.

Lackenby J.A., Chambers C.B., Ernst I. & Whittington I.D. 2007. Effect of water temperature on reproductive development of *Benedenia seriolae* (Monogenea: Capsalidae) from *Seriola lalandi* in Australia. *Diseases of Aquatic Organisms* **74**: 235–242.

Mansell B., Powell M.D., Ernst I. & Nowak B.F. 2005. Effects of the gill monogenean *Zeuxapta seriolae* (Meserve, 1938) and treatment with hydrogen peroxide on pathophysiology of kingfish, *Seriola lalandi* Valenciennes, 1833. *Journal of Fish Diseases* **28**: 253–262.

McHugh, S.E., Shinn, A.P. & Kay, J.W. 2000. Discrimination of *G. salaris* and *G. thymalli* using statistical classifiers applied to morphometric data. *Parasitology* **121**: 315–323.

McIlwraith I. 2010. Clean Seas takes a bath on kingfish loss. The Age (September 8, 2010). http://www.theage.com.au/business/clean-seas-takes-a-bath-on-kingfish-loss-20100907-14zq0.html

Mooney A.J., Ernst I. & Whittington I.D. 2006. An egg-laying rhythm in *Zeuxapta seriolae* (Monogenea: Heteraxinidae), a gill parasite of yellowtail kingfish (*Seriola lalandi*). *Aquaculture* **253**: 10–16.

Mooney A.J., Ernst I. & Whittington I.D. 2008. Egg-laying patterns and *in vivo* egg production in the monogenean parasites *Heteraxine heterocerca* and *Benedenia seriolae* from Japanese yellowtail *Seriola quinqueradiata*. *Parasitology* **135**: 1295–1302.

Rohde K. 1978. Monogenean gill parasites of the kingfish Seriola grandis Castlenau (Carangidae) from the Great Barrier Reef. *Publications of the Seto Marine Biological Laboratory* **24**: 369–376.

Shinn, A.P., Kay J.W. & Sommerville C. 2000. The use of statistical classifiers for the discrimination of species of the genus *Gyrodactylus* (Monogenea) parasitizing salmonids. *Parasitology* **120**: 261–269.

Shinn A.P., Gibson D.I. & Sommerville C. 2001. Morphometric discrimination of *Gyrodactylus salaris* Malmberg (Monogenea) from species of *Gyrodactylus* parasitising British salmonids using novel parameters. *Journal of Fish Diseases* **24**: 83–97.

Shinn A.P., Hansen H., Olstad K., Bachmann L. & Bakke T.A. 2004. The use of morphometric characters to discriminate specimens of laboratory-reared and wild populations of *Gyrodactylus salari*s and *G. thymalli* (Monogenea). *Folia Parasitologica* **51**: 239–252.

Tubbs L.A., Poortenaar C.W., Sewell M.A. & Diggles B.K. 2005. Effects of temperature on fecundity in vitro, egg hatching and reproductive development of *Benedenia seriolae* and *Zeuxapta seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriola lalandi*. *International Journal for Parasitology* **35**: 315–327.

Whittington I.D. 1996. Benedeniine (capsalid) monogeneans from Australian fishes: pathogenic species, site-specificity and camouflage. *Journal of Helminthology* **70**: 177–184.

Whittington I.D. 2005. Monogenea Monopisthocotylea (ectoparasitic flukes). In: *Marine Parasitology*. Rohde K. (Ed.), pp. 63-72. CSIRO Publishing, Melbourne, Australia.

Whittington I.D. & Chisholm L.A. 2008. Diseases caused by Monogenea. In: *Fish Diseases Volume 2*. Eiras J.C., Segner H., Wahlii T. & Kapoor B.G. (Eds.), pp. 683-816 (Chapter 13). Science Publishers, Inc., New Hampshire, USA.

Whittington I.D., Corneillie S., Talbot C., Morgan J.A.T. & Adlard R.D. 2001. Infections of *Seriola quinqueradiata* Temminck & Schlegel and *S. dumerili* (Risso) in Japan by *Benedenia seriolae* (Monogenea) confirmed by morphology and 28S ribosomal DNA analysis. *Journal of Fish Diseases* **24**: 421–425.

Williams R.E. 2009. *Oral treatments for monogenean parasites of farmed yellowtails,* Seriola *spp. (Carangidae)*. PhD thesis, School of Earth & Environmental Sciences, The University of Adelaide, 174 pp.

Williams R.E., Ernst I., Chambers C.B. & Whittington I.D. 2007. Efficacy of orally administered praziquantel against *Zeuxapta seriolae* and *Benedenia seriolae* (Monogenea) in yellowtail kingfish *Seriola lalandi*. *Diseases of Aquatic Organisms* **77**: 199–205.

Appendices

Appendix 1: Intellectual Property

All intellectual property rights relating to this research project are outlined in Section 11 and Schedule 1 of the signed „Agreement for Services" between the Minister for Agriculture, Food & Fisheries (of the South Australian Government) and The University of Adelaide (dated 18th May 2010).

The deliverable is the automated (computerised) system BEAST for counting flukes from yellowtail kingfish in South Australia described in this report.

To enquire about or obtain BEAST, see Appendix 7.

Appendix 2: Staff

Principal Investigator:

Ian D. WHITTINGTON[1,2,3]

Co-investigators:

Andrew P. SHINN[4]
James E. BRON[4]
Marty R. DEVENEY[5]

PhD students whose sample collection activities, although not directly associated with this project, contributed to its outcomes:

Kate S. HUTSON[1,6]
Rissa E. WILLIAMS[1,7]

[1]Marine Parasitology Laboratory, School of Earth & Environmental Sciences (DX 650 418), The University of Adelaide, North Terrace, Adelaide, South Australia 5005, Australia

[2]Monogenean Research Laboratory, Parasitology Section, The South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia

[3]Australian Centre for Evolutionary Biology & Biodiversity, The University of Adelaide, North Terrace, Adelaide, South Australia 5005, Australia

[4]Institute of Aquaculture, The University of Stirling, Stirling FK9 4LA, Scotland

[5]SARDI Aquatic Sciences, South Australian Research & Development Institute, PO Box 120, Henley Beach, South Australia 5022, Australia

[6]Present address: Marine Parasitology Laboratory, Discipline of Aquaculture, School of Marine & Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia

[7]Present address: Investigation & Diagnostic Centre–Wallaceville, Biosecurity New Zealand, Ministry of Agriculture & Forestry, PO Box 40742, Ward Street, Upper Hutt 5018, New Zealand

Email addresses of project investigators:
Ian Whittington: ian.whittington@samuseum.sa.gov.au
Andrew Shinn: a.p.shinn@stir.ac.uk OR aps1@stir.ac.uk
James Bron: j.e.bron@stir.ac.uk OR jeb1@stir.ac.uk
Marty Deveney: marty.deveney@sa.gov.au

Appendix 3: Industry Workshop

Date: 15th February 2011
Time: 13.00 h for lunch followed by presentations at 14.00 -16.00 h
Venue: Lincoln Marine Science Centre, Port Lincoln, South Australia

Presenters:

- Ian Whittington (University of Adelaide / South Australian Museum) — *Introduction*
- Andrew Shinn (University of Stirling, Scotland, U.K.) — *The BEAST Program*
- James Bron (University of Stirling, Scotland, U.K.) — *Some Background on How BEAST Works*

Discussion involving all persons present

Attendees:

- Tony Barton (Cleanseas Aquaculture, Whyalla)
- Joe Ciura (Cleanseas Aquaculture, Port Lincoln)
- Damian Critchley (Cleanseas Aquaculture, Port Lincoln)
- Dr Marty Deveney (SARDI Aquatic Sciences, West Beach)
- Sam Feige (Cleanseas Aquaculture, Port Lincoln)
- Luke Fraser (PIRSA Fisheries & Aquaculture, Adelaide)
- Professor Barbara Nowak (National Centre for Marine Conservation & Resource Sustainability, School of Aquaculture, University of Tasmania-Launceston; also representing the FRDC Aquatic Animal Health Subprogram)
- Ben Underdown (Cleanseas Aquaculture, Whyalla)

Appendix 4: Media

The project has been featured in *Fish Farming International* (February 2007).

Dependent on the success of the stakeholder trial and modification period, once BEAST is officially released, there may be scope for further media in consultation with FRDC and PIRSA Fisheries & Aquaculture.

Appendix 5: Presentations

Presentations at international conferences

1. Shinn A, Bron J., Chambers C., Ernst I. & Whittington I. 2005. Benedeniines and the BEAST: benedeniine enumeration and segmentation techniques. Fifth International Symposium on Monogenea (ISM5), August 8-12 2005, Sun Yat-Sen University, Guangzhou, China. (Abstract with program, P1, p. 129).

2. Shinn A.P., Whittington I.D. & Bron J.E. 2007. BEAST: Benedeniid Enumeration And Segmentation Technique. Training School on Fish Welfare EU COST ACTION 867 in collaboration with SCOFDA (Sustainable Control of Fish Diseases in Aquaculture), Department of Veterinary Pathobiology, University of Copenhagen, Denmark, 10-12 April.

3. Mooney A.J., Lackenby J.A. & Whittington I.D. 2009. Tales about Monogenea in kingfish (*Seriola lalandi*) culture from South Australia. The 6th International Symposium on Monogenea, August 2-7 2009, Cape Town, South Africa. (Abstract with program; presentation C24). Invited presentation.

4. Shinn A.P., Whittington I.D., Christison K.W. & Bron J.E. 2009. Man, monsters and machines: managing malicious monogenean miscreants. The 6th International Symposium on Monogenea, August 2-7 2009, Cape Town, South Africa. (Abstract with program; presentation C25).

Presentation at a national conference

1. Shinn A.P., Whittington I.D. & Bron J.E. 2008. Man, monsters and machines: managing malicious miscreants. The Annual Scientific Meeting of the Australian Society for Parasitology Inc. and the Australian National Network for Parasitology Annual Conference, July 6-9 2008, Stamford Grand, Glenelg, South Australia. (Abstract with program; contribution S17). Invited presentation.

Appendix 6: Public Outreach

1. Biodiversity Gallery, South Australian Museum, 2010. Contributions of specimens, photographs, textual information and models of parasites and host kingfish to inform the public about parasites in kingfish farming in the „Biodiversity Gallery", a permanent exhibition at the South Australian Museum, Adelaide, South Australia (opened 11th February 2010).

2. Whittington I.D. 2010. Discussions with members of the public about marine parasites during the opening weekend of the „Biodiversity Gallery", South Australian Museum, Adelaide, South Australia, 13-14th February 2010.

Appendix 7: A One-Page Flyer About BEAST