Enabling Land-based Production of Juvenile Yellowtail Kingfish in NSW

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Abbreviations

AFM – Activated Filter Media

AGR – Absolute Growth Rate

ANOVA – Analysis of variance

C – Cochran's test

CRC – Cooperative Research Centre

DO – Dissolved oxygen

DPH – Days post hatch

FAME –

F1 – First generation hatchery-reared

FCR – Food conversion ratio

FL – Fork length

FT - Flowthrough

g - grams

K – Condition factor

Kg – kilograms

K4P – FRDC Project No. 2016-200.30

L – litres

L2 – larval rearing tank #2

LED – Light emitting diode

MBBR – Moving bed bioreactor

NS – Not significant

NSW – New South Wales

NSW DPI – New South Wales Department of Primary Industries

PPT – parts per thousand

PSFI – Port Stephens Fisheries Institute

R&D – Research and Development

RAS – Recirculating Aquaculture System

Reps – replicates

SA – South Australia

SARDI – South Australian Research and Development Institute

SD – Standard deviation

SOP – Standard Operating Procedure

TAFE – Technical and Further Education

TAN – total ammonia nitrogen

WA – Western Australia

YTK – Yellowtail Kingfish

µm - micrometer

Wt – Weight

Executive Summary

NSW DPI conducted a series of experiments and commercial-scale production to investigate the viability of producing advanced juvenile yellowtail kingfish (YTK, *Seriola lalandi*) at the Port Stephens Fisheries Institute (PSFI) during March 2016 - December 2018. There is a significant shortfall of white-flesh fish at the Sydney fish market and NSW DPI has been doing research for approximately 10 years to develop technology for aquaculture of YTK in NSW. The reliable production of advanced juvenile YTK is a fundamental requirement to enable development of a viable farming industry for this species. To advance this objective, the suitability of intensive, land-based flow-through systems, recirculating aquaculture systems and outdoor raceways for production of advanced YTK were tested in NSW conditions.

Background

The Australian aquaculture industry has grown in volume at an average rate of around 12% per annum since 1992-93 and in 2017-18, the gross value of aquaculture was $1.42 billion and accounted for 44% of the gross value of fisheries production. The majority of this value came from marine production systems producing high-value species such as salmonids, tuna, and oysters and was dominated by Tasmania (62%) and South Australia (10.4%) which both have strong marine finfish sea cage based aquaculture sectors. In contrast, NSW aquaculture production by value represents about 4% of the Australian industry (Steven et al., 2020) and was mostly due to oyster production. NSW currently does not have an operating marine seacage industry.

NSW imports approximately 85% of its seafood and needs a substantial increase in investment and production, most notably, new marine-based aquaculture development is required. However, marine aquaculture development is fundamentally constrained by the lack of background biological and economic information. To develop marine finfish production, the NSW government has invested significantly in establishing a 20 ha Marine Aquaculture Research Lease (MARL) situated 3.5 km off Hawks Nest in Providence Bay near Port Stephens. The MARL will be viable for a period of five years to June 2021with approval to produce up to 998 t fish/annum. Yellowtail Kingfish (YTK) was the primary species of interest. In 2016, the Tasmanian-based salmonid farm, Huon Aquaculture (HA) entered into a collaborative R&D project with NSW DPI to install purpose-built seacages on the MARL. The aim of the project was to evaluate the viability of growing YTK in seacages in a high wave climate in NSW. Huon Aquaculture provided all day-to-day management of the growout operation and NSW DPI marine fish hatchery at the PSFI provided the YTK fingerlings produced in this FRDC project.

Since the early 1990s, research at PSFI has developed hatchery production technology and feeds for Australian snapper and mulloway and most recently for Southern Bluefin Tuna and YTK. This includes technology for intensive, biosecure hatchery production as well as extensive methods using plastic-lined, outdoor ponds. Methods for production of high-quality juvenile YTK were developed at an experimental-scale and techniques for commercial-scale production remained to be identified at PSFI. Also, tank-based research developed a production model that predicts a significant economic advantage by farming YTK in mid NSW waters because the time to reach a market size of 4 kg is approximately 446 days compared with approximately 757 days in the cooler South Australian waters.

On-going supply of high-quality juvenile fish from viable hatcheries is paramount to the success of any seacage growout business. Typically, juvenile fish are transferred from land-based hatcheries to seacages as quickly as possible and as relatively small fish (~5-10g YTK; 35+g Atlantic salmon smolt). This is mostly for logistical and perceived economic reasons as it is easier to transport large quantities of small fish. However, stocking of small fish into seacages can result in management issues including the need to use fine mesh nets and subsequent biofouling and regular cleaning and/or net-changing. Post-stocking mortality of juvenile fish can also be high due to the inability for small fish to acclimatise to a seacage environment. There is now a movement in the seacage industry to extend the land-based component of fish production and to stock larger, advanced, more robust fish (~100g+) and thus reduce the period of time in which the fish are ongrown at sea. The major challenge with this strategy is to have economically

and logistically viable technology for land-based production of larger size fish, especially considering the need for year-round production. Management of water quality including temperature and waste products is paramount to successful, intensive fish production. The suitability of intensive, land-based recirculating aquaculture systems and novel Biogill® filters on outdoor raceways for production of advanced YTK remain to be tested in NSW conditions.

Aims/objectives

1. to validate the feasibility of the PSFI hatchery to produce commercial quantities of YTK fingerlings

2. to determine the feasibility and logistics of large-scale, land-based systems for production of advanced juvenile YTK

Methodology

The applied research project focussed on a series of objectives designed to cover aspects of fingerling supply chain, fish growth, and economics and logistics. Research on production of YTK had been done in SA and WA and NSW to improve larval rearing methods to produce high-quality fingerlings (e.g. Seafood CRC project: 2011/740) and to understand the performance of YTK in SA seacages (e.g. Seafood CRC project: 2008/903 Understanding YTK). This project adopted the outcomes of these projects while answering questions that were unique to YTK production in NSW. Strategies for production of high-quality, advanced juvenile YTK in NSW were consequently needed to be adapted or developed.

We intended to focus on detailing the supply chain from the hatchery to the market and quantifying the costs of the venture through the identification of the input logistics and costs, both biological and economic.

Evaluation of large-scale land-based YTK fingerling production was divided into two components: hatchery and nursery.

Hatchery Experiments

The Port Stephens Fisheries Institute (PSFI) marine fish R&D unit has a long history with the development of technology for production of juvenile marine fish including snapper, mulloway and YTK and commercial production of hundreds of thousands of juvenile mulloway and snapper at the PSFI has been proven. PSFI has developed and improved broodstock management and larval rearing techniques for year-round production of high-quality YTK (Fielder and Heasman, 2011) at an experimental scale, however, the capacity of the PSFI hatchery to reliably produce commercial quantities of juvenile YTK remained to be demonstrated. This was evaluated by repeated operation of the commercial-scale intensive hatchery. The standard larval rearing tanks used at PSFI were 2000L with conical-bottoms and were operated on single pass seawater systems (Fielder & Heasman, 2011).

The rearing regime or ‘recipe” for rearing of YTK larvae (Fielder and Heasman, 2011) was attempted to be improved through replicated commercial-scale (n=3 reps/treatment) experiments designed to determine optimal abiotic factors including, tank colour, salinity and photoperiod and light source. The experimental treatment was compared with the current best-practice recipe in the commercial-scale tanks and quality of fingerlings produced was evaluated in terms of swimbladder inflation, larval survival and growth; and the degree of jaw and spinal deformation.

It should be noted that due to demand from Huon Aquaculture Ltd from the PSFI hatchery to supply 25,000 YTK fingerlings (20g) every 3 months for seacage growout, the scope of the hatchery studies was limited. Experiment treatments were selected conservatively and based on the likelihood that they would not result in poor larval growth and survival, compared with the current best-practice larval rearing regime.

Nursery experiments

Large-scale production of advanced juvenile YTK (up to 100g) was evaluated in land-based facilities including recirculation tank systems (RAS) with high-tech water management (including temperature, dissolved oxygen, particulate and dissolved nutrient filtration) and polyhouse-covered, plastic-lined raceway ponds. Growout of juvenile YTK was done at high-density (up to 30kg/m^3) in indoor, controlled-environment recirculating aquaculture systems (20,000L; n=4) and compared with production of YTK in outdoor raceway systems (350,000L; n=4 ponds), which were covered in a poly-house for heat retention.

The use of novel Biogill® filters (www. Biogill.com) to manage water quality in the raceway ponds was evaluated during growth cycles throughout the year. Ponds were each stocked with up to 500 YTK (100 - 500g) and ongrown using standard feeding protocols. Two ponds had Biogill® filters installed on the pond bank. Water was pumped (up to 200%/day) from the bottom of the pond into the Biogill® filter and returned to the pond. Two control ponds were managed according to standard protocols of water exchange with filtered estuary seawater. Solid waste and dissolved nutrient loads and filamentous algae growth were compared between the two operating systems. YTK were grown for 6-8 weeks and then harvested. Performance of outdoor raceway systems was compared with indoor recirculating systems in terms of fish quality and survival and cost of production. Assessment of Biogill® filter performance to improve water quality in raceways was combined with an assessment of the suitability of polyhouse covers over outdoor raceways to improve (increase) water temperature and to optimise juvenile YTK growth during winter. Performance was evaluated by comparing fish growth and survival, FCR as well as general health. Identification of optimal methods for removing and handling large numbers of advanced juvenile YTK from outdoor raceways for transport to seacages was also determined.

Results/key findings

Hatchery

- Internal tank colour (black or silver) did not affect growth or survival of YTK larvae to 30 dph; however larvae grown in silver-lined tanks were darker in colour than those larvae grown in black tanks. Differences in fish skin colour were transient and all fish became the same colour once removed from the treatment tanks and placed into common nursery tanks. Based on these results, the standard PSFI black tank colour was deemed optimal for YTK culture and remained as control tanks for future experiments.

- Light source (Hibay mercury vapour or full spectrum LED lights) did not affect growth, survival, swimbladder inflation or first feeding of YTK larvae to 18 dph. Hibay lights were approximately twice as bright as LED lights at the surface and at all depths through the water column. Either Hibay or full spectrum LED lights can be used for YTK larval rearing.

- Six commercial production runs were done with variable survival of larvae to metamorphosis (~30dph) ranging from 0-10.5%. Issues which likely contributed to poor survival of larvae in some batch's included excessive water temperature due to environmental heatwave conditions, decrease in influent salinity due to major rainfall event, poor egg quality due to abnormally frequent (3 monthly) spawning of broodfish, and potential uncontrolled establishment of deleterious bacterial communities in larval rearing tanks.

Nursery

- Advanced juvenile YTK (mean maximum 166g) were successfully cultured in RAS tanks (10 and 30 m^3; 1m and 2m depth, respectively) and flow-through tanks (FT; 5-10 m^3; 1m depth) for up to 192 days. Maximum stocking densities in both RAS and FT tanks were approximately 25 kg fish/m^3 with maximum feed rate of 0.5 kg food/day/m^3.
- FT tanks used up to 17x more influent seawater than RAS tanks to maintain high water quality, especially low suspended solid matter, and were reliant on ambient water temperature compared

with RAS which had temperature control. FT tanks had few components and were easier to manage than RAS which had mechanical, biological and UV filters.

- Advanced juvenile YTK were harvested quickly and easily from RAS and FT tanks using handheld environets. In both RAS and FT tanks, the bulk of the fish population was captured initially with tanks filled to full volume. Fish in RAS were encouraged to the tank surface with small amounts of feed, where they were captured over time (up to 95% of population within 5-10 minutes) by 1 technician. The remaining fish were captured following drainage of the tank. Fish in FT tanks were captured by two technicians working together to crowd the fish in the tank.
- It was possible in RAS to restrict the growth with no loss of condition of an older cohort of juvenile YTK by reducing temperature (15-16^{o}C) and feed rates to allow a younger cohort to attain the same weight by a specific date. This enabled amalgamation of the groups as a single cohort for stocking to seacages.
- Advanced juvenile YTK (up to ~1kg) were successfully cultured with high survival in outdoor polyhouse-covered, plastic-lined ponds supplied with compressed air only. Polyhouse covered ponds had a more stable water temperature than uncovered ponds. Covered ponds were typically 2^{o}C warmer than uncovered ponds in winter. During summer, covered ponds were approximately up to 1.5 ^{o}C cooler on hot days and 1.5 ^{o}C warmer on cold days. Final biomass was 7860 kg fish/ha. Maximum daily feed rate was 274.5 kg/day/ha. Harvesting of fish from the ponds was relatively easy with 5 staff taking approximately 45-60 minutes per pond. After three shots of the seine net only 12 fish (0.4%) of the population remained in the pond. These were captured once the pond water volume was drained.

Implications for relevant stakeholders

This project complemented the DoA project "Growing a profitable, innovative and collaborative Australian YTK aquaculture industry: bringing ‘white’ fish to the market - RnD4Profit-14-01-027" that was abbreviated to “kingfish for profit” (K4P). The end users are the Public, Regulators and Industry. Research addressed short term needs to develop marine fish farming in eastern Australia and to provide a platform for ongoing research.

Industry: YTK production within Australia has been challenging and further research was needed, particularly when entering new farming environments including high wave-climate, offshore sites. It was essential to identify supply chains from broodstock to market. In particular, culture of the largest juveniles possible on land before transfer to seacages was essential to optimise survival and production. Land-based techniques using simple flow-through tanks, sophisticated RAS and outdoor, polyhouse-covered ponds were evaluated and developed for viable production of advanced juvenile YTK. Each system offers benefits and challenges and optimal culture strategies will vary from site to site; the most likely scenario being incorporation of all methods to provide scope and control of fish production, especially during winter, when water temperature is too cold and peak summer, when water temperature is too hot for optimal growth of juvenile YTK.

Public: Extensive public consultation identified key concerns with respect to the sustainable operation of the MARL. Many aquaculture ventures have failed after relatively short periods of time from establishment, and one contributing factor was often due to inadequate understanding of the biological requirements for reliable, cost-effective production of seed stock.This project successfully identified that advanced juvenile YTK could be produced sustainably at the PSFI hatchery, which is a fundamental requirement to operation of a viable marine fish farming industry.

Regulators: There was a need for NSW DPI to develop a Marine Waters Sustainable Aquaculture Strategy for NSW (MWSAS) to streamline investment pathways and promote sustainable seafood production. Data from this project was incorporated in development of a MWSAS.

Recommendations

Several areas of research are needed to improve our understanding of larval rearing and nursery culture of YTK, including:

- Further understand the interactions of salinity and temperature, particularly in the first 12 days post-hatching. YTK larvae may have low tolerance of small variations in salinity and this is vital information for hatchery operators, especially if estuarine seawater with variable salinity is used in the hatchery

- Investigate the development of bacterial communities in the PSFI hatchery tanks and methods to avoid r-strategist bacteria populations from proliferating, including maturation of influent seawater and use of commercial probiotics

- Investigate improved methods to provide dissolved oxygen in nursery tanks used for high-density culture of YTK, including nannobubble technology

Keywords

Yellowtail kingfish, Seriola lalandi, hatchery, nursery, juvenile, RAS

Introduction

Background

NSW DPI has an approved 20 hectare offshore sea cage Research Lease 3.5 km off Hawks Nest in Providence Bay near Port Stephens, NSW for a period of five years until June 2021. The lease allowed the NSW Government to extend its successful marine hatchery research at PSFI to offshore sea cage research to validate the commercial potential of a number of marine finfish species and trial the latest production technologies in the high energy coastal waters of NSW. In 2016, the Tasmanian-based salmonid farm, Huon Aquaculture (HA) entered into a collaborative R&D project with NSW DPI to install purpose-built seacages on the MARL. The aim of the project was to evaluate the viability of growing YTK in seacages in a high wave climate in NSW. Huon Aquaculture provided all day-to-day management of the growout operation and NSW DPI marine fish hatchery at the PSFI provided the YTK fingerlings produced in this FRDC project.

The Australian aquaculture industry has grown in volume at an average rate of around 12% per annum since 1992-93 and in 2017-18, the gross value of aquaculture was $1.42billion and accounted for 44% of the gross value of fisheries production. The majority of this value came from marine production systems producing high-value species such as salmonids, tuna, and oysters and was dominated by Tasmania (62%) and South Australia (10.4%) which both have strong marine finfish sea cage based aquaculture sectors. In contrast, NSW aquaculture production by value represents about 4% of the Australian industry (Steven et al., 2020) and was mostly associated due to oyster production. NSW currently does not have an operating marine seacage industry.

The farming of Yellowtail kingfish (YTK) is one of the fastest growing aquaculture sectors in Australia due to the demand for this seafood product and the potential economic advantages of marine-based aquaculture. Since the early 1990s, research at PSFI has developed hatchery production technology and feeds for Australian snapper and mulloway and most recently for Southern Bluefin Tuna and YTK. This includes technology for intensive, biosecure hatchery production as well as extensive methods using plastic-lined, outdoor ponds. Methods for production of high-quality juvenile YTK were developed at an experimental-scale and techniques for commercial-scale production remain to be identified at PSFI. Also, tank-based research developed a production model that predicts a significant economic advantage by farming YTK in mid NSW because time to reach a market-size of 4 kg is approximately 446 days compared with approximately 757 days in the cooler South Australian waters.

On-going supply of high-quality juvenile fish from viable hatcheries is paramount to the success of any seacage growout business. Typically, juvenile fish are transferred from land-based hatcheries to seacages as quickly as possible and as relatively small fish (~5-10g YTK; 35+g Atlantic salmon smolt). This is mostly for logistical and perceived economic reasons as it is easier to transport large quantities of small fish. However stocking of small fish into seacages can result in management issues including the need to use fine mesh nets and subsequent biofouling and regular cleaning and/or net-changing. Post-stocking mortality of juvenile fish can also be high due to the inability for fish to acclimatise to a seacage environment. There is now a movement in the seacage industry to extend the land-based component of fish production and to stock large, advanced, robust fish (~100g+) and thus reduce the period of time in which the fish are ongrown at sea. The major challenge with this strategy is to have economically and logistically viable technology for land-based production, especially considering the need for year-round production. Management of water quality including temperature and waste products is paramount to successful, intensive fish production. The suitability of intensive, land-based recirculating aquaculture systems and novel Biogill® filters on outdoor raceways for production of advanced YTK remain to be tested in NSW conditions.

Objectives

1. to validate the feasibility of the PSFI hatchery to produce commercial quantities of YTK fingerlings

2. to determine the feasibility and logistics of large-scale, land-based systems for production of advanced juvenile YTK

Method - Hatchery

The applied research project focussed on a series of objectives designed to cover aspects of fingerling supply chain, fish growth, and economics and logistics. Research on production of YTK had been undertaken in SA and WA and NSW to improve larval rearing methods to produce high-quality fingerlings (e.g. Seafood CRC project: 2011/740) and to understand the performance of YTK in seacages in SA (e.g. Seafood CRC project: 2008/903 Understanding YTK). This project adopted outcomes of these projects to develop strategies for production of high-quality, advanced juvenile YTK in NSW.

Evaluation of large-scale land-based YTK fingerling production was divided into two components: hatchery and nursery.

Hatchery Experiments

General

The Port Stephens Fisheries Institute (PSFI) marine fish R&D unit has a long history with development of technology for production of juvenile marine fish including snapper, mulloway and YTK and commercial production of hundreds of thousands of juvenile mulloway and snapper at the PSFI has been proven. PSFI has developed and improved broodstock management and larval rearing techniques for year-round production of high-quality YTK (Fielder and Heasman, 2011) at an experimental scale, however, the capacity of the PSFI hatchery to reliably produce commercial quantities of juvenile YTK remained to be demonstrated. This was evaluated by repeated operation of the commercial-scale intensive hatchery. The standard larval rearing tanks used at PSFI were 2000L with conical-bottoms and were operated on single pass seawater systems (Fielder & Heasman, 2011).

The rearing regime or ‘recipe” for rearing of YTK larvae (Fielder and Heasman, 2011) was attempted to be improved through replicated commercial-scale (n=3 reps/treatment) experiments designed to determine optimal abiotic factors including, tank colour, salinity, photoperiod and light source. The experimental treatment was compared with the current best-practice recipe in the commercial-scale tanks and production was evaluated in terms of swimbladder inflation, larval survival, growth and fish quality, especially the degree of jaw and spinal deformation.

It should be noted that due to demand from Huon Aquaculture Ltd from the PSFI hatchery to supply 25,000 YTK fingerlings (20g) every 3 months for seacage growout, the scope of the hatchery studies was limited. Experiment treatments were selected conservatively and based on the likelihood that they would not result in poor larval growth and survival, compared with the current best-practice larval rearing regime.

Statistical Analyses

Data were assessed for homogeneity of variance using Cochran’s test (C; Winer et al., 1991). Experiments were designed for analysis using single-factor analysis of variance (ANOVA). Where significant differences were found, means were compared with the Student-Newman-Keuls test (SNK). Statistical analyses were performed using NCSS Professional Version 8.0.23 (Hintze, 2012)

Broodstock and egg supply

YTK broodstock were held at PSFI in 4 independent 25,000 L recirculating systems (RAS; described in Fielder & Heasman, 2011). Briefly, each RAS consisted of a main fish holding tank, a 200 L sump for collecting eggs, a rotating drum screen-filter (20 um; Hydrotech 501), a moving bed bioreactor (biofilter) and reverse cycle refrigeration unit used to control water temperature (range 16-22 °C). Tanks were fitted with lids and fluorescent light was provided to control photoperiod (Fielder & Heasman, 2011). Each tank was supplied with filtered estuarine seawater (10µm) at

approximately 10% of the total volume each day. A 2.1 kW centrifugal pump circulated the tank water through the filters at a flow rate of 300-400 L min^{-1}. The bottom of each tank was siphoned weekly to remove build-up of organic material.

Each tank contained different numbers of broodstock. Two tanks contained wild broodstock (Tanks 1 and 8) and two tanks contained offspring of wild broodstock (Tanks 5 and 6) from the PSFI hatchery (i.e. Fl generation; Table 1). The broodfish were inducted into a wider feeding and nutrition study as a component of the K4P project with the aim to determine if changing YTK broodstock from a best practice' "natural" feeding regime of sardines and squid to a commercial "pellet" feeding regime affected reproductive output and egg quality. Detailed methods and results are discussed elsewhere (in Stone and Booth, 2019) but briefly, reproductive output was measured in several ways including fertilization rate and enumeration of eggs and hatched eggs. Morphometric indices such as egg size and oil droplet size were also assessed (n=20). In addition, the chemical composition of eggs was examined to determine if the feeding regime affected nutrient or fatty acid methyl ester (FAME) content. The YTK broodstock at PSFI had been genotyped, therefore the genetic diversity within different tanks was inferred using DNA extracted from eggs and PCR amplification in an attempt to relate these outcomes to diet selection.

At the commencement of the experiment, two broodstock tanks (Tanks 5 and 8) were allocated to the new "pellet" regime (Ridley Pelagica diet + Breed-M broodstock diet (Inve Aquaculture)); and 2 tanks (Tanks 1 and 6) were maintained on the current best-practice "natural" feeding regime (i.e. natural regime; sardines + squid). Fish were fed to apparent satiation once on Monday, Wednesday and Friday of each week at approximately 1300 h. This feeding frequency followed current hatchery practices at PSFI. Spawning cycles where fish were held at constant water temperature of 16°C and photoperiod of 10L:14D (hours of light:dark) for approximately three months followed by a rapid increase in water temperature from 16°C to 22°C over 24h (Fielder and Heasman, 2011) were established in an attempt to spawn eggs from all tanks every three months. Four spawning cycles were attempted:

Cycle 1 23 March 2016 to 5 June 2016 (74 days)

Cycle 2 6 June 2016 to 4 September 2016 (90 days)

Cycle 3 5 September 2016 to 4 December 2016 (90 days)

Cycle 4 5 December 2016 to 12 March 2017 (97 days)

Tank 5 and Tank 8 were returned to natural feed sources in cycle 4 (and thereafter). When the feed experiment was finished, all tanks of broodfish were returned to a diet of fresh squid and pilchards for ongoing spawning cycles.

The last week of each cycle was dedicated exclusively to egg collection and enumeration and fish were not fed during that week. All tanks of broodstock were induced to spawn spontaneously in the last week of each cycle by increasing the water temperature from 16 °C to 22°C within 24 to 48 h. Spawning typically occurred 3 to 4 days after the start of thermal manipulation (Fielder and Heasman, 2011).

Table 1. PSFI YTK broodstock inventory.

	Tank l	**Tank 5**	**Tank 6**	**Tank 8**
Fish type	Wild	Fl	Fl	Wild
Number of fish	7	5	8	9
Est. size of fish (kg)	25-26	12-13	10-11	10-12
Est. biomass (kg)	182	65	88	108
Food type	fresh	pellet	fresh	pellet

Effect of tank colour

Two commercial-scale hatchery experiments were done to determine the effect of tank colour (black [control] v reflective silver) on growth and survival of YTK larvae. We know that YTK larvae require high light intensity for early-stage development (Stuart and Drawbridge, 2011; Fielder and Heasman, 2011) and that YTK larvae typically occupy the upper depths of the water column which can potentially induce higher larvae density and subsequent reduced feeding efficiency and conspecific contact and cannibalism. The aim of the experiments was to determine if coating the tank wall with a reflective, silver lining would increase light intensity through the water column and encourage homogeneous dispersal of YTK larvae through the water column. The experiments were done in 6 x 2000L conical-bottom upwelling tanks situated in a controlled environment room. Three tanks had black sides and a white bottom (the standard PSFI hatchery design) and three tanks had the entire wall and floor covered with a silver contact material (Platino silver self-adhesive lining paper, DC Fix). Larvae stocking density (20-60/L), larval feeding and water management were consistent with standard hatchery procedures used for YTK larval rearing at PSFI (Fielder and Heasman, 2011). Light intensity provided to all tanks was approximately 1500 Lux at the centre of the tank surface with photoperiod of 12L:12 D. Because the number of replicate tanks was low (n=3), the experiment was repeated in time. Juvenile fish produced in Experiment 1 and Experiment 2 contributed to nursery production of Batch 1 and Batch 2, respectively.

Effect of light source

Two experiments were done to investigate the effects of LED floodlights (Voltex Electrical, 100W LED High Bay Light - cool white; Experiment 1) and Full spectrum LED lights (ViparSpectra LED light model R900, www.vipaspectra.com; Experiment 2) compared with the standard mercury vapour HiBay lights (HB400MV 400W Mercury Vapour Crombay High Bay Light Fitting) used in the PSFI hatchery. The mercury vapour lights are aging and new LED technology has entered the market. Halogen lights are expensive to operate in terms of power consumption, and produce a lot of heat which can be detrimental to maintaining optimal temperatures in intensive hatcheries. In contrast LED lights are energy efficient, long-lived and produce little heat. All other hatchery operations followed the standard operating procedures used at PSFI (Fielder & Heasman. 2011). Fertilised eggs were obtained from wild, captive YTK broodfish, and newly-hatched larvae stocked at 20 larvae/L (Experiment 1) and 5 larvae/L (Experiment 2) to each of six 2000L tanks. Each tank was surrounded by a black plastic curtain to prevent illumination from adjacent tanks. The larvae were cultured for 18 days and then harvested and stocked to nursery tanks.

Commercial-scale production (Batch's 3b, 4a, 4b, 5a, 5b, 6)

Six commercial-scale hatchery experiments were done to evaluate the PSFI intensive hatchery for reliable production of YTK fingerlings. The need to ensure the target number of juvenile YTK (25,000 fish/3months and 20g/fish) for supply to Huon Aquaculture to stock the seacages was a significant driver for the way in which the hatchery was operated and the degree of risk that we were able to take with respect to experimental treatments. Following the results of the first two experiments which showed the standard black-sided tanks at PSFI were optimal for YTK larval production, we ran all 6 hatchery tanks using standard protocols described in Fielder & Heasman (2011).

Assessment of microbiome development in YTK

A study was done by Jackson Wilkes-Walburn in fulfilment of an Honours thesis (UNSW) to investigate issues of the diversity of larval YTK intestinal microbiome and how it develops in a larviculture system. Next-generation DNA sequencing of the 16S rRNA gene were used to show that the intestinal microbiome experienced variation in the relative abundance of bacteria and shifts in both diversity and key bacterial taxa throughout the development of *S. lalandi* larvae. The thesis abstract is furnished in Appendix 1.

Results - Hatchery

Spawning

Fecundity and hatch rate

Collectively, the broodstock held on the natural feed regime or changed back to the natural feed regime in cycle 4 produced a total of approximately 7.4 million eggs over 4 cycles, whereas fish fed the pellet regime produced about 0.75 million eggs (Figures 1 and 2). Note that Tank 5 and Tank 8 were returned to natural feed sources in cycle 4 (and thereafter). Apart from a minor single spawn in cycle 1 (<12,000 eggs total), Tank 8 did not spawn again until being returned to natural feeds in cycle 4, where five spawnings occurred that collectively produced more than 400,000 eggs. The fecundity of the main breeding tank (Tank 1) decreased steadily from cycle 2 indicating the high frequency of spawning might have influenced the fecundity of fish in this tank.

Mean fertilisation rate of eggs varied between spawning event within tanks and between tanks and ranged from averages of 30-80%. Mean fertilisation rate for Tank 1 decreased from cycle 1 of approximately 70% to cycle 3 of approximately 40% but showed some improvement in cycle 4 of 65%. Fertilisation rate in Tank 5 was high at 70-80% in three of the spawning cycles and was low but similar to other tanks in cycle 2. Mean fertilisation rate of eggs in Tank 6 was lowest in the first spawning cycle (30%) but increased steadily with each subsequent spawning cycle to approximately 80%. Tank 8 only spawned during the last spawning cycle 4 with mean fertilisation rate of 60% (Figure 3)

In general, mean hatching rates were similar between tanks within spawning cycles and were 70-80%. Mean hatching rate in Tank 1 however showed a steady decline from approximately 60% in spawning cycle 1 to 35% in cycle 3 (Figure 4). The number of larvae successfully hatched from viable (ozonated) eggs derived from broodstock fed natural feeds was approximately 3,064,645 and derived from pellet fed regimes was 411,178.

The mean diameter of eggs and oil droplet did not differ between tanks or broodstock feeding regimes (Figures 5 and 6).

Genetic diversity of offspring

Eggs collected from broodstock tanks during cycle 1 and cycle 2 were linked to individual parent stock within each tank. This allowed identification of the individuals that were making a contribution to different spawning events. However, although broodstock had been genotyped, the sex of the animals was unknown. Enumeration of the data indicated there were greater numbers of offspring groups identified in the wild (Tank 1) and Fl (Tank 6) broodstock fed sardines and squid than in the wild (Tank 8) and Fl (Tank 5) broodstock fed pelletised feeds (Table 1). When the data was compared using feed type as the fixed factor, ANOVA found a highly significant difference ($F_{1,2}=50.89$; $P=0.019$) between the mean number of offspring groups identified in the natural fed group (138.0±2.8) as opposed to the pellet fed group (26.5±21.9).

Figure 1. Total number of eggs spawned by YTK broodstock in each tank during each spawning cycle.

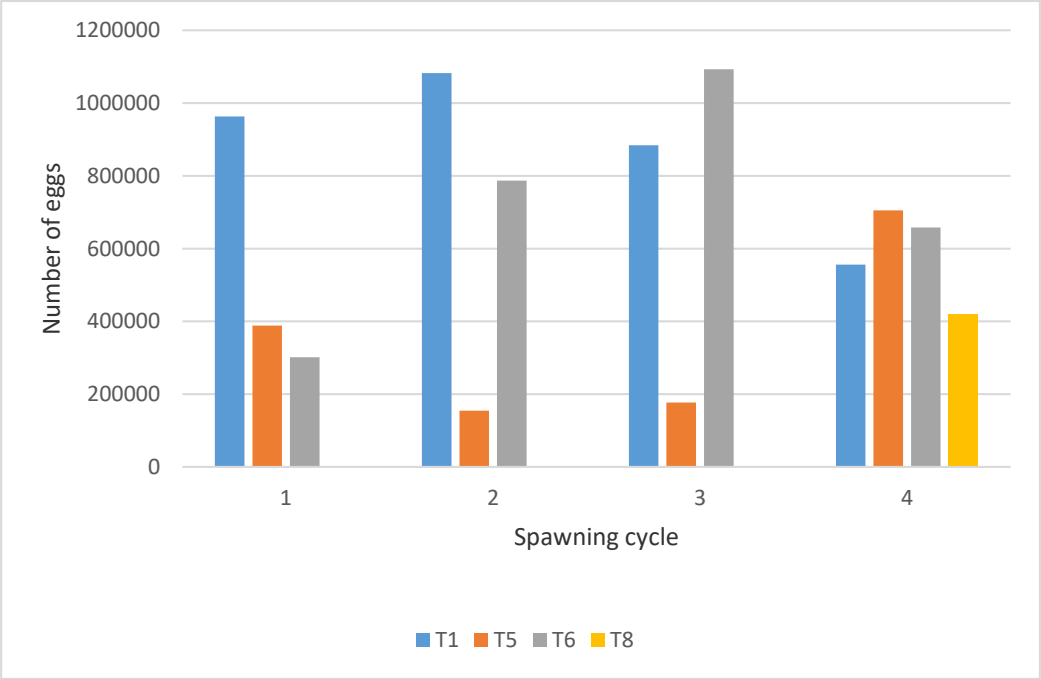


Figure 2. Total number of viable eggs for each diet for each spawning cycle.

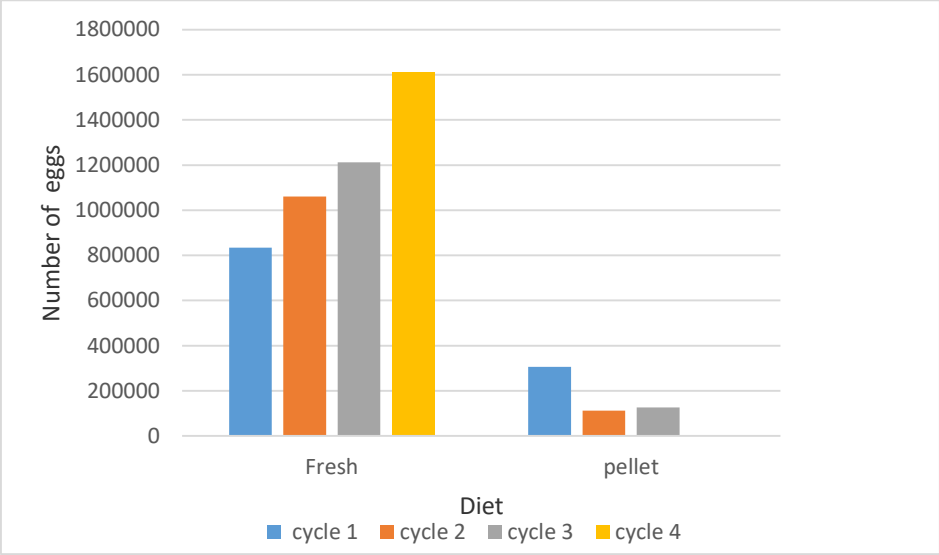


Figure 3. Mean ± SD fertilisation rate for each spawning cycle.

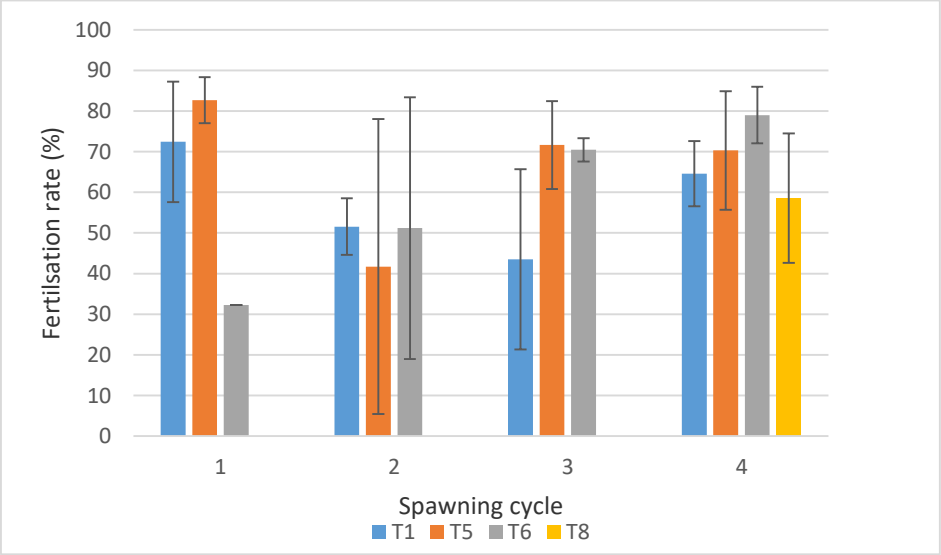


Figure 4. Mean ± SD hatch rate (%) for each spawning cycle.

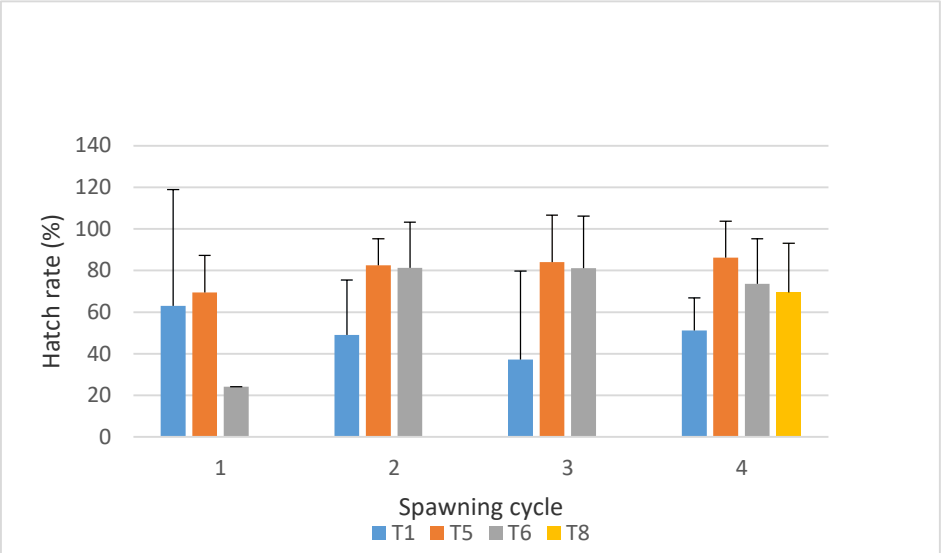


Figure 5. Mean ± SD egg diameter of viable eggs.

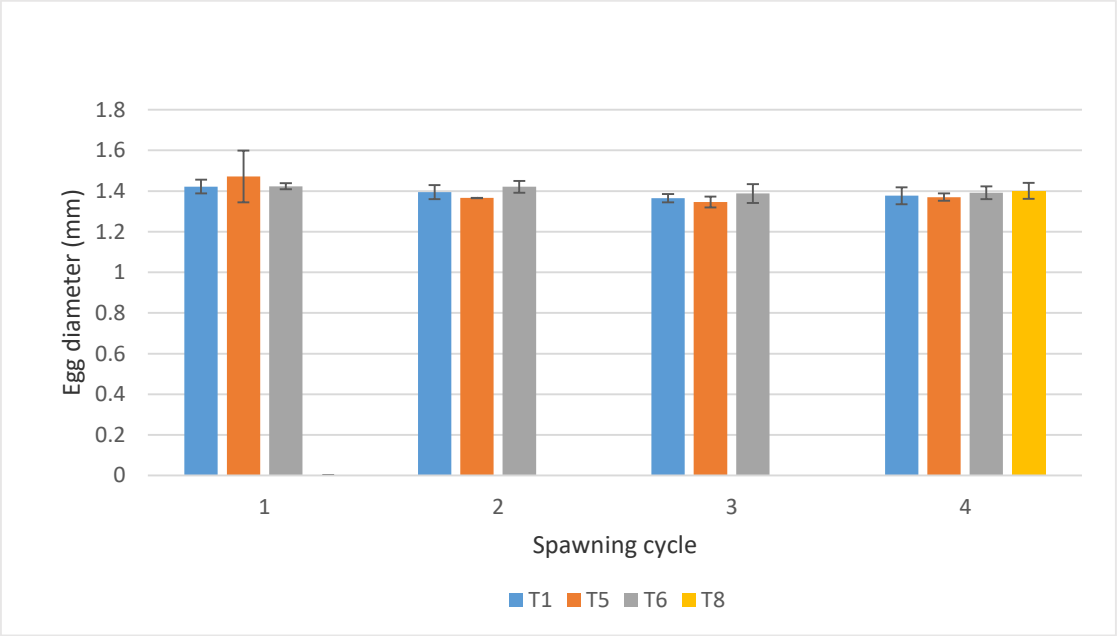
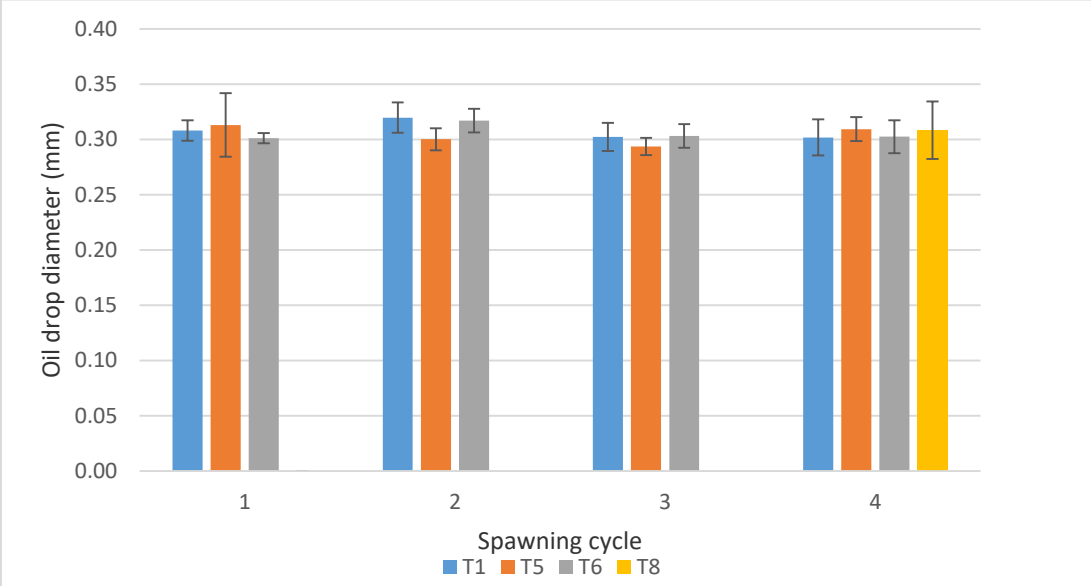


Figure 6. Mean ± SD oil drop diameter of viable eggs.



Hatchery experiments.

Effect of tank colour

Tank colour did not affect survival (Figures 7 and 8) or growth (Figures 9 and 10) of YTK larvae in both Experiment 1 and Experiment 2, despite larvae in silver-lined tanks appearing to use more of the water column. Light intensity ranged from approximately 5000 lux at the water surface to approximately 800 lux at the bottom of the tank (~ 1m deep) with only minor differences between the tank colour treatments. However tank colour affected the external appearance of larvae with fish from silver–lined tanks being darker than those grown in black tanks (Figure 11), indicating that silver-lined tanks likely reflected more light from the tank bottom compared with black tanks. Differences in fish skin colour were transient and all fish became the same colour once removed from the treatment tanks and placed into common nursery tanks.

Based on these results, the standard PSFI black tank colour was deemed optimal for YTK culture and remained as control tanks for future experiments.

Figure 7. Survival of YTK larvae grown for 30 days in 2000L tanks with different internal colour. Data are means ± SD (n=3 tanks). Experiment 1.

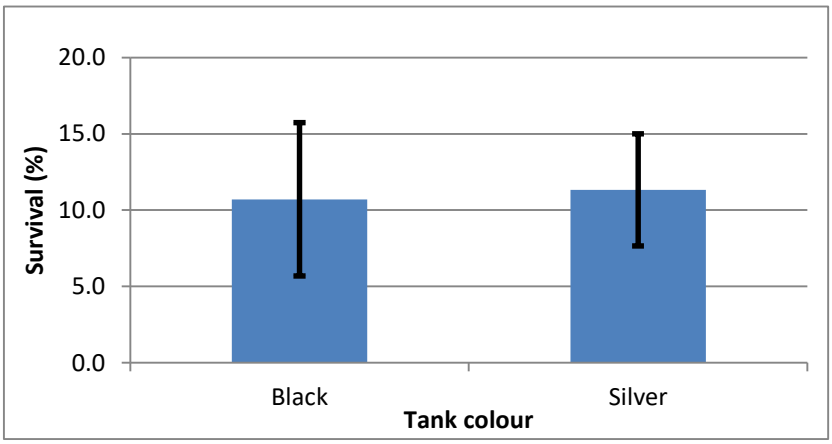


Figure 8. Survival of YTK larvae grown for 30 days in 2000L tanks with different internal colour. Data are means ± SD (n=3 tanks; NS P=0.87). Experiment 2.

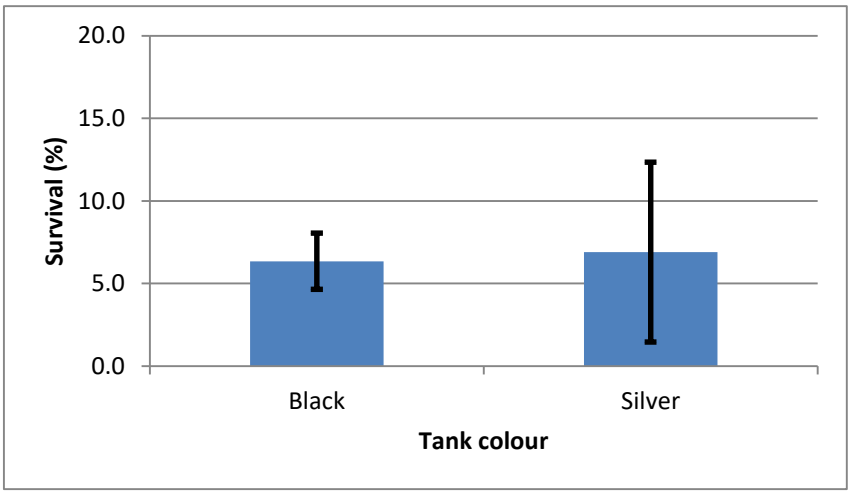


Figure 9. Standard length of YTK larvae grown for 30 days in 2000L tanks with different internal colour. Data are means (n=3 tanks). Experiment 1.

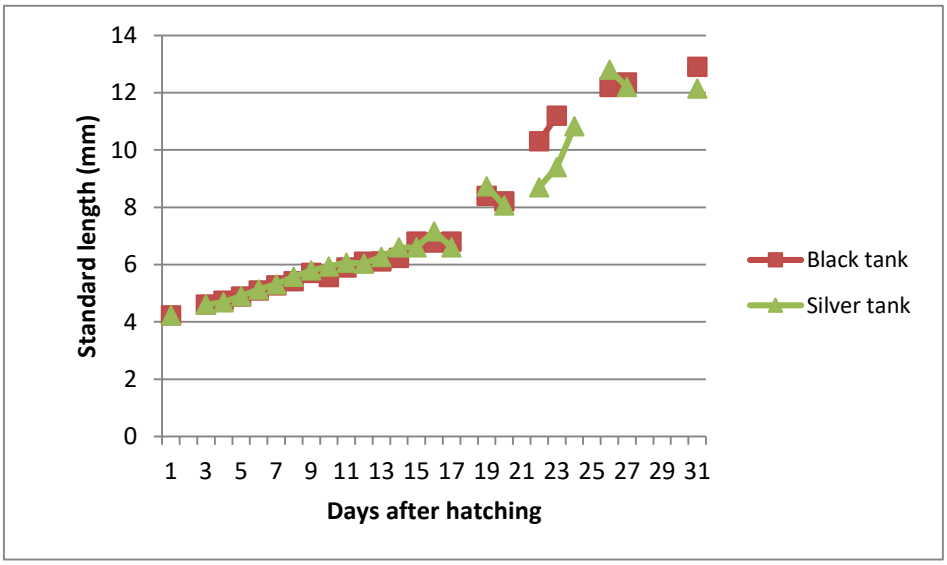


Figure 10. Standard length of YTK larvae grown for 25 days in 2000L tanks with different internal colour. Data are means (n=3 tanks). Experiment 2.

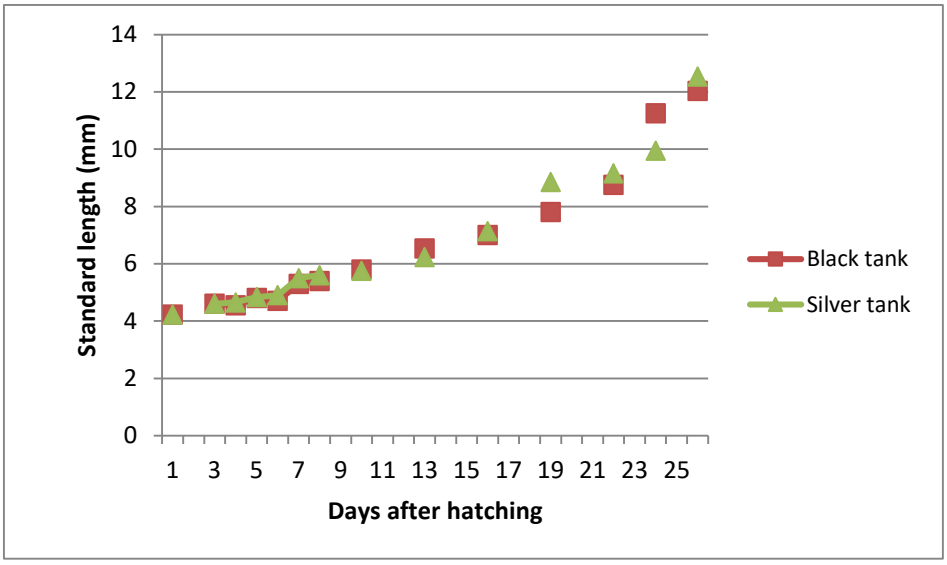
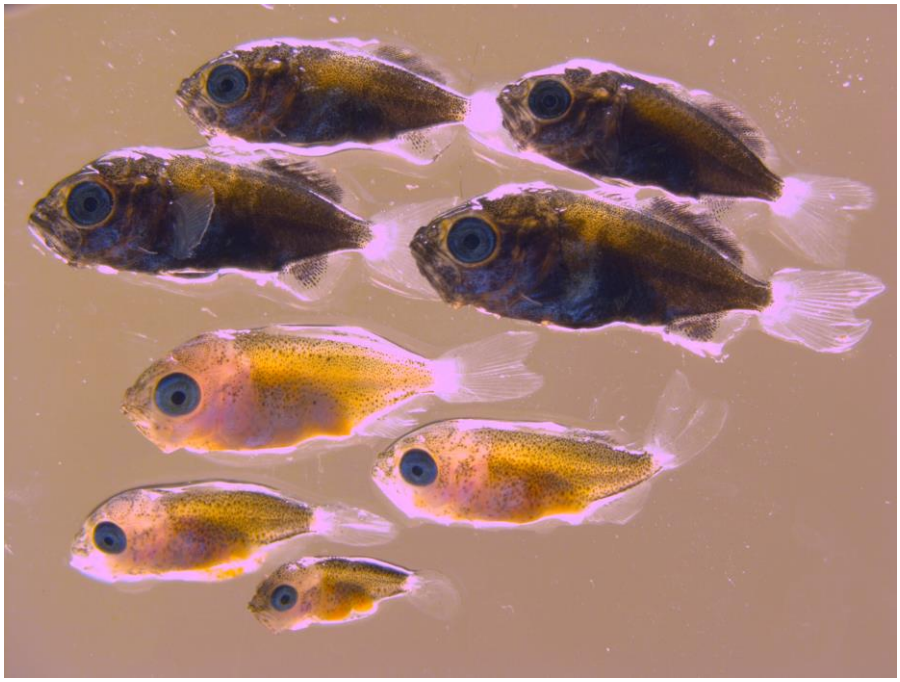


Figure 11. External skin colour of 21 dph YTK larvae reared in silver-lined tanks (top 4 dark fish) and black tanks (bottom 4 pale fish).



Effect of Light

Experiment 1. Effect of LED floodlights

Significant mortality occurred in all tanks between 7-9 dph, however, there was a trend that higher mortality occurred in the LED treatment tanks and the LED lights were removed. The experiment was terminated, Hibay lights were reinstalled on all tanks and the hatchery was operated following normal hatchery rearing protocols for commercial-scale production (Batch 3a). Unfortunately, continued mortality occurred in most hatchery tanks and survival was low at 0.4% resulting in production of 5338 juvenile YTK.

A further hatchery run (Batch 3b) was started as soon as the hatchery was emptied in an attempt to produce target numbers of fingerlings for stocking to seacages for the third stocking cycle (Reported below in commercial hatchery runs).

Experiment 2. Effect of full spectrum LED lights

The Hibay lights were almost twice as bright as the LED's at the surface and brighter at all depths through the water column; however, due to large within-treatment variation, light intensity was not statistically different (Figure 12). The light source did not affect survival (Figure 13), growth (Figure 14), swimbladder inflation (Figure 15) or first feeding (Figure 16). Results demonstrate that either Hibay or full spectrum LED lights are suitable for YTK larval rearing.

Figure 12. Light intensity produced by Hibay and LED lights measured at the tank surface and at depth. Data are means (n=3 tanks).

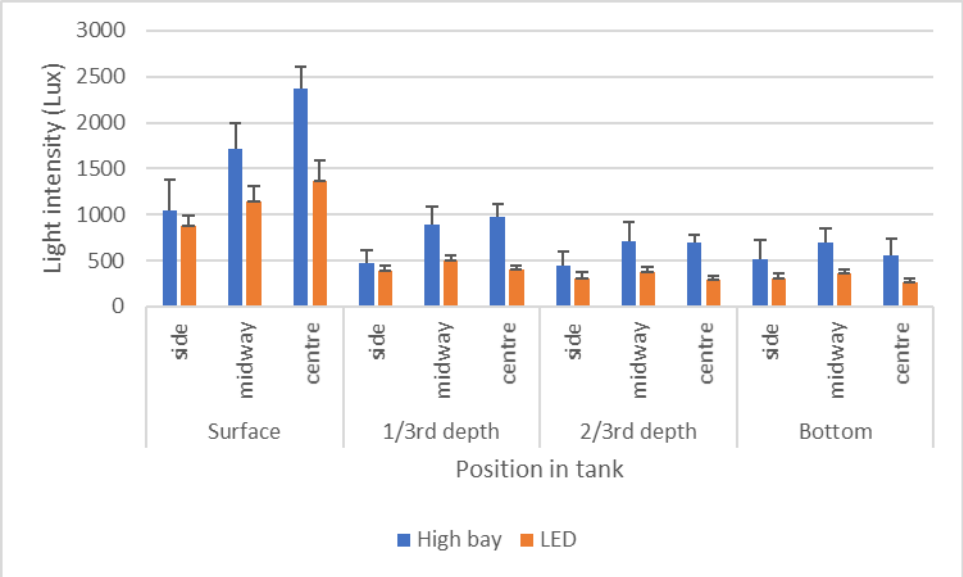


Figure 13. Survival of YTK larvae grown for 18 days in 2000L tanks and illuminated with different lights. Data are means ± SD (n=3 tanks). NS, P=0.106

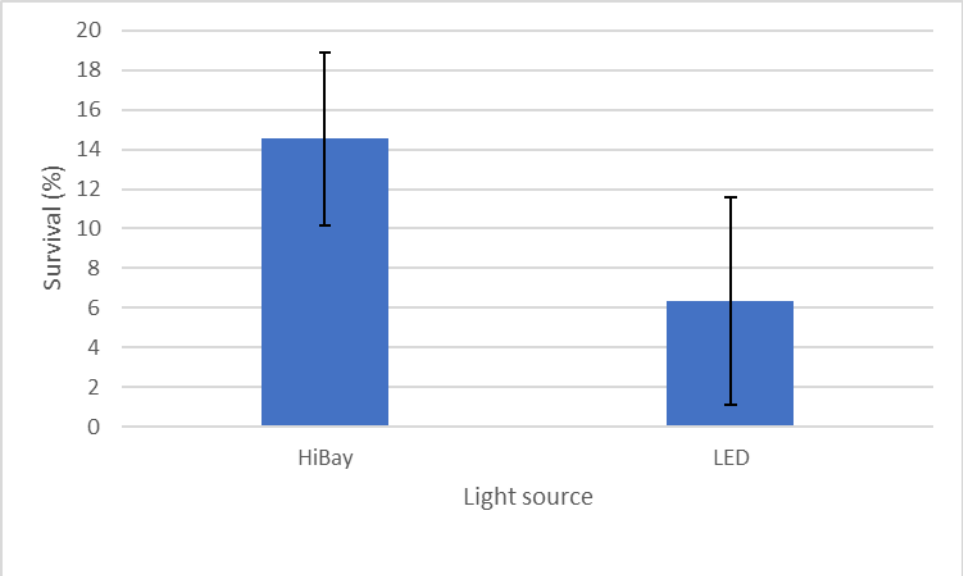


Figure 14. Standard length of YTK larvae grown under Hibay or LED light. Data are means ± SD (n=20 larvae for 3 tanks).

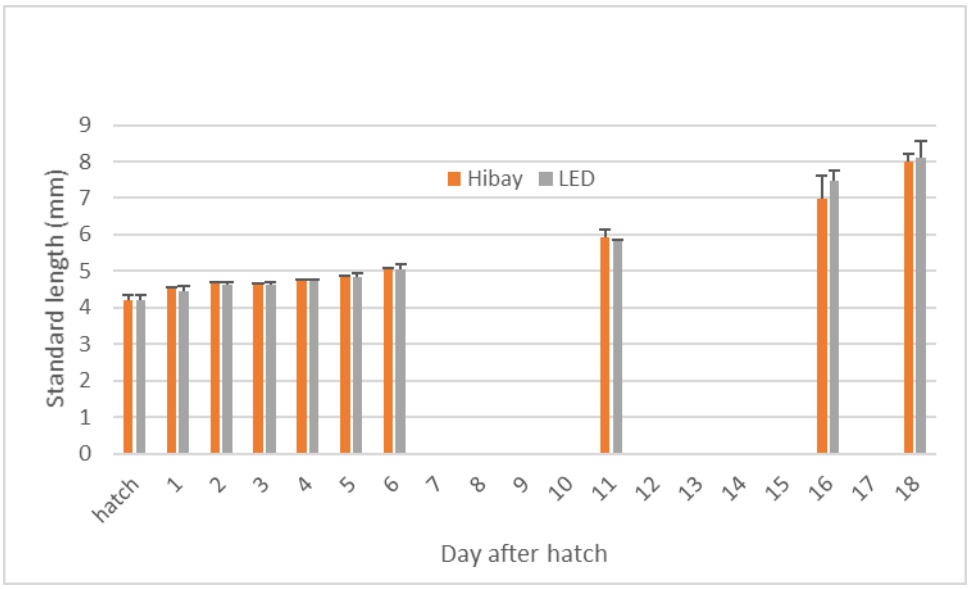


Figure 15. Swimbladder inflation of YTK larvae grown under Hibay or LED light. Data are means ± SD (n=20 larvae for 3 tanks).

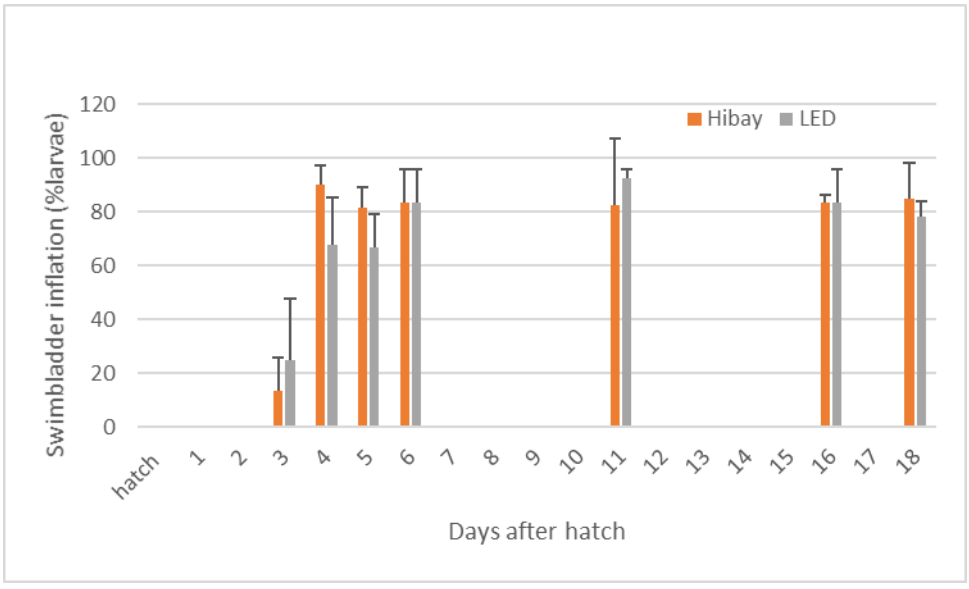
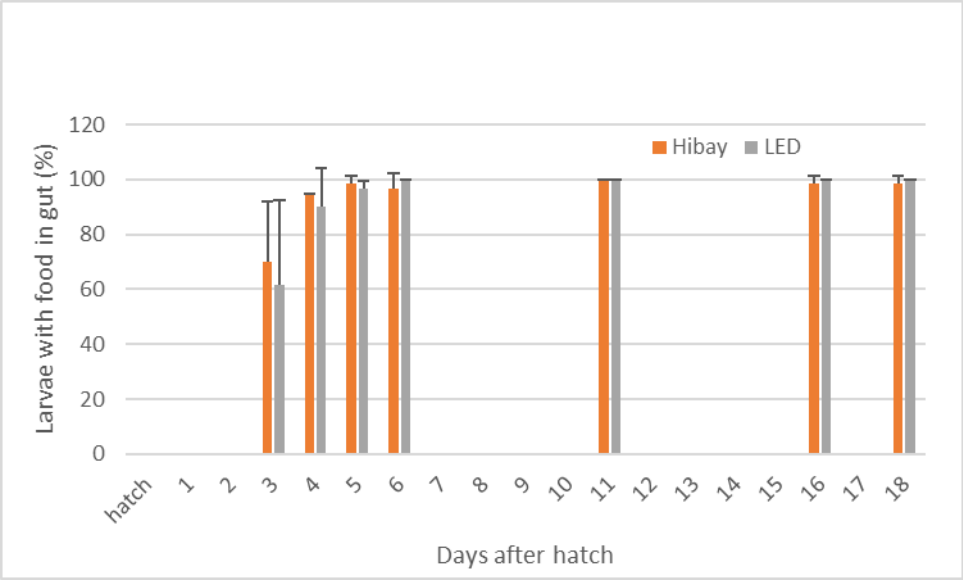


Figure 16. Number of YTK larvae with food observed in the gut when grown under Hibay or LED light. Data are means ± SD (n=20 larvae for 3 tanks).



Commercial Hatchery production runs

Commercial batch 3b Results

Following the failure of the experiment to investigate the effect of LED floodlights on YTK larval production, a further hatchery run (Batch 3b) was started as soon as the hatchery was emptied in an attempt to produce target numbers of fingerlings for stocking to seacages.

Management of water temperature with room air-conditioning was a significant challenge during extreme heatwaves experienced at Port Stephens and water temperature in the hatchery tanks occasionally exceeded the target temperature (21ºC) by 5ºC. Compared with previous hatchery batches, the survival of larvae to metamorphosis appeared to be compromised by the higher temperature and ranged from 1.9% to 6.6%. A total of 17,500 juvenile YTK were produced (Figures 17 and 18). Both Batch 3a and 3b juvenile YTK were ongrown separately but growth was managed to allow amalgamation of similar size fish for stocking to seacages (reported below).

Figure 17. Survival of YTK larvae in individual hatchery tanks (6 tanks/batch). Bars with the same colour are the same tank.

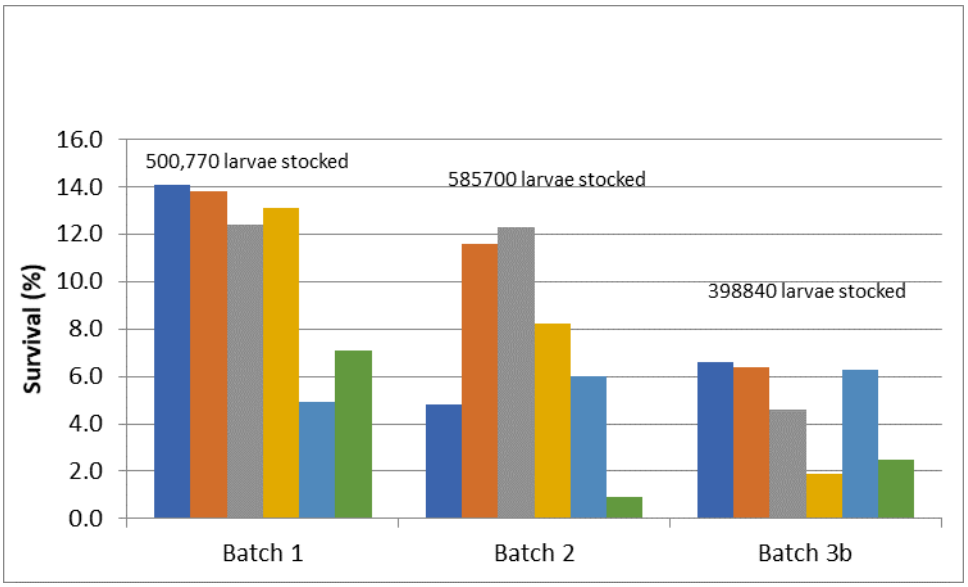
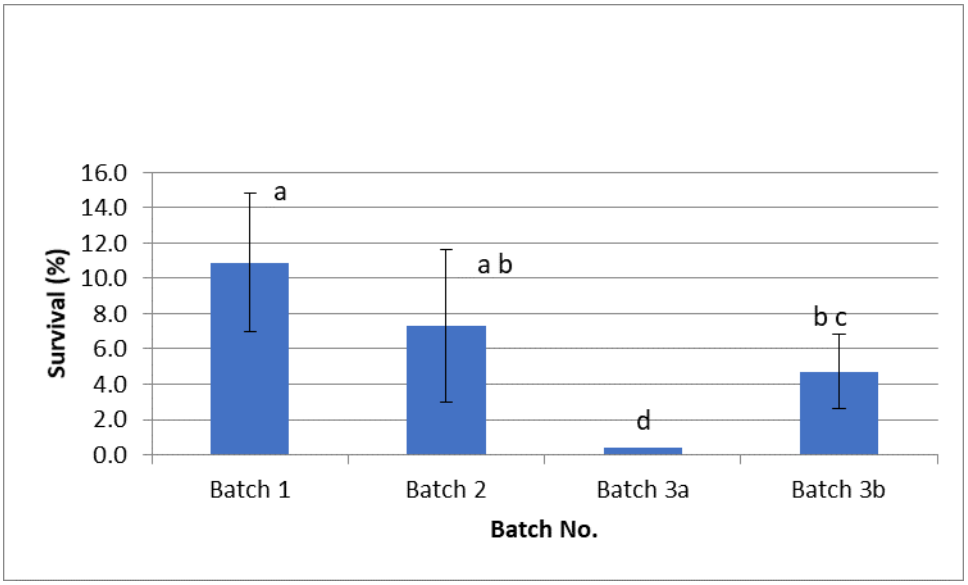


Figure 18. Mean ± SD survival of YTK cultured in the PSFI hatchery 2016/2017 (n=6 tanks/batch).



Commercial Hatchery production run Batch 4a and 4b results

Batch 4 production consisted of two hatchery runs, Batch 4a and Batch 4b. Based on problems experienced with hatchery production with Batch 3, a decision was made to attempt to optimise larval rearing conditions for Batch 4a. Significant larval mortality occurred in all tanks at approximately 7-12 dph, and was coincidental with an extreme rainfall event which resulted in deterioration of estuarine water quality, and especially a decrease in salinity from 35ppt to 29ppt (Figures 19 and 20). Survival was low (2.0%) resulting in production of 12,000 YTK fully weaned fry.

Figure 19. Representative larval rearing tank water quality for Batch 4a

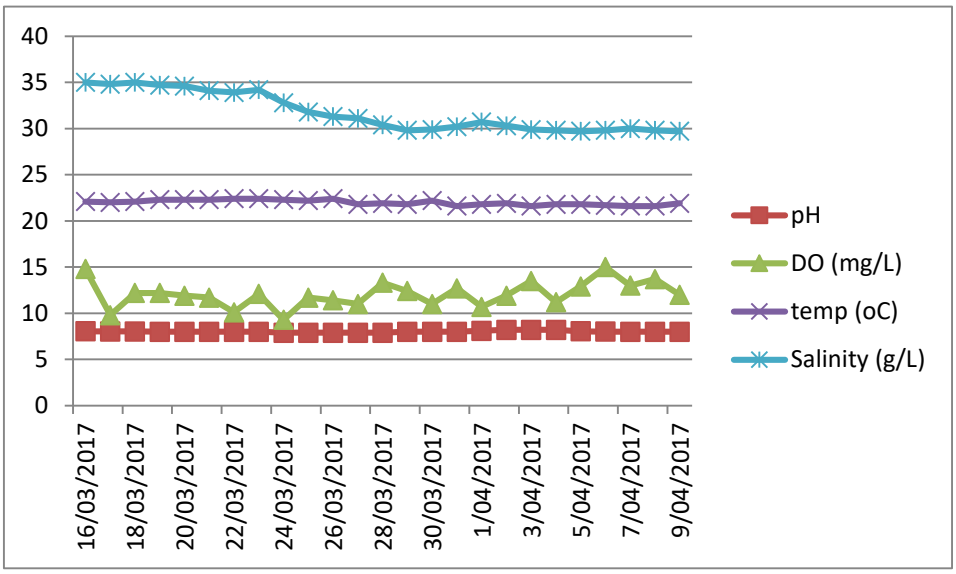
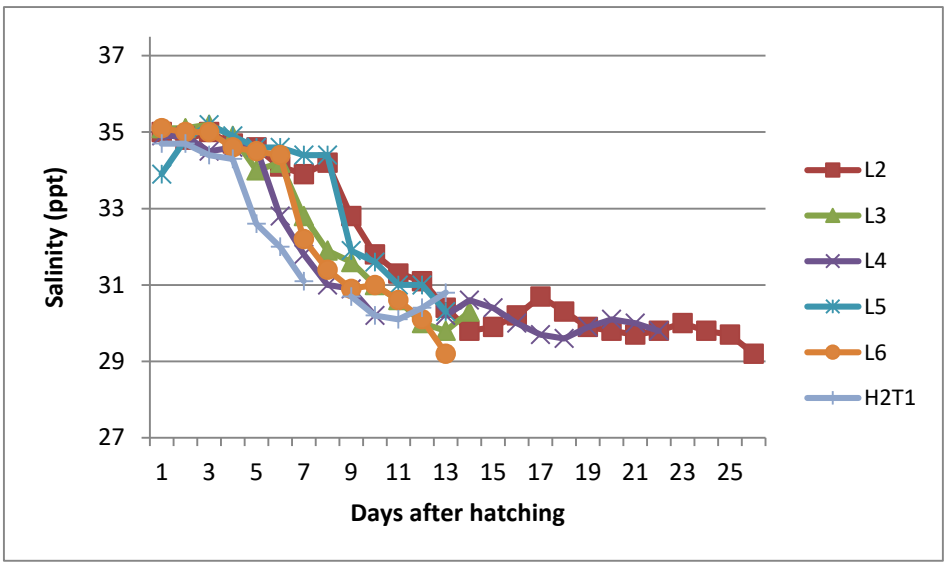


Figure 20. Salinity for all hatchery tanks (L2, L3, L4, L5, L6 and H2T1) during production of Batch 4a.



A further production hatchery run, Batch 4b, was done as soon as the hatchery was cleaned and prepared for stocking. Attention was made to the salinity of the influent seawater and if lower than 35 ppt, adjusted to 35 ppt by addition of artificial sea salt (see Figure 21). In addition, once larvae had finished swimbladder inflation at 6/7 dph, the photoperiod was increased from 12:12 L:D to 24:0 L:D to promote continued swimming and to potentially avoid night-time larval sinking. This hatchery run progressed without incident and survival of 9.1% was equivalent to the previous best Batch 1 (Figure 22). Further experimentation is required to confirm the interaction of salinity and photoperiod on the effect of early-stage larval YTK sinking. A factorial experiment has been designed to compare the combined effect of salinity and photoperiod on survival and growth of post-swim bladder inflation YTK larvae and will be done in 32x100L experiment tanks at PSFI.

Figure 21. Water quality of a representative larval rearing tank for Batch 4b.

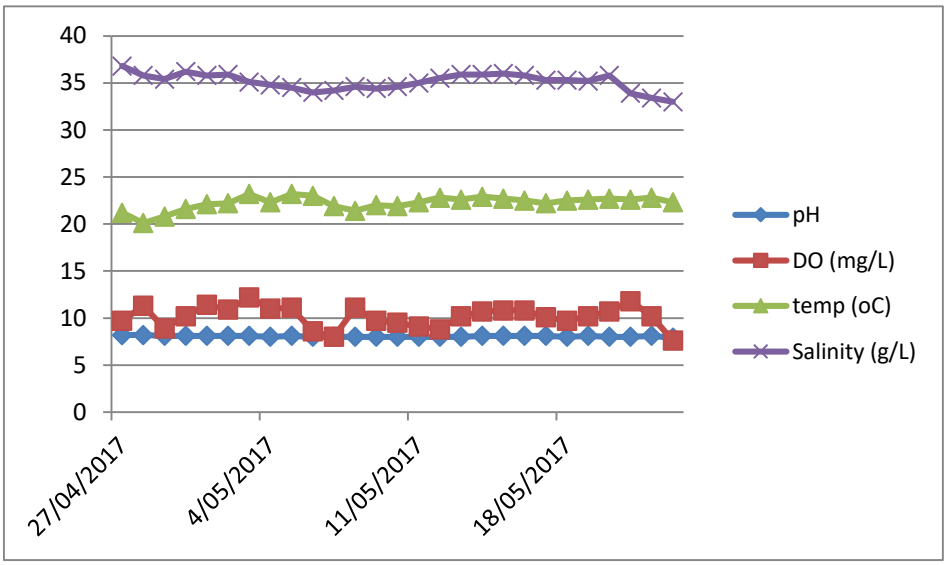
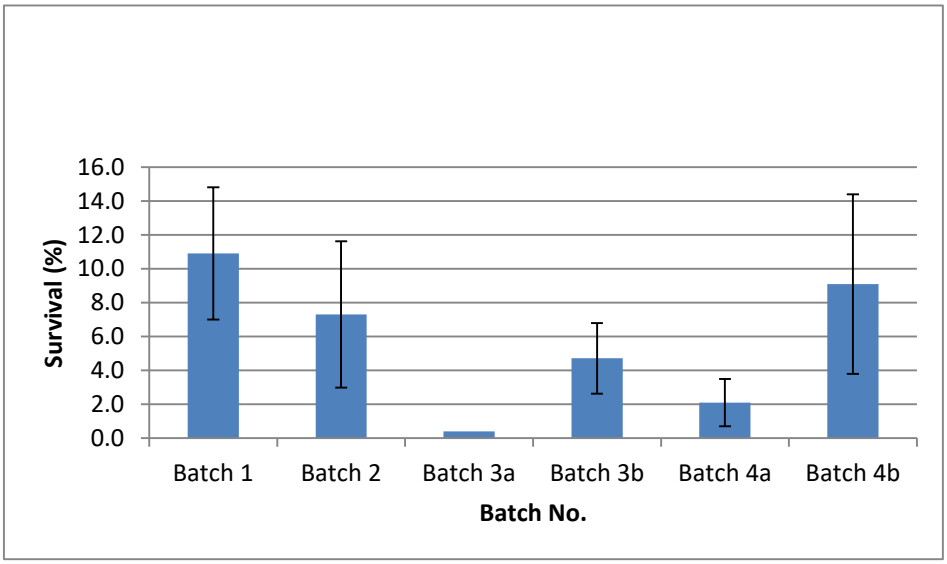


Figure 22. Survival of YTK larvae in all larval Batch's completed at PSFI. Data are means ± SD (n=6 tanks/batch).



Commercial production runs batch 5a, 5b and 6

Batch 5a and 6 resulted in complete failure of the run. Deformed larvae and swimbladder hyperinflation of 6-12 dph larvae were experienced in both runs and 100% mortality occurred. This was despite our efforts to avoid previous issues with water quality by using trucked ocean seawater and adding artificial sea salt to ensure salinity was 35ppt. No pathology was reported following intensive investigation of both batches of larvae.

A total of 17000 YTK were produced in batch 5b. These fish were successfully ongrown in various nursery systems (reported below).

Discussion – Hatchery

Broodstock

A diet of high-quality frozen pilchards and squid fed to broodfish YTK resulted in higher fecundity and hatching rates in both wild-caught and F1 broodfish compared with a diet of commercial pellet feeds. Broodfish diet did not affect egg morphology. A diet of pilchards and squid also resulted in more cohorts of progeny in wild-caught and F1 broodfish compared with that of broodfish fed commercial diets. These results confirmed that natural feeds support better outcomes in YTK broodfish maintained at PSFI and this feeding regime will continue to be used as the 'best-practice' diet (Fielder and Heasman, 2011).

The PSFI 'best-practice' feeding regime entails feeding broodfish to apparent satiation three times a week (Monday, Wednesday and Friday) at approximately 1300 each day. Mature, wild-caught YTK spawn spontaneously in captivity after 1 to 2 years domestication. In our hatchery, year-round, on-demand spawning has been achieved for many years by exposing wild-caught broodfish to truncated photo-thermal regimes (Fielder and Heasman, 2011). In contrast, we have little knowledge of the effect of this management strategy on F1 broodfish or indeed the effects that regular and repeated manipulation of the photo-therm to stimulate spawning has on reproductive success of wild-caught and F1 YTK broodfish. The choice of feed-type, feeding strategy and spawning frequency used by the hatchery needs to account for biological factors which control which control gonad development, oogenesis, spermatogenesis, duration of the vitellogenic cycle and oocyte maturation. The fecundity and fertilisation rates of the main breeding tank (Tank 1) decreased steadily with each successive spawning cycle, suggesting that the frequency of spawning every three months, compared with a natural, single-season (Spring-Summer) spawning event per year was excessive, and negatively influenced egg/sperm quality and fecundity. The short and repetitive spawning cycles adopted due to the need to supply juvenile YTK for commercial growout R&D may have placed undue reproductive stress on the brood YTK and/or their ability to undergo effective gamete recrudescence. Alternative methods to manage broodfish including maintenance of more tanks of mature YTK which are held at different stages of photo-thermal manipulation allowing individual tanks to be spawned once each year and then returned to a long-term period of recrudescence, will need to be considered in future of multiple spawning events per year are required.

Hatchery

Effect of tank colour

Yellowtail kingfish (YTK) larvae are sight-feeders and require high intensity of light to optimise survival in hatchery tanks (Stuart and Drawbridge, 2011; Fielder and Heasman, 2011). Early-stage yellowtail kingfish larvae at PSFI typically occupy the top 1/3 of the water column. This behaviour is suboptimal in terms of larval density as fish are not homogeneously distributed through the water

column. Inefficient feeding can occur as live feeds including rotifers and artemia are distributed homogeneously throughout the water column, thus ostensibly rendering unavailable approximately 2/3 of the feed population. In addition, higher larval density can result in increased conspecific contact and subsequent cannibalism. We tested, in two repeated experiments, whether changing the tank colour from the standard black sides (we assumed had high light absorbance) and a white bottom to highly reflective silver sides and silver bottom affected YTK larvae performance. We were unable to measure any differences in light intensity through the water column and there were no differences in survival or growth of YTK larvae. However, the larvae cultured in the silver tanks were significantly darker in colour than those cultured in the black-sided tanks with white bottoms and demonstrates a response to different light reflection/refraction from the tank bottom.

Changes in external fish colour due to melanosome migration, variations in melanophore size and density and skin melanin content, are well understood as a means for background adaptation or camouflage (Leclercq et al., 2010). The major factor which influences fish colour change is the ratio of the intensities of light from above and below the fish (Sugimoto, 2002). The penetration of light in the aquatic environment together with the substrate reflectance is, therefore, an important factor, which varies with water spectral absorbance and turbidity. Typically, fish exposed to a white background become pale in colour, while the opposite is observed for fish held on a dark background (Leclercq et al., 2010). Interestingly, YTK larvae held in tanks with silver bottoms were significantly darker in colour than larvae held in tanks with white bottoms. This suggests that silver-bottom tanks reflected less light than white-bottom tanks and is counterintuitive to our hypothesis that silver would be more reflective than white. The silver film which we used in the tanks had a slightly matt surface which may have caused incident light to scatter randomly rather than reflect directly back into the water column, resulting in the fish recognising the silver-bottom tank as a dark bottom.

Based on these results we determined that the standard black-side and white-bottom tanks at PSFI were better than silver tanks for YTK larval rearing.

Effect of Light

Light is a key environmental factor that synchronizes all life-stages of fish, from embryo development to sexual maturation. The underwater photo-environment is complex since light characteristics (i.e. intensity, photoperiod and spectrum) depend on the absorbance properties of the water column (Villamizar et al., 2011).

Marine fish larvae including YTK, are generally sight feeders and their retinas possess visual pigments that respond to specific wavelengths facilitating object detection (Kjorsvik et al., 2004). Fish species that occupy different ecological environments have different visual sensitivity to different wavelengths (Bowmaker, 1990; Lythgoe, 1979). There is, therefore, the possibility that the larvae visual system could be predisposed to perform best under spectral conditions most frequently encountered in its particular ecological niche. YTK larvae are pelagic and oceanic and are known to require high light intensity conditions of 14,000 to 30,000 Lux to perform best in cultured conditions (Stuart and Drawbridge, 2011; Fielder and Heasman, 2011). Providing high light intensity with artificial lighting is a challenge, and available lights may vary in spectrum. PSFI has used mercury vapour hibay lights since 2010 for YTK larvae production, but they are expensive to operate, produce a lot of heat and being 240volt are dangerous in a wet, salty environment of a marine fish hatchery. New LED technology has emerged on the market that needed evaluation for its suitability for YTK larval rearing. In particular, full-spectrum 12 volt LED lighting systems provide wavelengths in the visible spectrum of light that range from 360 nm (violet) to 750 nm (red) (Bohren and Huffman, 1983), are being used successfully in the marine aquarium fish industry. The 12 volt lights offer an added advantage of being relatively safe to operate in a hostile, wet environment.

We attempted two experiments to investigate the suitability of LED lighting for YTK larval rearing. The first experiment was terminated at 9 dph due to excessive mortality in all tanks, but it appeared mortality was higher in the LED floodlit tanks. Given the commercial pressure to produce YTK

fingerlings for Huon Aquaculture seacages, we removed the LED lights and reinstalled the mercury vapour hibay lights in an effort to improve the fingerling production outcome.

The second experiment investigated the suitability of Full-spectrum, 12 volt LED lights for YTK larval rearing. The standard mercury vapour hibay lights produced a light intensity that was almost twice as bright at the surface and at all depths through the water column as that produced by the LED lights; however there was no difference between treatments in larval survival, growth, swimbladder inflation or percentage of larvae that were feeding.

Our results demonstrate that the quality of light including intensity and spectrum, produced by mercury vapour hibay lights and full-spectrum LED lights is suitable for YTK larvae. YTK larvae are positively phototactic and tend to preferentially occupy shallow water depths in our culture tanks, particularly from the water surface to 30-50cm depth, where all wavelengths including the shortest violet and the longest, red, orange and yellow persist before they are absorbed quickly as depth is increased in water (Villamizar et al., 2011). This may suggest that YTK larvae are sensitive to the entire visible light spectrum however improvement in YTK larval culture may be elicited with further investigation of the sensitivity of larvae to specific wavelengths.

Commercial production

Six hatchery runs were done to produce a target of 25,000 YTK fingerlings/run (~20g/fish) at 3 monthly intervals between runs. In general, the production targets were met, however, we experienced intermittent problems with inconsistent survival between runs and between larval tanks within runs.

A common bottleneck to our production was high mortality of larvae at 6-12 dph. High mortality of YTK larvae is experienced by research and commercial hatcheries (Woolley et al., 2012; Stuart and Drawbridge, 2011) and has been attributed to poor initial swimbladder inflation, sinking of larvae at night, failure to feed, suboptimal light intensity, and supraoptimal water temperature. Despite our problems with low survival they were similar to survival rates reported by other hatcheries and were typically 5-10% to pre-weaning age of 16^{+} dph (Stuart and Drawbridge, 2011).

Prior to starting this project, we had completed many years of research in association with other Australian Research and commercial facilities including Challenger TAFE and SARDI, to develop a standard operating procedure (SOP) for YTK larval rearing at PSFI (Fielder and Heasman, 2011). The SOP which outlines optimal tank operating regimes including temperature range, light source, photoperiod, aeration/oxygenation levels, seawater exchange, and live feed type/density/enrichment, had resulted in consistent production of YTK fingerlings in our commercial-scale hatchery. We attempted to adhere to the SOP during all commercial hatchery runs during this project; however, we experienced two episodes of abnormal conditions which resulted in poor larval production.

The first episode occurred during Batch 3 when an extreme, prolonged heatwave during January 2017 caused failure of the hatcheries air-conditioning system to maintain the target tank water temperature of 21ºC. At times during the hatchery run, water temperature reached 26ºC which was 5ºC hotter than optimal water temperature. Temperature tolerance of marine fish larvae is species-specific and can vary with ontogeny (Fielder et al., 2005). Previous studies have shown that the optimal temperature range for YTK larvae is 21-24ºC (Fielder and Heasman, 2011; Ma, 2014). It appears that YTK larvae are stenothermal, especially within the first 10 dph, with growth and survival significantly reduced when larvae are cultured outside of the optimal temperature range. Ma (2014) showed that survival of YTK larvae when reared at 25ºC was significantly lower than those larvae reared between 21-23ºC and that 100% of YTK larvae died when they were cultured at 27ºC. The temperature of 26ºC reached during our YTK production run was therefore highly likely the reason why survival was lower than expected ranging from 1.9-6.6%. Maintenance of water temperature within a narrow range is critical in YTK hatcheries and cost-effective, reliable methods are fundamentally important for sustainable, commercial production of juvenile YTK.

The second episode occurred during batch 4a when an extreme rainfall event, which lasted for more than 7 days, resulted in the salinity of our hatchery intake seawater to reduce from 35 ppt to 29 ppt within two days. The larvae were 6 dph at the start of the salinity decline and significant mortality occurred. Similarly to temperature, the tolerance of marine fish larvae to changes in salinity is species-specific and can change with ontogeny (Fielder et al., 2005). Generally, younger less-developed larvae are less tolerant of change in salinity. The salinity tolerance of YTK larvae is unknown; however, YTK larvae are pelagic and oceanic, which suggests strongly that they are stenohaline, at least as early-stage larvae. The buoyancy of YTK larvae is controlled by the swimbladder, which inflates between 3-5dph (Fielder and Heasman, 2011; Woolley et al., 2012). Failure to inflate the swimbladder usually results in larvae dying as they cannot maintain buoyancy within the water column, swim continuously and become stressed. In addition, body-density of early-stage YTK larvae increases as larvae age is increased, and peaks with the highest body density at 12 dph (Woolley et al., 2012). In our production run, YTK larvae from 6-12 dph showed high rates of sinking out of the water column due to a decrease in salinity and subsequent reduction in larval buoyancy. Larvae that sink, often come in contact with the tank bottom, which is covered with a bacterial community, and likely contains potential pathogens including Vibrio spp. (Woolley and Qin, 2010; Vadstein et al., 2018). A reduced salinity and therefore environmental density will also have resulted in the need for larvae to expend more energy to maintain their position in the water column, and hence become stressed and die. Mean survival of larvae was low with an average of 2.0% to metamorphosis. Further research is needed to determine the optimal salinity range for larval YTK and to improve culture systems that can manage reduction in influent seawater salinity in the event of rainfall events.

Production run Batch 4b was started immediately following the completion of Batch 4a, and in an attempt to avoid problems caused by a potential reduction in salinity we added artificial seasalt to maintain salinity at 35 ppt. We did not experience any abnormal larval YTK mortality during the run and average survival of 9.1% was the same as the best production in Batch 1.

We experienced differences in larval survival between tanks within production batches which is common in hatchery operations, and often for unknown reasons. Trained fish pathologists were unable to detect any pathology in samples of moribund larvae which we supplied routinely for examination. The differences in performance of larvae held in different tanks may be explained by the operating system employed at PSFI and resultant detrimental fish-microbe interactions (Vadstein et al, 2018). Like many marine fish hatcheries throughout the world, we employ ostensibly a flow-through system where tank water is replaced with influent seawater on a daily basis and without being recycled. The influent seawater is treated to meet the requirements of the fish larvae including particle removal, temperature regulation and importantly disinfection to remove pathogenic bacteria and viruses. The treated water is used in all facets of larval rearing including egg incubation, larval rearing tanks, and live feed cultures.

In flow-through systems, re-colonisation of the bacterial community is uncontrolled and tends to select for r-strategist bacteria which are known to cause poor larval performance due to their ability to over-colonize the larval hosts and create detrimental fish-microbe interactions. Importantly this uncontrolled establishment of r-strategist bacterial populations also results in lack of reproducibility and can explain varied performance between tanks stocked with larvae from the same cohort of eggs and treated in the same manner during the larval production (Vadstein et al., 2018). Alternative larval rearing systems to flow-through systems, employ methods to provide matured seawater by use of biofilters which encourage formation of k-strategist bacterial populations. K-strategists are non-opportunistic, suppress r-strategists, and promote healthy host-microbe interactions (Vadstein et al., 2018). It is likely therefore that the variable YTK larvae survival we experienced between and within larval batches was due to promotion of r-strategist microbial populations. This should be investigated in future production runs of YTK larvae.

Predictable fingerling production is fundamentally important for management of a viable growout industry and we need to investigate methods to control and manage the microbial populations

including systems to mature disinfected seawater before use in the hatchery and application of commercially available probiotics.

A further confounding factor which may have caused intermittent, poor hatchery survival relates to the 3 month frequency with which our YTK broodfish were induced to spawn in order to satisfy commercial demands from the seacage growout facility. YTK broodfish are serial spawners during Spring/Summer or Autumn months when the water temperature is 17-24^{o}C (Fielder and Heasman, 2011). After spawning, broodfish then have approximately 6-9 months to undergo gonadal recrudescence through winter when water temperature is low (<17^{o}C), in preparation for the following season spawning event. Our broodfish are held under controlled environmental conditions. The fish are mostly held at 16^{o}C and induced to spawn by rapidly increasing water temperature from 16^{o}C to 22^{o}C. Spawning of individual fish occurs approximately every second day over a 7-10 day period to provide suitable numbers of eggs to stock the hatchery. Water temperature of broodfish tanks is then returned to 16^{o}C. The tank of broodfish that contributed eggs predominantly to our production hatchery batches (Tank 1) every three months clearly showed a steady decline in fecundity and fertilisation rate of eggs with successive spawning events. This suggests strongly that gonadal recrudescence was inadequate within a 3 month period, and quality of eggs spawned was negatively affected. It is well known that poor fish egg quality, as evidenced by low fertilisation among other parameters, can have a profound negative effect on larval development, growth and survival (Bobe, 2015). Methods to avoid the need to spawn frequently YTK broodfish should be investigated and include expanding the number of tanks of mature YTK and maintain them in out-of-season environmental conditions, offset by three monthly intervals.

Method - Nursery

General

Large-scale production of advanced juvenile YTK (up to 100g) was evaluated in land-based facilities including recirculation tank systems (RAS) with high-tech water management (including temperature, dissolved oxygen, particulate and dissolved nutrient filtration) and polyhouse-covered, plastic-lined raceway ponds. Growout of juvenile YTK was done at high-density (up to 30kg/m^{3}) in indoor, controlled-environment recirculating aquaculture systems (20,000L; n=4) and compared with production of YTK in outdoor raceway systems (350,000L; n=4 ponds), which were covered in a poly-house for heat retention.

The use of novel Biogill® filters (www. Biogill®.com) to manage water quality in the raceway ponds was evaluated during growth cycles throughout the year. Ponds were each stocked with up to 500 YTK (100 - 500g) and ongrown using standard feeding protocols. Two ponds had Biogill® filters installed on the pond bank. Water was pumped (up to 200%/day) from the bottom of the pond into the Biogill® and returned to the pond. Two control ponds were managed according to standard protocols of water exchange with filtered estuary seawater. Solid waste and dissolved nutrient loads and filamentous algae growth was compared between the two operating systems. YTK were grown for 6-8 weeks and then harvested. The performance of outdoor raceway systems was compared with indoor recirculating systems in terms of fish quality and survival and cost of production. Assessment of Biogill® performance to improve water quality in raceways was combined with an assessment of the suitability of polyhouse covers over outdoor raceways to improve (increase) water temperature and to optimise juvenile YTK growth during winter. The performance was evaluated by comparing fish growth and survival, FCR as well as general health. Identification of optimal methods for removing and handling large numbers of advanced juvenile YTK from outdoor raceways for transport to seacages was also determined.

Experiment 1

RAS were used which had been constructed as demonstration facilities and used successfully to maintain relatively low densities of marine fish including snapper, mulloway and YTK. The systems had not been used to culture large biomasses of YTK. Two RAS were used and each System (1 and 2) consisted of 2x10m^3 flat-bottom tanks which were connected to mechanical drum filtration and 3m^3 biological moving bed bioreactor filtration, and a heater/chiller unit. System 1 included RAS 1 and RAS 2 and System 2 included RAS 3 and RAS 4. Approximately 100% of the tank volume was passed through the filters every 2 hours. In addition, exchange of new disinfected seawater was done daily on an as-needs basis according to water quality, especially TAN concentration and dissolved / suspended organics and was generally 25% of the tank volume/d. A further two, 5m^3 tanks were operated on flowthrough (FT) of 10µm filtered seawater with approximately 50-70% of the tank volume exchanged each hour. Each tank was stocked with advanced YTK (approximately 15-21g; Table 2) and cultured for 32 days (RAS tanks) and 24 days (FT) using standard procedures including feeding with Ridley Pelagica diet (1.5 amd 3mm) twice/day at approximately 2.5% biomass/d. Water quality (pH, salinity, temperature and DO) was monitored daily in the morning. It should be noted that it was not the intention to maximise growth of YTK but rather to maintain feeding and growth within manageable limits and to obtain production data and to reduce potential for stock loss. This was done by maintaining water temperature at target 16ºC in the RAS and to restrict feed ration as opposed to satiation feeding. FT tanks were operated with ambient seawater temperature.

Experiment 2

This experiment was done in two, purpose-built RAS each consisting of the following components: 30m^3 production tank, 1m^3 sump, rapid sand filter, foam fractionator, heater/chiller unit, UV filter and a biofilter (moving bed bioreactor, MBBR, RAS 1; Biogill® RAS 2) (Figure 23). Influent water was filtered through sand and cartridge filters (10µm nominal), a UV filter and then heater/chiller to manage water temperature. Compressed pure oxygen was supplied initially to both tanks via immersed diffusion stones, however these were deemed inefficient as a result of declining and unstable DO concentrations. On 24 November 2016, both RAS were fitted with individual oxygen saturation systems.

The aim of the experiment was to compare the performance of standard MBBR filtration with Biogill® and to determine any operational and logistical issues that could preclude the use of RAS for production of advanced YTK in land-based systems. Both MBBR and Biogill® were new and biological activity for nitrification was limited prior to the start of the experiment. The systems had been operated for approximately 1 month with low stocking density of fish (~20kg) in an attempt to initiate *Nitrosomonas* and *Nitrobacter* populations for nitrification. Total ammonia nitrogen (TAN) was monitored and exchange of influent seawater was adjusted accordingly to ensure potentially toxin unionised ammonia was low. In general, approximately 100% of new, influent seawater was exchanged eac day through both RAS.

Figure 23. Thirty (30) m^3 RAS tanks at PSFI for nursery culture of juvenile YTK. RAS 1 (with black MBBR filter) and RAS 2 (with Biogill® filter). Note a foam fractionator below Biogill® of RAS 2.

Experiment 3 (Batch 3a and 3b)

Evaluation of the suitability of RAS and flow-through systems at PSFI for intensive culture of advanced juvenile YTK continued and two experiments were completed for ongrowing of Batch 3 and Batch 4 fingerlings.

The experiment was done in two, purpose-built RAS which each consisted of the following components: 24.1m^3 production tank, 1m^3 sump, rapid sand filter, foam fractionator, heater/chiller unit, UV filter and a biofilter (moving bed bioreactor, MBBR, RAS 1; Biogill® RAS 2) and oxygen saturation systems. Influent water was filtered through sand and cartridge filters (10µm nominal), a UV filter and then heater/chiller to manage water temperature.

The aim of the experiment was to compare the performance of standard MBBR filtration with Biogill® and to determine any operational and logistical issues that could preclude the use of RAS for production of advanced YTK in land-based systems. Both MBBR and Biogill® had active biological activity for nitrification having been successfully used to culture the previous Batch 2 YTK. In general, approximately 40-70% of new, influent seawater was exchanged and approximately 700% of the tank water was recirculated through the filtration system for both RAS each day. Batch 3a was approximately 30 days older and significantly larger than Batch 3b therefore in order for us to amalgamate the batch's as one stocking to seacages we attempted to manage growth (i.e. slow down) of Batch 3a by manipulation of water temperature and feeding.

Each tank was stocked with juvenile YTK (3660 fish at 11.4g, Batch 3a, RAS 2; 13325 fish at 5.8g, Batch 3b, RAS 1) and cultured for 68 days using standard procedures including feeding with Ridley Pelagica diet (1.5 and 3mm) twice/day at approximately 2.5% biomass/d. Water quality (pH, salinity, temperature and DO) was monitored daily in the morning. It should be noted that it was not the intention to maximise growth of YTK but rather to maintain feeding and growth within manageable limits and to obtain production data and to reduce potential for stock loss. This was done by maintaining water temperature at target 15-16ºC in the RAS2 and to restrict feed ration as opposed to satiation feeding. RAS 1 was operated at target temperature of 22ºC to optimise growth of YTK.

After 70 days, all fish were harvested and divided evenly between both RAS tanks (8200 fish/RAS tank) and ongrown for a further 150 days. After approximately 45 days, the biomass in each RAS was

reduced by removing fish for ongrowing in Flowthrough tanks as in the previous studies to provide data for management of the different systems.

Experiment 4 Batch 4

The aim of experiment was to continue to evaluate the suitability of a range of RAS and flowthrough tanks for ongrowing of advanced juvenile YTK. All of Batch 4 was ongrown at PSFI in 15 land-based tanks of varying sizes and consisting of RAS or simple flowthrough design. All tanks were provided with compressed oxygen either delivered via an oxygen saturation cone (RAS 1 and 2) or ceramic diffusers (all other tanks). General husbandry of the fish followed standard operating protocols as described previously. Fish were transferred from the hatchery at 32 dph (~0.1g) and then size graded to prevent cannibalism every 2-3 days until fish were 1-2 g. Fish were allocated to various tanks and biomass reduced by dividing fish into other empty tanks on an ad hoc basis when water quality (Dissolved oxygen, suspended solids) was deemed to be suboptimal. The fish were held in the nursery tanks for a further 192 days as Huon had continued delays with installation of a seacage for the batch of fish.

Experiment 5

Evaluation of the suitability of RAS, flow-through systems and polyhouse ponds at PSFI for intensive culture of advanced juvenile YTK continued and two studies were completed for ongrowing of batch 5b fingerlings.

The first study followed previous investigations where production of juvenile YTK was done in two, purpose-built RAS or simple flow-through tanks. Each RAS tank consisted of the following components: 33m^3 production tank, 1m^3 sump, rapid sand filter, foam fractionator, heater/chiller unit, UV filter and a biofilter (moving bed bioreactor, MBBR, RAS 1; Biogill® RAS 2) and oxygen saturation systems. Influent water was filtered through sand and cartridge filters (10µm nominal), a UV filter and then heater/chiller to manage water temperature; and 5000L tanks operated on flow-through water exchange.

All tanks were provided with compressed oxygen either delivered via an oxygen saturation cone (RAS 1 and 2) or ceramic diffusers (all other tanks). General husbandry of the fish followed standard operating protocols as described previously. Fish were stocked into 8 flowthrough tanks (mean weight 4.9±2.0 g) and 2 x RAS tanks (mean weight 16.6 g) to provide an initial stocking density of 1.9±0.7 kg/m^3 (flowthrough tanks) and 0.5 kg/m^3 (RAS tanks). The study was run for 135 days from 4/04/18 to 08/09/18 during winter, after which time the fish were harvested and stocked to Botany Bay and Lake Macquarie as part of a stock enhancement program.

Outdoor nursery and polyhouse experiments

Experiment 6

Evaluation of the suitability of polyhouse-covered 250 m^2 ponds for culture of advanced YTK was done during the winter of 2017 (Figure 24). Two ponds were each stocked with 50, advanced juvenile YTK and managed following standard culture practices similar to those used in the nursery tanks (above). Filtered seawater was supplied to each pond at approximately 25% exchange each day. In addition water was pumped from each pond via a submerged automatic vacuum pool cleaner (Rebel®2, Pentair, Dandenong South, Australia) and through a sand filter to remove particulate waste and biological filter (MBBR, Pond 3; Biogill® Pond 4) before being returned to the pond at an exchange

rate of approximately 50%/day. An uncovered pond was run in concert with filtered estuarine seawater to provide comparative data on water quality, at a particular temperature. Water quality measurements were taken once each day in the morning.

Figure 24. Four, 250m^3 plastic-lined ponds covered with polyhouse at PSFI with Biogill® installed for water quality management.

Experiment 7

Evaluation of the suitability of polyhouse-covered 250m^3 ponds for culture of advanced YTK was done during October 2018. Two ponds were each stocked with 500, advanced juvenile YTK (133.8±7.4 g/fish) and managed following standard culture practices similar to those used in the nursery tanks (described above). Filtered seawater was supplied to each pond at approximately 100% exchange each day. In addition, water was drawn from the pond pumped through a sand filter to remove particulate waste before being returned to the pond via a Mazzei venturi at an exchange rate of 100%/day. Air was supplied to each pond through three 30cm diameter submerged airstones. An uncovered pond was run in concert with filtered estuarine seawater to provide comparative data on water quality, in a particular temperature. Water quality measurements were taken once each day in the morning (0800-0830). In addition, temperature was logged every two hours with temperature loggers. Data was downloaded at the end of the study. Phytoplankton densities were estimated every 2-5 days using a haemocytometer. After approximately 20 days, a submerged automatic vacuum pool cleaner (Rebel®2, Pentair, Dandenong South, Australia) was installed into each pond and operated by 1 hp swimming pool pump for 8-10h/day until the end of the study. Waste water and detritus were discharged directly from the pond. At the end of the study, fish were harvested by pulling a 4mm seine net through the pond to crowd fish at one end, fish were then netted by hand into transporting tanks.

Results - Nursery

Experiment 1

YTK grew in all tanks over the culture period with Absolute Growth Rates (AGR) for RAS tanks of 0.4±0.1 g/d and flowthrough tanks 1.0±0.04 g/d. The AGR for RAS systems was likely lower than FT due to lower operating temperatures of ~16ºC compared with ambient of ~20ºC in the FT (Table 2). Maintenance of saturated dissolved oxygen (DO) concentration was not possible with simple air diffusion and it was necessary to infuse pure oxygen at 1-3L/min to avoid DO crashes, especially within 30-60 minutes after feeding. It was necessary to initiate 25-50% daily exchange of new disinfected seawater in RAS systems to reduce the concentration of dissolved organic material which resulted from leachate of faeces and/or the pellet diet. Improvements needed for the RAS systems were identified and included the installation of foam fractionators to remove dissolved organics and oxygen saturating cones to optimise DO saturation. FT systems were relatively easy to maintain compared with RAS, however, they used large volumes of new, influent seawater to maintain high water quality; 60,000-84,000 L/d/tank (12-17 times tank volume/day) compared with RAS which used 2500-5000L/d/tank. Moreover, the FT tanks used only filtered seawater, whereas, RAS used filtered, disinfected and temperature-controlled seawater.

Table 2. Tank specifications, growth and feeding data for Experiment 1 that compared culture of juvenile YTK in four RAS and two Flowthrough (FT) tanks at PSFI.

	Tank					
	RAS 1	RAS2	RAS3	RAS4	FT1	FT2
Tank volume (m^3)	10	10	10	10	5	5
Seawater exchange (%/h)	50	50	50	50	50-70	50-70
Temperature (ºC)	16±1	16±1	16±1	16±1	20±1	20±1
Days of culture	32	32	32	32	24	24
Number of fish	4927	5417	4670	5087	1211	1634
Mean initial fish weight (g)	17.9	16.3	15.6	14.5	21.1	15.0
Mean final fish weight (g)	27.5	31.2	30.3	28.8	44.6	40.4
Starting biomass (kg)	88.2	88.3	72.9	73.8	25.6	24.5
Harvest biomass (kg)	135.5	169.0	141.5	146.5	54.0	66.0
Biomass increment (kg)	47.3	80.7	68.6	72.7	28.5	41.5
Absolute growth rate (g/day)	0.3	0.5	0.5	0.4	1.0	1.1
Initial stocking density (kg/m^3)	8.8	8.8	7.3	7.4	5.1	4.9
Final stocking density (kg/m^3)	13.5	16.9	14.2	14.7	10.8	13.2
Amount feed (kg)	67.6	68.4	66.8	67.6	30.7	30.7
FCR	1.4	0.8	1.0	0.9	1.1	0.7

Experiment 2

YTK grew well in both RAS systems and with AGR of 0.3 and 0.5 g/d for RAS 1 and RAS 2, respectively, and FCR in both RAS was <1.0 (Table 3). The higher AGR in RAS 2 was likely due to an increased average water temperature (21.6±1.0 ºC) compared with that of RAS 1 (19.7±2.2 ºC) for the duration of the experiment. Two mortality events occurred during the experiment. The first was on 25 November 2016 in RAS 1 when 2998 fish died following an overnight DO crash due to failure of a

new pressure pump which was operating the oxygen saturation system. The second mortality event occurred in RAS 2 when 2916 fish died, again for DO crash due to human error. Water quality was relatively stable for the duration of the experiment and as fish biomass and daily feed increased, water pH was decreased. No buffering of pH was done and final pH was approximately 6.5 in both RAS (Figures 26 and 27). No deleterious effects of low pH were observed with fish growth (Figure 25) and the concentration of unionised ammonia was negligible as a consequence.

Nitrification performance of biofilters appeared to occur earlier in the Biogill® compared with the MBBR (Figures 26 and 27) when TAN was recorded as 1 mg/L on 9 December 2016 in RAS 2. TAN remained at 2 mg/L in the MBBR, RAS 1 tank until fish were harvested from the tank.

Harvest of the YTK proved very simple from the 30m^3 tanks, as we experienced in Experiment 1 in 10m^3 tanks, using the same dipnetting method. Fish from both RAS were encouraged to school up at the water surface by feeding small quantities of feed (1-2 g), and the unanaesthetised fish were captured and transferred to a transporting bin. Approximately 98% of the fish population were captured in a timely manner using this method. The remaining 2% of fish were captured once the tank was drained down to allow a technician to wade in the tank and catch the fish with a dipnet.

Issues that were identified for rectification of the RAS operating system for further investigation included: channelling and inefficient operation of the sand filter with standard sand media. The sand media was exchanged for a new charged glass media, Activated Filter Media (AFM) and tested in subsequent experiments. Installation of alarm system for low DO issues was recognised as an essential component.

Table 3. Tank specifications, growth and feeding data for Experiment 2 at PSFI that compared juvenile YTK grown in RAS using MBBR or Biogill® to provide water treatment.

	Tank	
	RAS 1 (MBBR)	RAS 2 (Biogill)
Tank volume (m^3)	30	30
Seawater exchange recirc (%/h)	30	30
Seawater exchange influent (%/h)	5	5
Temperature (°C)	19.7±2.2	21.6±1.0
Days of culture	52	38
Number of fish stocked	15041	14140
Number of fish harvested	12043	11224
Mean initial fish weight (g)	3.6	2.8
Mean final fish weight (g)	19.7	19.9
Starting biomass (kg)	54.1	39.6
Harvest biomass (kg)	237.2	223.4
Biomass increment (kg)	183.1	183.8
Absolute growth rate (g/day)	0.3	0.5
Initial stocking density (kg/m^3)	1.8	1.3
Final stocking density (kg/m^3)	7.9	7.4
Amount feed (kg)	174.0	154.3
FCR	1.0	0.8

Figure 25. Mean wet weight (g) of YTK grown in RAS tanks with different biological filtration systems. Batch 2, 2016. Experiment 2.

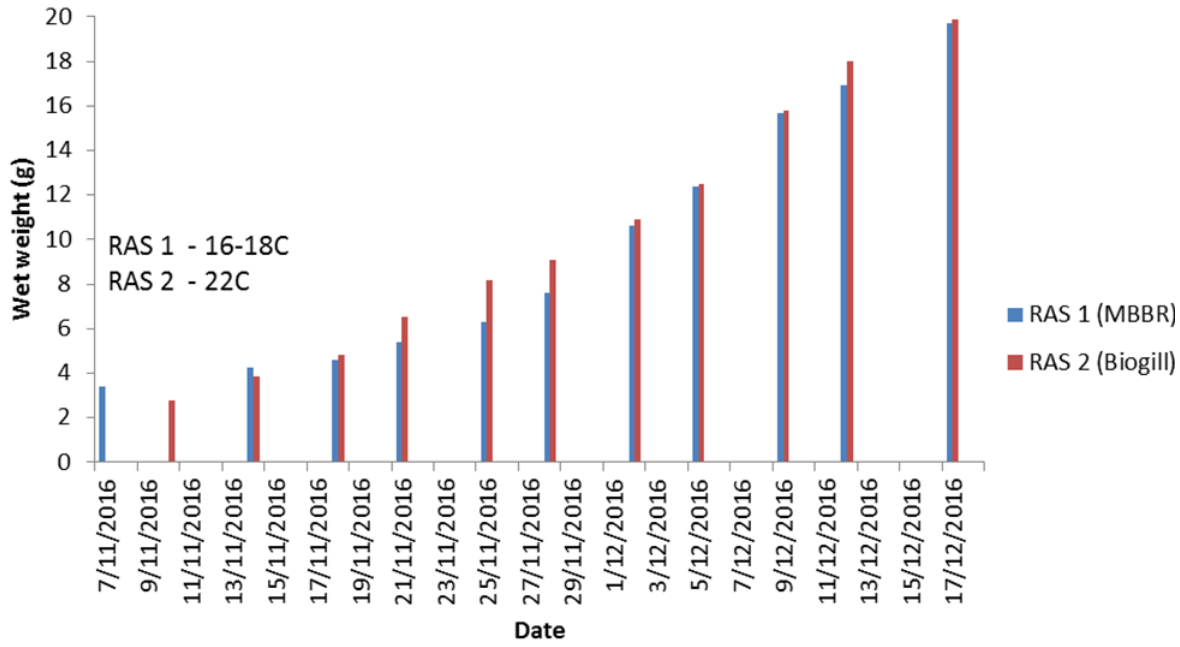


Figure 26. Water quality (DO, temperature, salinity, pH and TAN) of RAS 1 (MBBR) with juvenile YTK cultured for 52 days. Experiment 2.

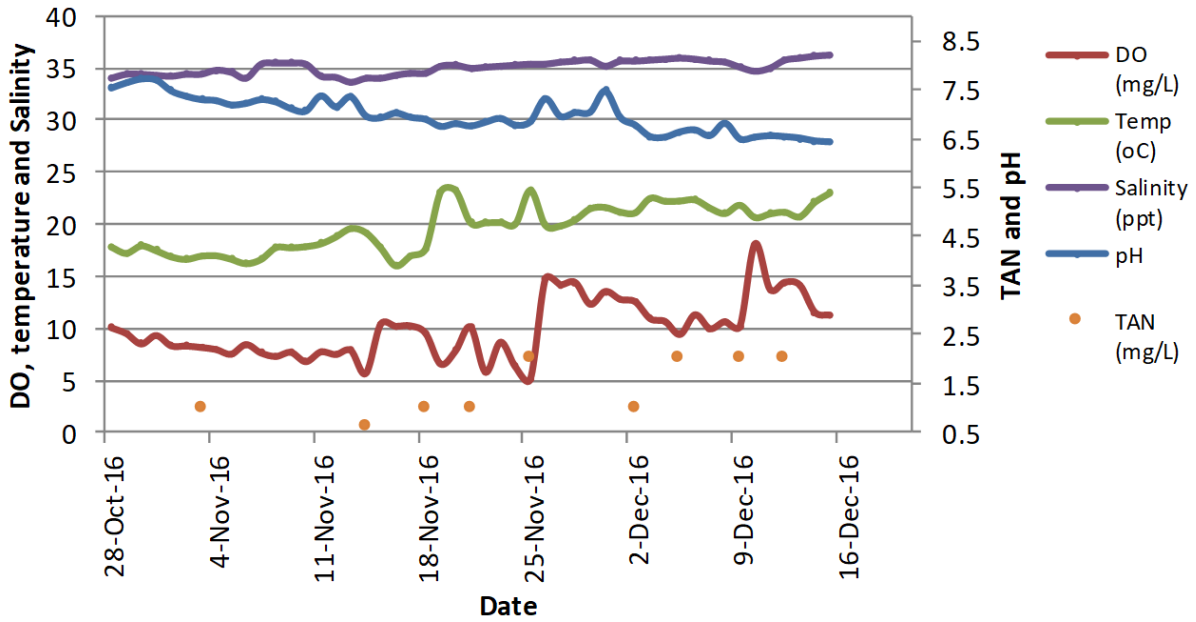
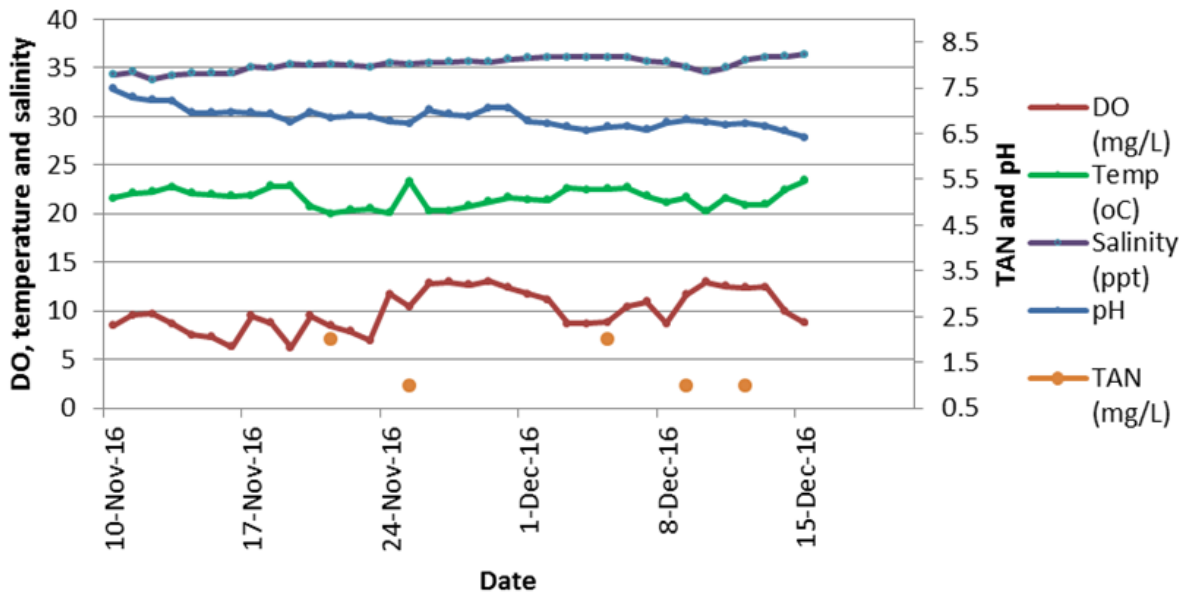


Figure 27. Water quality (DO, temperature, salinity, pH and TAN) of RAS 2 (Biogill) with juvenile YTK cultured for 38 days. Experiment 2.



Experiment 3 (Batch 3a and 4)

Growth of Batch 3a (0.23 g/d AGR) was lower than Batch 3b (0.28 g/d AGR) over a 68 day period and both batch's had reached the same mean target weight of ~26g/fish (Figure 28) thus demonstrating that growth of an older batch of YTK can be successfully arrested by maintenance of water temperature at 15-16^{o}C (Figure 29) and feeding restricted rations without compromising the fish condition or health. The fish were destined to be transported to a seacage on the MARL at this time, however, delays by Huon Aquaculture to install a seacage required fish to be ongrown in the RAS and subsequently flowthrough tanks. Fish continued to grow well in the RAS tanks at a target temperature of ~18^{o}C (Figures 29 and 30) and flowthrough tanks and fish attained a mean weight of 120g by 30 September 2017 (0.63 g/d AGR). Total biomass of fish held in tanks ranged from 9.9 to 16.8 kg/m^3 (Figure 31). A total of 10902 (1308kg) fish were successfully transported by helicopter from PSFI to the MARL in a specially designed 500L bin at a maximum stocking density of 300 kg fish/m^3 (Figure 32).

Figure 28. Mean weight and fork length of Batch 3a and 3b grown in RAS tanks.

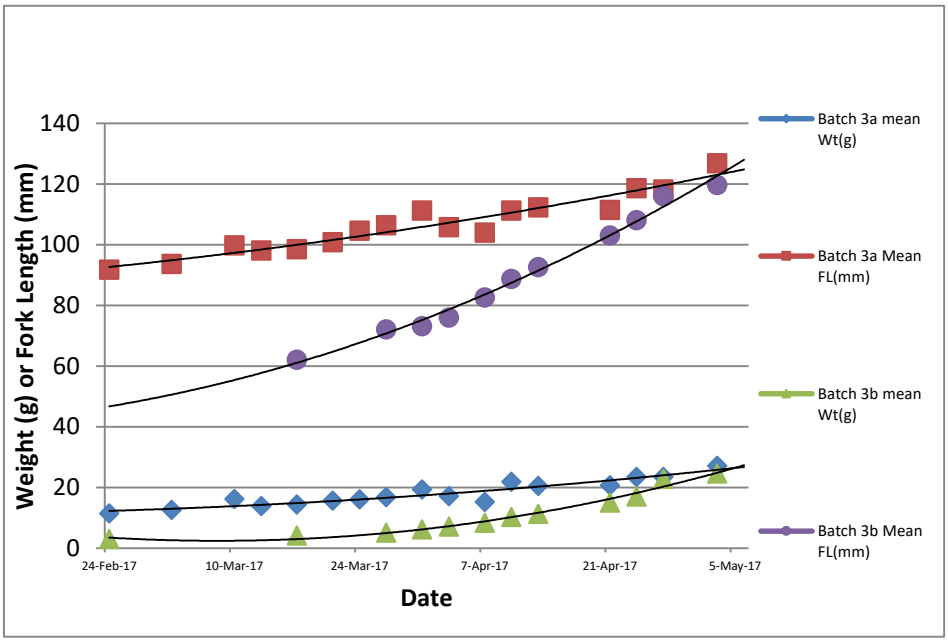


Figure 29. Mean wet weight, fork length, and water temperature of Batch 3a YTK held in RAS 2 and PSFI (n=30 fish).

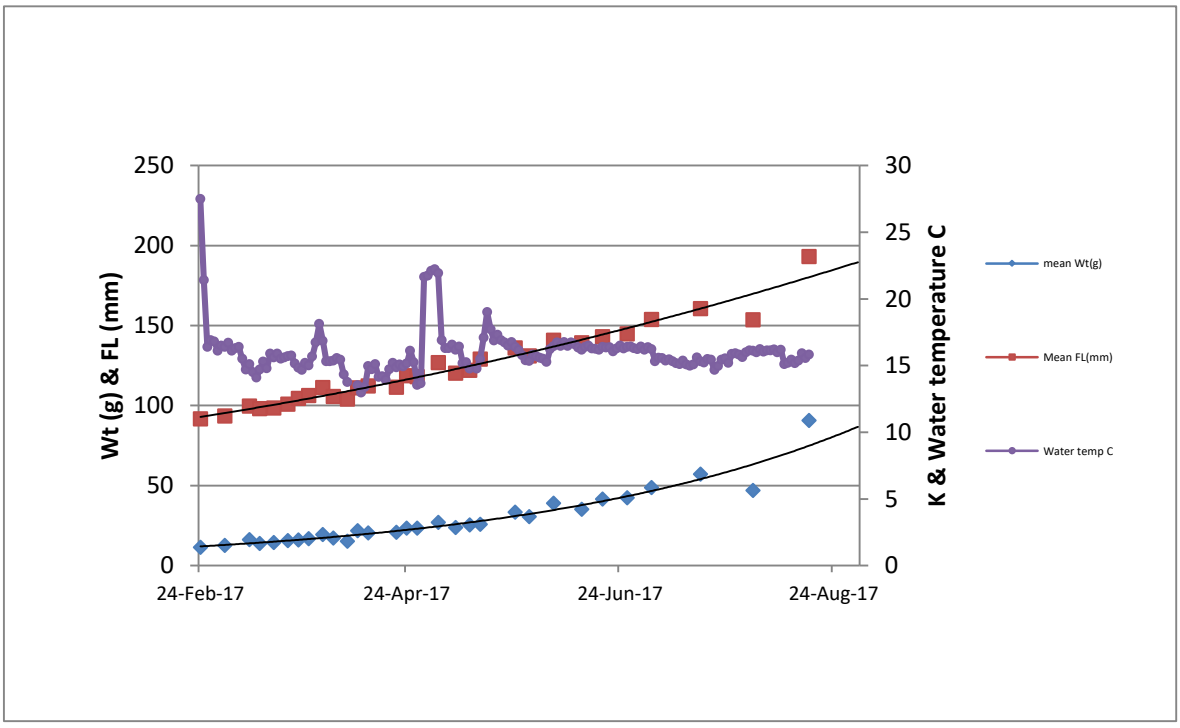


Figure 30. Mean wet weight, fork length, condition factor (K) and temperature of Batch 3b grown in RAS at PSFI.

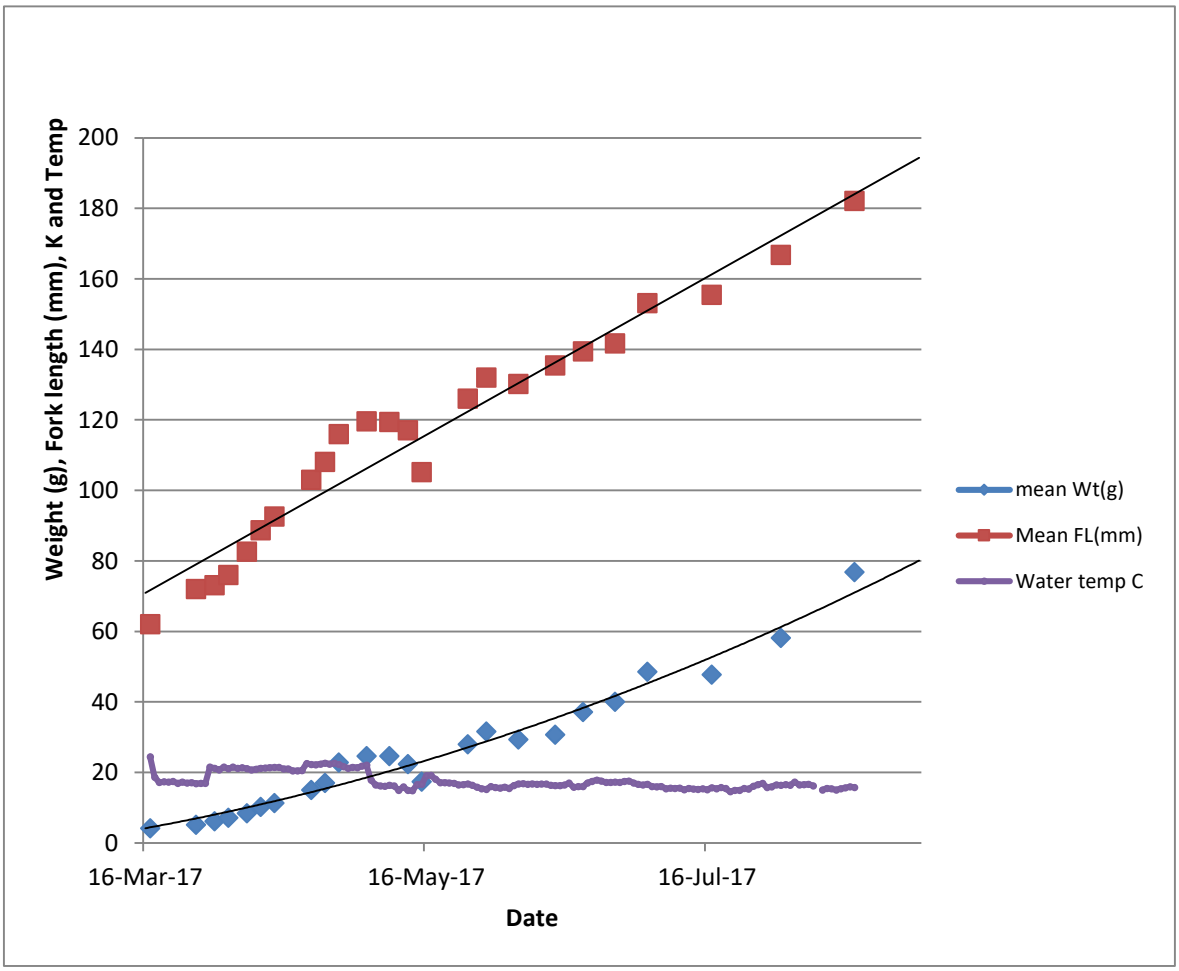


Figure 31. Final stocking density for Batch 3, September 30 2017.

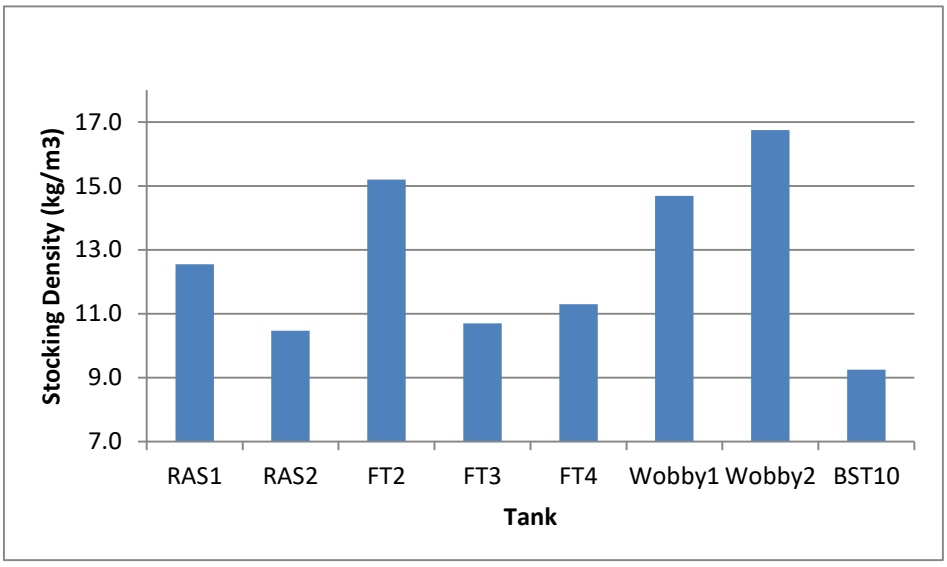
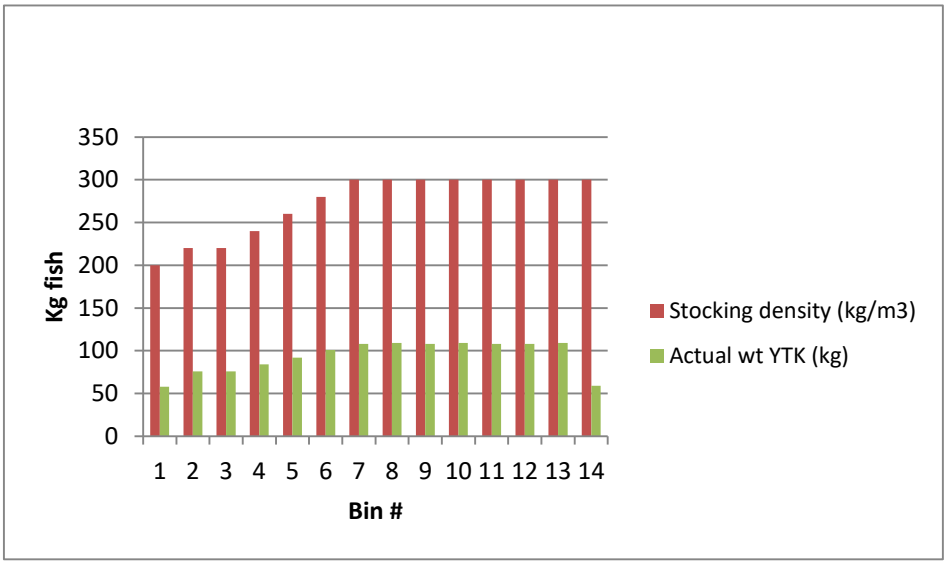


Figure 32. Target stocking density and weight of Batch 3 YTK flown in each transport bin.



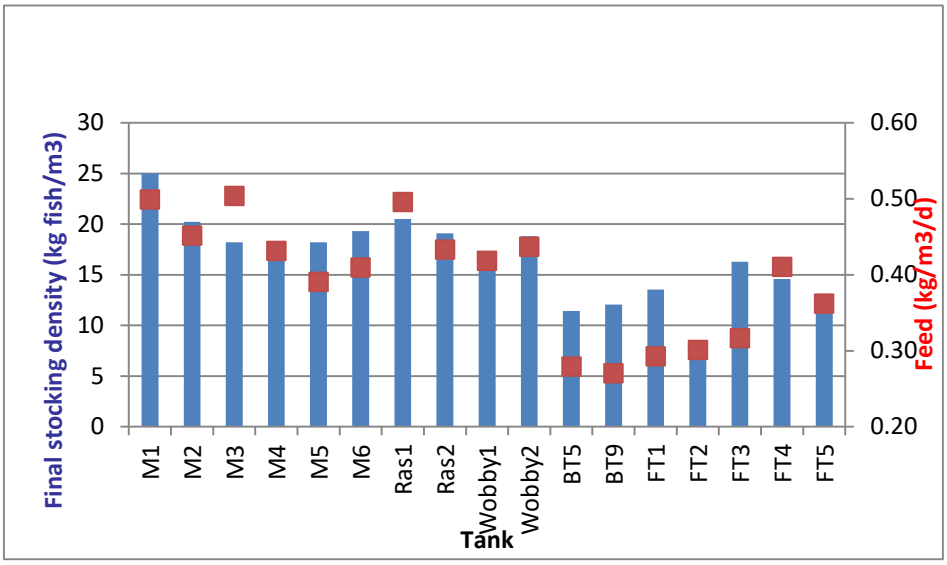
Experiment 4 (Batch 4)

YTK grew well in all tanks during the 192 day period and reached a final mean weight of 166g/fish representing an AGR of 0.87g/d. As fish increased in size, some fish randomly jumped out of the 4.9m^3 tanks, mostly at night. This was stopped with the installation of mesh fence around the top of each tank.

Final stocking density of fish stocked ranged from 7.5 kg/m^3 to 25 kg/m^3 and daily feed rates ranged from 0.3 to 0.5 kg feed/m^3/day (Figure 33). Maintenance of high water quality was possible at these stocking densities and feed rates and fish were in excellent condition at harvest.

An issue identified in previous trials of poor mechanical filtration due to channelling of the sand media in the RAS systems mechanical filers was addressed by changing standard sand media with Activated Filter Media (AFM). The AFM did not channel as much as sand, however, it was difficult to backwash and clean. Weekly physical breaking up of the AFM by technicians improved the filtering efficacy and tank water quality was deemed good. Future investigation to improve the RAS mechanical filtration included installation of air-assisted backwash, and addition of a swirl particulate separator before the AFM filter.

Figure 33. Final stocking density (kg/m^3) and feed/m^3/d, Batch 4, 9/10 December 2017.

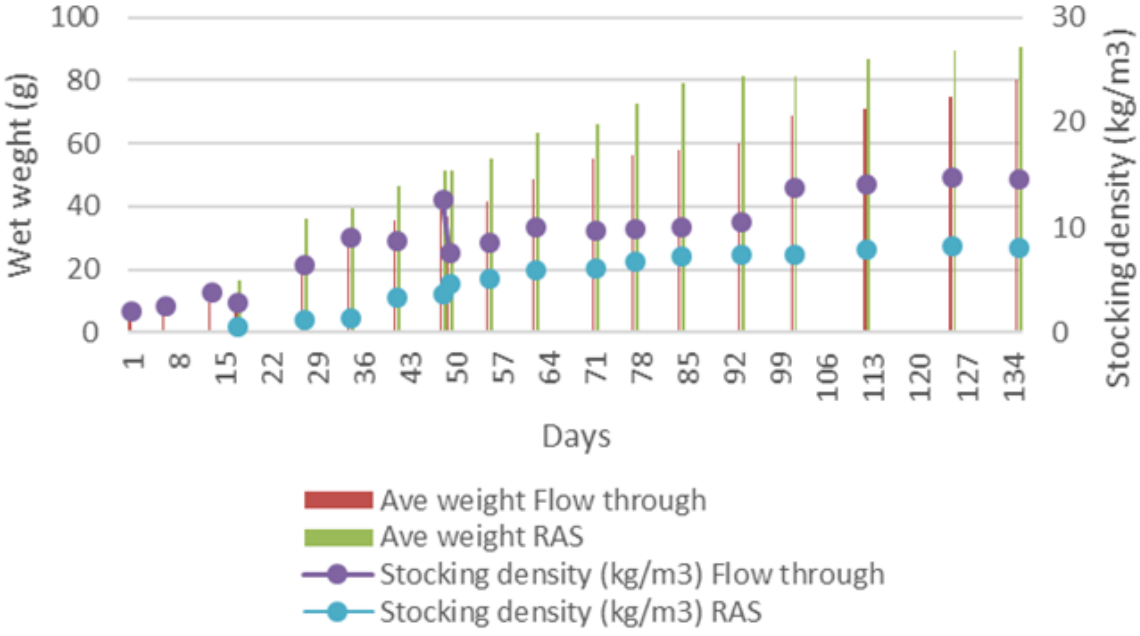


Experiment 5

YTK grew well in all tanks during the 135 day period and reached a final mean weight of 80.4±8.7g/fish (Flowthrough) and 90.9±13.4g/fish (RAS) and representing an Absolute Growth Rate (AGR) of 0.6g/d and 0.7g/d, respectively. Final stocking density of fish was 14.6±1.0 kg/m^3 and 8.1±0.2 kg/m^3 for flowthrough and RAS tanks, respectively (Figure 34).

Maintenance of high water quality was possible at these stocking densities and feed rates, and fish were in excellent condition at harvest.

Figure 34. Wet weight and stocking density of juvenile YTK grown in 5000L flowthrough tanks (n=8) and 33,000L RAS tanks (n=2).



Outdoor nursery and polyhouse experiments

Experiment 6

Water temperature of the polyhouse-covered ponds (range 14.2 – 19.5 ºC) was on average 2.0 ± 0.8 ºC warmer than the uncovered pond (range 11.3 – 17.1 ºC) over the 114 day culture period (Figure 35). Growth of YTK was high with FCR of approximately 2.0:1 (Table 4; Figure 36). Unexplained mortality of all fish in Pond 1 occurred after 77 days of culture.

Table 4. Growth and feed consumption of YTK cultured in 250m^2 polyhouse covered ponds at PSFI.

	Pond		
	1		2
Pond volume (m^3)	250		250
Seawater exchange recirc (%/h)	50		50
Seawater exchange influent (%/h)	25		25
Temperature range (ºC)	14.2-19.5		14.2-19.5
Days of culture	77		114
Number of fish stocked	50		50
Number of fish harvested	50	mortality event	50
Mean initial fish weight (g)	280.9		286.4
Mean final fish weight (g)	579.8		1094.1
Starting biomass (kg)	14.0		14.3
Harvest biomass (kg)	29.0		54.7
Biomass increment (kg)	14.9		40.4
Absolute growth rate (g/day)	3.9		7.1
Initial stocking density (kg/m^3)	0.1		0.1
Final stocking density (kg/m^3)	0.1		0.2
Amount feed (kg)	31.6		76.4
FCR	2.1		1.9

Figure 35. Water temperature of greenhouse-covered and ambient 250m^3 ponds at PSFI, 2017.

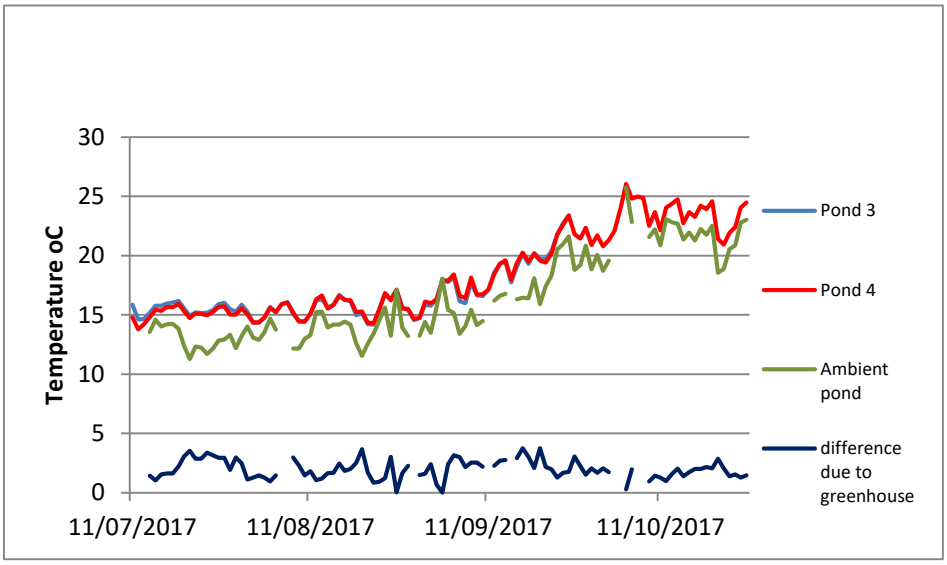


Figure 36. YTK harvested after 114 days (July-November 2017) of culture in polyhouse-covered, plastic-lined ponds at PSFI. Fish were 1.0 kg.

Outdoor nursery and polyhouse experiments

Experiment 7

Water temperature of the polyhouse-covered ponds (range 18.0 – 27.4 ºC) was more stable than that of an uncovered pond being approximately up to 1.5ºC cooler on hot days and 1.5ºC warmer on cold days (Figures 37 and 38). pH and salinity were stable for the 35 day period (Figure 39). Morning Dissolved Oxygen was initially 100% saturated but showed a steady decrease to approximately 80% saturation once daily feed rates were approximately 200 kg/ha/day (Figure 40). Maximum daily feed rate was 274.5 kg/ha/d (= 274 g feed/m^3/d). Growth of YTK was high with AGR of 7.4±1.4 g/d and FCR of 0.9±0.2 (Figure 40). Mean final weight was 401±41.8g/fish. Final biomass was 196.5±20.4 kg/pond (=7860 kg/ha). Survival was also high 97.8% for the 35 day growout period. A phytoplankton bloom was observed to develop in both ponds on 26/10/18 and continued to increase steadily in density until the ponds were harvested (Figure 41). No external parasites were observed in weekly samples of fish and their skin and gills.

Harvesting of fish was relatively easy with 5 staff taking approximately 45-60 minutes per pond. After three shots of the seine net only 12 fish (0.4%) of the population remained in the pond (Figure 42). These were captured once the pond water volume was drained.

Figure 37. Water temperature of two polyhouse-covered ponds and an uncovered ambient pond for 35 days.

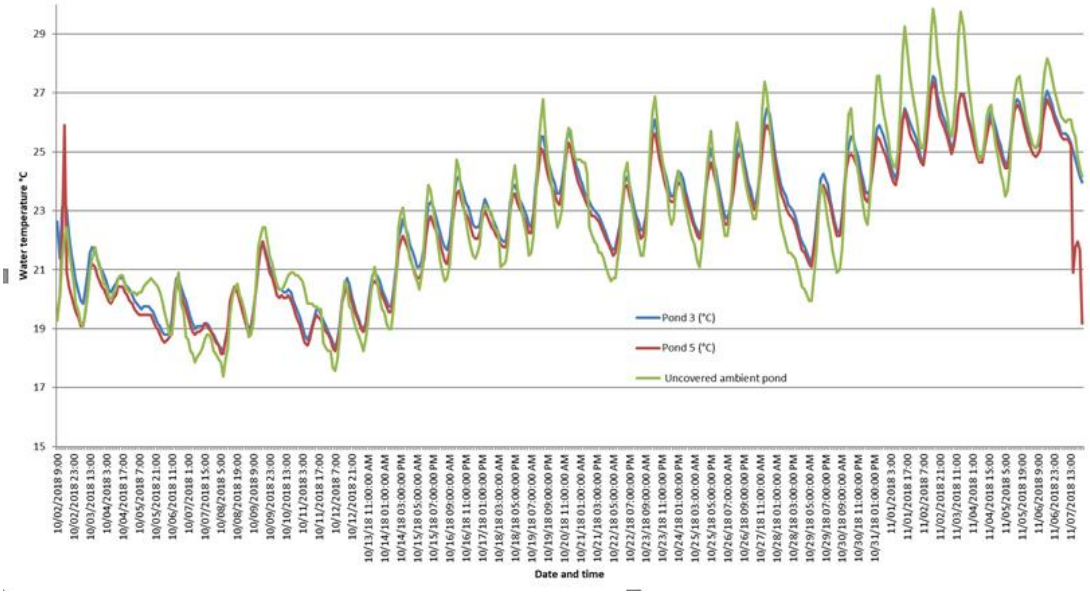


Figure 38. Mean water temperature of covered ponds (n=2) and an uncovered pond and the difference between the types of ponds for 27/10/18 to 4/11/18.

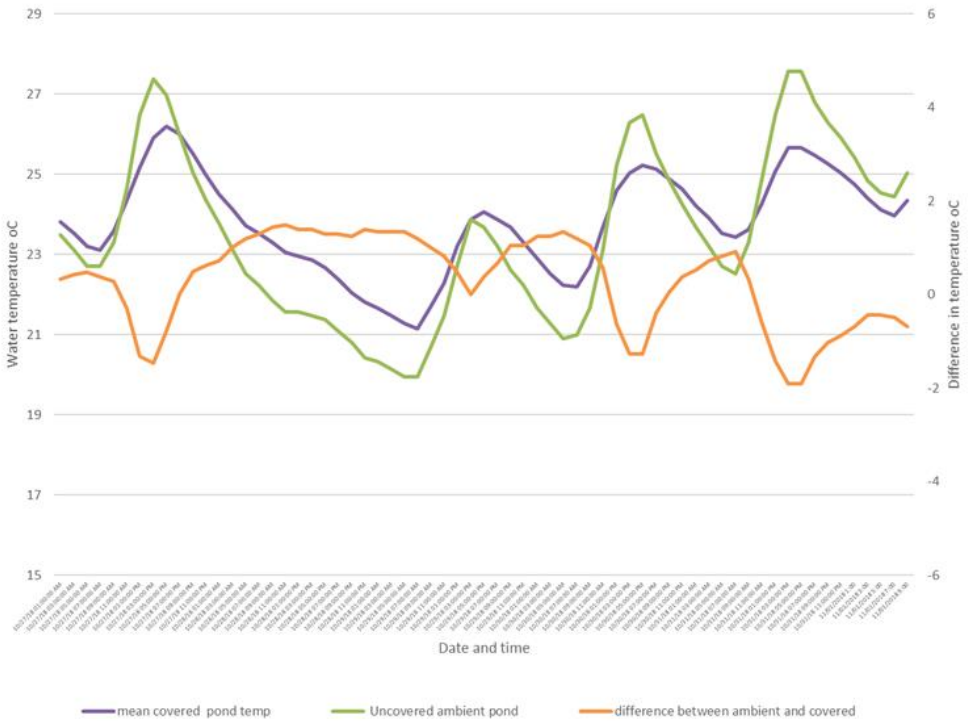


Figure 39. Water quality of polyhouse ponds. Data are means (n=2 ponds).

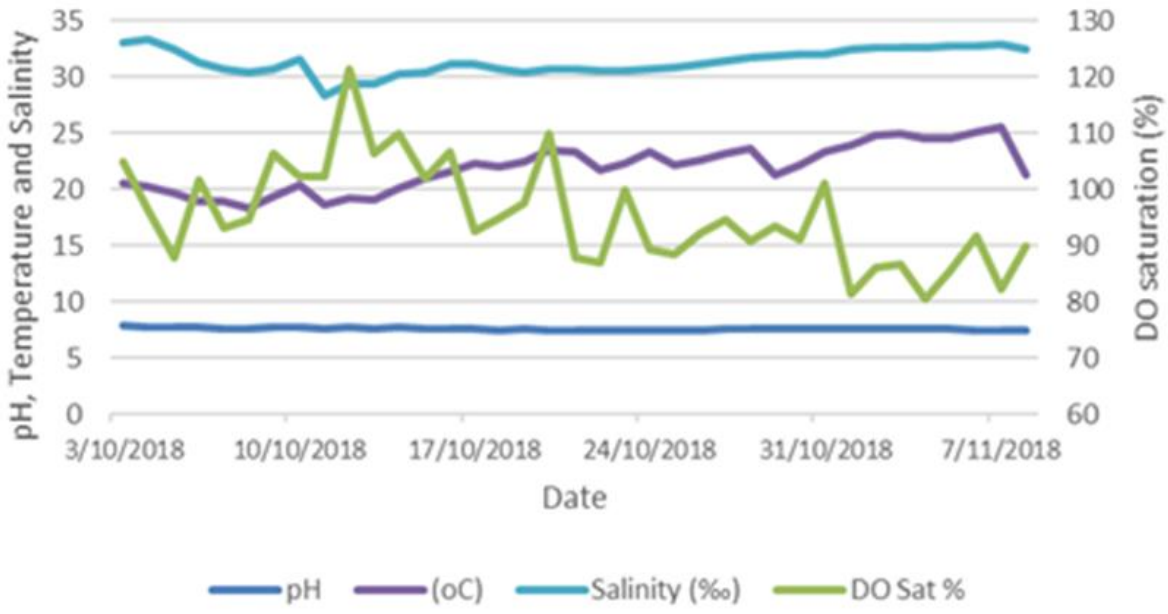


Figure 40. Estimated biomass, feed and DO of polyhouse-covered 250m^3 ponds stocked with YTK. Data are mean ± SD for n=2 ponds.

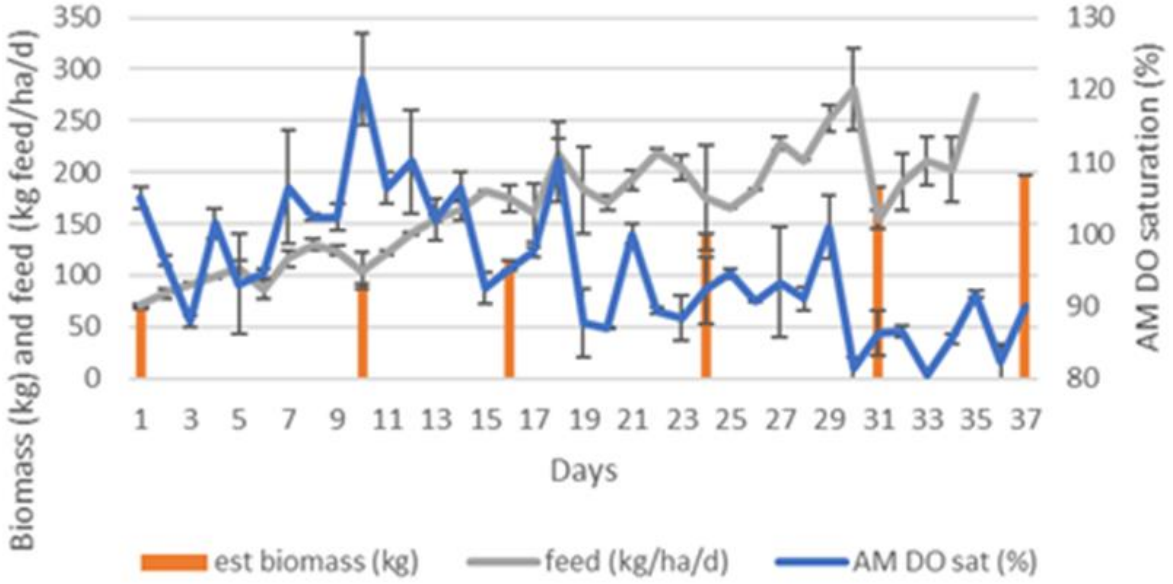


Figure 41. Algal cell density of polyhouse-covered ponds. Data are mean ± SD for n=2 ponds.

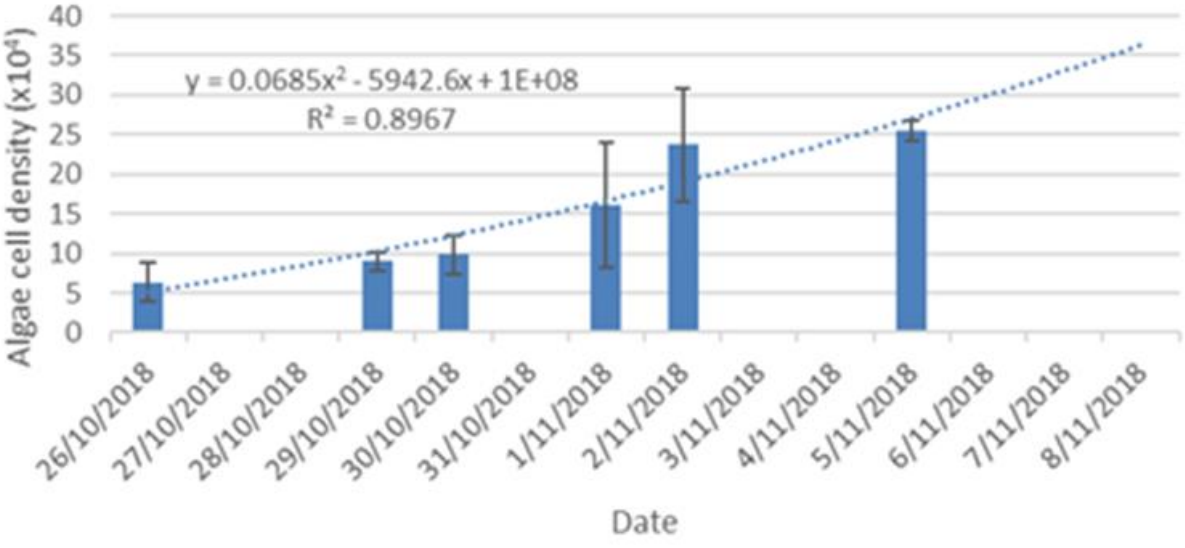


Figure 42. Seine harvest capture of 401g YTK grown in plastic-lined, polyhouse covered ponds for 35 days.

Discussion - Nursery

Advanced juvenile YTK (mean maximum weight 166g) were successfully cultured in RAS tanks (10 and 30 m^3; 1m and 2m depth, respectively) and flow-through tanks (FT; 5-10 m^3; 1m depth) for up to 192 days. Maximum stocking densities in both RAS and FT tanks was approximately 25 kg fish/m^3 with maximum feed rate of 0.5 kg food/day/m^3. RAS are typically operated with exchange rates of new, influent seawater in the order of 10% total volume/day. This is done mostly to maintain nitrate levels, which are produced during nitrification of TAN, at non-toxic concentrations (Espinal & Matulić, 2019), while 100% of the main tank water is recirculated through the mechanical and biological filters 12-24 times/day. In our studies, we generally exchanged up to 50% total volume/day of new influent seawater as biomass and stocking density of fish was increased. This high rate of seawater exchange was done to reduce the concentration of dissolved organic and micro-particulate waste from feeds and faeces, and resulted in the seawater being tainted brown in colour. It was not known whether long-term exposure to the dissolved organics was likely to cause any problems with health of the YTK, however we have observed, albeit rarely, in previous studies, YTK display unexplained jaw tetany, which appeared to be associated with poor water quality. The tetany was transient once tank water was exchanged and fish recovered completely within an hour or so.

The mechanical drum and sand filtration used in our RAS appeared adequate to remove the bulk of the suspended solids during all production runs, however, we observed an improvement in performance and management of sand filters when sand was replaced with the glass AFM. Sand media became ‘sticky’ presumably with a bacterial film (Espinal & Matulić, 2019) which made back-flushing to remove trapped waste inefficient. In addition, open channels formed over time within the sand media

which allowed direct passage of some seawater through the media and thus avoiding filtration. In contrast, the formation of channels was not observed in the AFM.

AFM is a positively charged media (see www.drydenaqua.com) and is reputed by the manufacturers to remain free of bacterial films. The back-flushing efficiency of both sand and AFM media in our systems was poor, as evidenced by the need to physically break up the media on a weekly basis and to flush with a freshwater hose to remove trapped detritus. We attempted to improve the back-flushing capability of the filters by installing air supply as well as water to de-compact the media but this had limited effect. The pressure of water supplied by the pumps may have been inadequate to lift and de-compact the sand/AFM media and this should be investigated in future.

Total ammonia nitrogen (TAN), comprising the toxic un-ionized ammonia (NH_3-N) and the relatively non-toxic ammonium ion (NH_4^+-N) (Bregnballe, 2015) was generally always detected in our RAS with a Biogill® or MBBR biofilter and especially as fish biomass and feed rates were increased; however, Biogill® appeared to be more efficient (at least twice as efficient in Experiment 2) than MBBR in removal of TAN. The pH in the RAS with either Biogill® or MBBR steadily fell to below 7, as fish biomass and feed rates increased. A reduction in pH in RAS is typical as a result of increasing concentration of CO_2 from fish respiration and the release of H^+ during the nitrifying process (Boyd, 1991). The low pH recorded in our studies likely had a direct benefit to fish husbandry due to the formation of ionised ammonia which has a low-toxicity to fish (Wurts, 2003). Contrary to Abbink et al. (2012) who showed that survival, growth and FCR of juvenile YTK were reduced when cultured in RAS with low pH of 6.58, we were unable to detect any obvious reduction in growth or survival of juvenile YTK for up to 190 days at pH 6.5-7.0.

It should be noted that due to the commercial pressure to supply YTK fingerlings to Huon Aquaculture to stock seacages, we took a conservative approach to water quality management in the nursery systems. As a consequence, we did not challenge the RAS in terms of reducing the volume of influent exchange seawater and then relying largely on the biological and mechanical filtration systems to remove dissolved and suspended solid waste, respectively from the system water. It is therefore possible that our RAS operated with both Biogill® and MBBR bioreactors were underperforming in their capacity to nitrify TAN from the system water. The fish carrying capacity of the systems may consequently have been lower than possible. The carrying capacity of the RAS at PSFI remains to be tested in a research environment with low pressure to produce fish for growout.

Flowthrough tanks (FT) used up to 17x more influent seawater than RAS tanks to maintain high water quality low in suspended solid bio-waste, when stocked with YTK at approximately 25kg/m^3. FT were also reliant on ambient water temperature compared with RAS which had the capacity to control water temperature. FT were simple to construct, had few operating components and were consequently easier to manage than RAS which had mechanical, biological and UV filters that required regular maintenance.

The addition of pure oxygen was necessary in RAS and FT to maintain dissolved oxygen concentrations at target 80-100% saturation. We attempted to achieve this goal by using ceramic diffusers (FT and RAS) and oxygen cones (RAS only). Ceramic diffusers are cheap and only require a source of compressed oxygen to operate, however, it is an inefficient method to provide dissolved oxygen due to relatively large bubble size and short contact time between oxygen bubbles and the surrounding seawater environment, especially in shallow tanks. Excess oxygen is wasted and this represents a cost to production. Oxygen cones, on the other hand, are significantly more efficient at oxygen transfer (approximately 40x), but require a pressurized system driven by a pump. The reliance on a mechanical system to provide dissolved oxygen adds risk to the RAS operation as mechanical failure can occur. We experienced pump failure on two occasions during the project, both of which resulted in mortality of YTK. The inclusion of systems to detect a reduction in water flow is essential for rapid response to address potential low dissolved oxygen concentration, and can be enhanced by having simple ceramic diffuser oxygen systems as a back up.

The ability to control environmental conditions in RAS allowed us to effectively manage the growth and condition of two different aged cohorts of juvenile YTK to enable merging of the two cohorts into a single cohort prior to stocking to seacage. Maintaining low water temperature (15-16^{o}C) and feed

rates of an older cohort of fish allowed a younger cohort of YTK held under optimal conditions for growth (~22ºC) to attain the same weight as the older by a specific date. This enabled amalgamation of the groups as a single cohort for stocking to seacages. This ability to manage growth while maintaining fish condition, provides opportunities to build stocks of similar sized fish from multiple spawning batches. This could be useful in small hatcheries with limited larval rearing capacity, or as in our case, to compensate for larval batches that not produce the target number of fish.

Advanced juvenile YTK were harvested quickly and easily from RAS and FT tanks using handheld nets. In both RAS and FT tanks, the bulk of the fish population was captured initially with tanks filled to full volume. Fish in RAS were encouraged to the tank surface with small amounts of feed, where they were captured over time (up to 95% of population within 5-10 minutes) by 1 technician. The remaining fish were captured following drainage of the tank. Fish in FT tanks were captured by two technicians working together to crowd the fish in the tank.

Advanced juvenile YTK (up to ~1kg) were successfully cultured with high survival in outdoor polyhouse-covered, plastic-lined ponds supplied with compressed air only. Polyhouse covered ponds had a more stable water temperature than uncovered ponds. Covered ponds were typically 2ºC warmer than uncovered ponds in winter. During summer, covered ponds were approximately up to 1.5 ºC cooler on hot days and 1.5 ºC warmer on cold days. Final biomass was 7860 kg fish/ha. Maximum daily feed rate was 274.5 kg/day/ha. Harvesting of fish from the ponds was relatively easy with 5 staff taking approximately 45-60 minutes per pond. After three shots of the seine net only 12 fish (0.4%) of the population remained in the pond. These were captured once the pond water volume was drained.

In the first study done in ponds, we experienced a mortality event in one pond which we attributed to rapid development of a phytoplankton bloom that became obvious several days leading up to the mortality event. We were unable to detect other obvious deleterious water quality parameters including water temperature, salinity, pH or dissolved oxygen, and we suggest that the increased turbidity may have been the causative factor. We have observed in previous studies at PSFI, abnormal, transient jaw tetany of YTK in tanks with high turbidity and this may indicate the YTK are intolerant of low water quality. We were exchanging small volumes (about 25%) of influent seawater each day and recirculation of water from the pond to the external Biogill® system was approximately 50% volume per day. This level of water exchange and treatment was likely insufficient to maintain suitable water quality for YTK, which are a pelagic species, and generally occupy high-quality ocean environments (Fielder and Heasman, 2011). In the second successful pond study mentioned above, we increased the pumping capacity of filtered seawater to the ponds to potentially avoid any issues with poor water quality for future experiments. Daily exchange of influent seawater was increased from 25% to 100%/day and recirculation of pond water through the Biogill® fiter was increased from 50% to 100%/day. We also improved the capacity to remove settled organic waste from the pond bottom by operating a swimming pool vacuum ‘creepy crawly’ and discharged the waste directly from the pond, rather than into the Biogill® as occurred in the first study. This pond management was effective in maintaining high water quality and juvenile YTK grew and survived well.

Conclusion

Hatchery

- Internal tank colour (black or silver) did not affect growth or survival of YTK larvae to 30 dph; however, larvae grown in silver-lined tanks were darker in colour than those larvae grown in black tanks. Differences in fish skin colour were transient and all fish became the same colour once removed from the treatment tanks and placed into common nursery tanks. Based on these results, the standard PSFI black tank colour was deemed optimal for YTK culture and remained as control tanks for future experiments.

- Light source (Hibay mercury vapour or full spectrum LED lights) did not affect growth, survival, swimbladder inflation or first feeding of YTK larvae to 18 dph. Hibay lights were approximately twice as bright as LED lights at the surface and at all depths through the water column. Either hibay or full spectrum LED lights can be used for YTK larval rearing.

- Six commercial production runs were done with variable survival of larvae to metamorphosis (~30dph) ranging from 0-10.5%. Issues which likely contributed to poor survival of larvae in some batch's included excessive water temperature due to environmental heat wave conditions, decrease in influent salinity due to major rainfall event, poor egg quality due to abnormally frequent (3 monthly) spawning of broodfish, and potential uncontrolled establishment of deleterious bacterial communities in larval rearing tanks.

Nursery

- Advanced juvenile YTK (mean maximum 166g) were successfully cultured in RAS tanks (10 and 30 m^3; 1m and 2m depth, respectively) and flow-through tanks (FT; 5-10 m^3; 1m depth) for up to 192 days. Maximum stocking densities in both RAS and FT tanks was approximately 25 kg fish/m^3 with maximum feed rate of 0.5 kg food/day/m^3.

- FT tanks used up to 17x more influent seawater than RAS tanks to maintain high water quality, especially low suspended solid matter, and were reliant on ambient water temperature compared with RAS which had temperature control. FT tanks had few components and were easier to manage than RAS which had mechanical, biological and UV filters.

- Advanced juvenile YTK were harvested quickly and easily from RAS and FT tanks using handheld environets. In both RAS and FT tanks, the bulk of the fish population was captured initially with tanks filled to full volume. Fish in RAS were encouraged to the tank surface with small amounts of feed, where they were captured over time (up to 95% of population within 5-10 minutes) by 1 technician. The remaining fish were captured following drainage of the tank. Fish in FT tanks were captured by two technicians working together to crowd the fish in the tank.

- It was possible in RAS to restrict the growth with no loss of condition of an older cohort of juvenile YTK by reducing temperature (15-16^{o}C) and feed rates to allow a younger cohort to attain the same weight by a specific date. This enabled the amalgamation of the groups as a single cohort for stocking to seacages.

- Advanced juvenile YTK (up to ~1kg) were successfully cultured with high survival in outdoor polyhouse-covered, plastic-lined ponds supplied with compressed air only. Polyhouse covered ponds had a more stable water temperature than uncovered ponds. Covered ponds were typically 2^{o}C warmer than uncovered ponds in winter. During summer, covered ponds were approximately up to 1.5 ^{o}C cooler on hot days and 1.5 ^{o}C warmer on cold days. Final biomass was 7860 kg fish/ha. Maximum daily feed rate was 274.5 kg/day/ha. Harvesting of fish from the ponds was relatively easy with 5 staff taking approximately 45-60 minutes per pond. After three shots of the seine net only 12 fish (0.4%) of the population remained in the pond. These were captured once the pond water volume was drained.

Several areas of research are needed to improve our understanding of larval rearing and nursery culture of YTK, including:

- Further understand the interactions of salinity and temperature, particularly in the first 12 days post hatching. YTK larvae may have low tolerance of small variations in salinity and this is vital information for hatchery operators, especially if estuarine seawater with variable salinity is used in the hatchery

- Investigate the development of bacterial communities in the PSFI hatchery tanks and methods to avoid r-strategist bacteria populations from proliferating, including maturation of influent seawater and use of commercial probiotics

- Investigate improved methods to provide dissolved oxygen in nursery tanks used for high-density culture of YTK, including nannobubble technology

Implications

This project complemented the DoA project "Growing a profitable, innovative and collaborative Australian YTK aquaculture industry: bringing ‘white’ fish to the market - RnD4Profit-14-01-027". The end users are the Public, Regulators and Industry. The research undertaken addressed the short term needs to develop marine fish farming in eastern Australia and to provide a platform for ongoing research.

Industry: YTK production within Australia has been challenging and further research was needed, particularly when entering new farming environments including high wave-climate, offshore sites. It was essential to identify supply chains from broodstock to market. In particular, the culture of the largest juveniles possible on land before transfer to seacages was essential to optimise survival and production. Land-based techniques using simple flow-through tanks, sophisticated RAS and outdoor, polyhouse-covered ponds were evaluated and developed for viable production of advanced juvenile YTK. Each system offers benefits and challenges and optimal culture strategies will vary from site to site; the most likely scenario being incorporation of all methods to provide scope and control of fish production, especially during winter (too cold) and peak summer (too hot).

Public: Extensive public consultation identified key concerns with respect to the sustainable operation of the MARL. Many aquaculture ventures have failed after relatively short periods of time from establishment, and one contributing factor was often due to inadequate understanding of the biological requirements for reliable, cost-effective production of seed stock.This project successfully identified that advanced juvenile YTK could be produced sustainably at the PSFI hatchery, which is a fundamental requirement to operation of a viable marine fish farming industry.

Regulators: There was a need for NSW DPI to develop a Marine Waters Sustainable Aquaculture Strategy for NSW (MWSAS) to streamline investment pathways and promote sustainable seafood production. Data from this project was incorporated in the development of a MWSAS.

Recommendations

The results of this project build on the SOP for the production of juvenile YTK as reported in the hatchery manual “ Hatchery Manual for the production of Australian Bass, Mulloway and Yellowtail Kingfish” (Fielder and Heasman, 2011). The manual is almost 10 years old and inclusion of the results generated in this project in a revised Manual will update the present SOP employed at PSFI.

Further development

Several areas of research are needed to improve our understanding of larval rearing and nursery culture of YTK, including:

- Further understand the interactions of salinity and temperature, particularly in the first 12 days post-hatching. YTK larvae may have low tolerance of small variations in salinity and this is vital information for hatchery operators, especially if estuarine seawater with variable salinity is used in the hatchery

- Investigate the development of bacterial communities in the PSFI hatchery tanks and methods to avoid r-strategist bacteria populations from proliferating, including maturation of influent seawater and use of commercial probiotics

- Investigate improved methods to provide dissolved oxygen in nursery tanks used for high-density culture of YTK, including nannobubble technology

Extension and Adoption

Results of the project have been directly conveyed to NSW DPI aquaculture managers through verbal discussion and supply of interim reports. This was criticial to development of the Marine Waters Sustainable Aquaculture Strategy for NSW (MWSAS).

Results have also been supplied intermittently to colleagues at Challenger TAFE, WA.

Revision of the Hatchery Manual for YTK production will incorporate results generated in this project. The manual is freely available on-line from the NSW DPI website.

We also embedded for the duration of the project an industry employed Fisheries Technician, Georgia Pember, to gain first-hand, timely knowledge of all hatchery and nursery operations and results. We believe technical transfer is likely the most efficient method to increase technical capacity and rapid dissemination of information.

Project coverage

Hatchery production of YTK was included in a report done by ABC Landline in 2018 which covered the seacage farm developments and aquaculture research in general at the PSFI.

Project materials developed

Jackson Wilkes Walburn Honours Thesis (UNSW) Title: Assessing microbiome development in commercially raised Yellowtail Kingfish (*Seriola lalandi*). See Appendix 1 for thesis abstract.

Publication originating from Thesis:

Wilkes Walburn, J., Wemheuer, B., Thomas, T., Copeland, E., O'Connor, W., Booth, M., Fielder, S. and Egan, S., 2019. Diet and diet-associated bacteria shape early microbiome development in Yellowtail Kingfish (*Seriola lalandi*). Microbial Biotechnology 12(2), 275–288 doi:10.1111/1751-7915.13323 https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/1751-7915.13323

References

Abbink, W., Garcia, A., Roques, J., Partridge, G, Kloet, K., Schneider, O., 2012. The effect of temperature and pH on the growth and physiological response of juvenile yellowtail kingfish *Seriola lalandi* in recirculating aquaculture systems. Aquaculture, 330-333, 130-135.

Bobe, J., 2015. Egg quality in fish: Present and future challenges. Animal Frontiers 5, 66-72, doi:10.2527/af.2015-0010

Bohren, C. F. and Huffman, D.R. "Absorption and Scattering of Light by Small Particles", Wiley, 1983, ISBN 0-471-29340-7

Bowmaker, J.K., 1990. Visual pigments of fishes. In: Douglas, R.H., Djamgoz, M.B.A. (Eds.), The Visual System of Fish. Chapman & Hall, London, pp. 81–107.

Boyd, C., 1991. Types of aeration and design considerations. Pages 39-47 in: L. Swarm (Editor), Proceedings of the Second Annual Workshop: Commercial Aquaculture Using Water Recirculating Systems. Illinois State University, Normal, IL, Nov. 15- 16,1991.14.

Bregnballe, J., 2015. A Guide to Recirculation Aquaculture. An introduction to the new environmentally friendly and highly productive close fish farming systems. Copenhagen: Eurofish 64 pp.

Espinal C.A., Matulić D, 2019. Recirculating Aquaculture Technologies. In: Goddek S., Joyce A., Kotzen B., Burnell G. (eds). Aquaponics Food Production Systems. Springer, Cham

Fielder, D.S. and Heasman, M.P., 2011. Hatchery manual for the production of Australian Bass, Mulloway and Yellowtail Kingfish. Industry and Investment NSW, 122pp., ISBN 978 1 74256 058 8.

Fielder, D, Bardsley, W., Allan, G., Pankhurst, P., 2005. The effects of salinity and temperature on growth and survival of Australian snapper, *Pagrus auratus* larvae. Aquaculture, 250, 201-214.

Kjorsvik, E., Pittman, K., Pavlov, D., 2004. From fertilisation to the end of metamorphosis—functional development. In: Moksness, E., Kjørsvik, E., Olsen, Y. (Eds.), Culture of Cold-Water Marine Fish. Blackwell Publishing Ltd., Oxford, pp. 230–231.

Leclercq, E., Taylor, J.F., Migaud, H., 2010. Morphological skin colour changes in teleosts. Fish and Fisheries, 11, 159-193.

Lythgoe, J.N., 1979. The Ecology of Vision. Clarendon Press, Oxford

Ma, Z., 2014. Food ingestion, prey selectivity, feeding incidence, and performance of yellowtail kingfish *Seriola lalandi* larvae under constant and varying temperatures. Aquaculture International 22(4), 1317-1330.

Steven, A.H., Mobsby, D. and Curtotti, R., 2020, Australian fisheries and aquaculture statistics 2018, Fisheries Research and Development Corporation project 2019-093, ABARES, Canberra, April. CC BY 4.0. https://doi.org/10.25814/5de0959d55bab

Stuart, K.R., Drawbridge, M., 2011. The effect of light intensity and green water on survival and growth of cultured larval California yellowtail (*Seriola lalandi*). Aquaculture 321, 152-156

Sugimoto, M., 2002. Morphological color changes in fish: regulation of pigment cell density and morphology. Microscopy Research and Technique 58, 496–503.

Vadstein, O., Attramadal, K.J., Bakke, I., Forberg, T., Olsen, Y., Verdegem, M., Giatsis, C., Skjermo, J., Aasen, I.M., Gatesoupe, F-J., Dierckens, K., Sorgeloos, P., Bossier, P., 2018. Managing the microbial community of marine fish larvae: A holistic perspective for larviculture. Frontiers in Microbiology, doi: 10.3389/fmicb.2018.01820.

Villamizar, N., Blanco-Vives, B., Migaud, H., Davie, A., Carboni, S., Sánchez-Vázquez, F.J., 2011. Effects of light during early larval development of some aquacultured teleosts: A review. Aquaculture 315, 86-94.

Woolley, L.D., Qin, J.G., 2010. Swimbladder inflation and its implications to the culture of marine finfish larvae. Reviews in Aquaculture 2, 181-190.

Wurts, W.A., 2003. Daily pH cycle and ammonia toxicity. World Aquaculture, 34, 20-21.

Appendix 1

Wilkes Walburn, Jackson, 2017. Assessing microbiome development in commercially raised Yellowtail Kingfish (*Seriola lalandi*). The University of New South Wales Faculty of Science School of Biological, Earth and Environmental Sciences, Honours Thesis.

Abstract
Land based fish larviculture is essential for the production of marine finfish for consumption by a growing global population. Yellowtail Kingfish (*Seriola lalandi*) is an economically significant species in the Asia-Pacific region. Yet there is a lack of knowledge surrounding the diversity of its intestinal microbiome and how it develops in a larviculture system. Therefore, we used next generation DNA sequencing of the 16S rRNA gene to show that the intestinal microbiome experienced variation in the relative abundance of bacteria and shifts in both diversity and key bacterial taxa throughout the development of *S. lalandi* larvae. Despite this variation a small, yet abundant, core microbiome was identified across all developmental stages examined. Furthermore we demonstrated that these changes in the *S. lalandi* intestinal microbiome reflected changes in the feed-associated bacterial community. The greatest microbiome shift occurred as the larvae transitioned from live feeds to commercially formulated pellet, characterised by a transition from *Proteobacteria* to *Firmicutes* as the dominant phyla. The larval microbiome was also similar to the rearing-water but only at the pre-feeding stage. These results suggest that diet rather than host-deterministic factors or the rearingwater strongly contributes towards shaping the microbiome in larval *S. lalandi*.