Improving hatchery production of Yellowtail Kingfish larvae and fingerlings

Dr Jennifer Cobcroft

Project No. 2010/753

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

August 2012

NSW DEPARTMENT OF PRIMARY INDUSTRIES

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ISBN: 978-1-925983-43-2

The Australian Seafood CRC is established and supported under the Australian Government's Cooperative Research Centres Program. Other investors in the CRC are the Fisheries Research and Development Corporation, Seafood CRC company members, and supporting participants.

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Australian Government
Fisheries Research and Development Corporation

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Non-Technical Summary

2010/753 Improving hatchery production of Yellowtail Kingfish larvae and fingerlings

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PROJECT OBJECTIVES:

1 To identify key factors which can be manipulated in the hatchery to increase Yellowtail Kingfish survival and swimbladder inflation rate (>97% in 5g fingerlings in 2010), and reduce malformations
2 To identify key factors which can be manipulated to increase production of high quality rotifers
3 To rapidly apply research findings to production scale systems for Yellowtail Kingfish at Clean Seas Tuna

OUTCOMES ACHIEVED

Three key issues were identified by Clean Seas Tuna Ltd (CST) with impacts on the efficiency of commercial Yellowtail Kingfish *Seriola lalandi*, including jaw malformation, swimbladder inflation rate and survival. These were investigated in this project by the same research teams involved in the complementary project on Southern Bluefin Tuna (SBT) hatchery research (SfCRC 2010/750).

The CST Standard Operating Procedures for Yellowtail Kingfish (YTK) hatchery processes were reviewed and updated during the YTK season in 2010, prior to the SBT season. CST had a successful YTK production season in 2010, although survival between runs was variable (6 to 11%), and all fingerlings adhered to the quality control standards. The low swimbladder inflation rates seen in 2009 were rectified and many protocols were tightened, updated and improved. Jaw malformation remained a significant issue in commercial production, with an average of 35% of juveniles affected. Three feeding strategies were compared at a proof-of-concept scale, as well as artificial versus natural lighting. Meal feeding resulted in lower survival and growth than the standard practise of feeding multiple times during the day to a target density. There was an indication that jaw malformation was lower in YTK grown in indoor tanks under artificial light compared with outdoor tanks and sunlight, and temperature control was more consistent in inside tanks, although growth and survival were similar.

Two experiments were conducted in the newly commissioned R&D system in Arno Bay. The first on commercial rotifer enrichment products found that S.presso®, S.pirit®, Origreen® and N-rich® all provided for good survival, growth and

swimbladder inflation, with no significant differences at 9 days post-hatching (DPH) when the experiment was terminated due to low swimbladder inflation (~60%). However at 9 DPH, swimbladder inflation in N-rich fed larvae was significantly lower (35%), around half that of the other diets (65%). The second experiment on light intensity revealed that survival, swimbladder inflation and the incidence of jaw malformation at 9 DPH were all lower at 1,000 lux, than at the higher levels 7,000 and 12,000 lux. There was no significant effect of light on growth up to 9 PDH. Improvements in the operation of the R&D system are required to enable increased swimbladder inflation and performance values comparable to the commercial systems.

Four experiments conducted at PSFI, identified the optimal water temperature (24.5^{o}C), live feed enrichment protocols (S.presso), light intensity (32,000 lux) and dissolved oxygen (saturated) parameters for promotion of optimal swimbladder inflation rates, growth, survival and quality of YTK larvae. The results contributed to the understanding of the roles of abiotic and biotic factors in the larviculture of *Seriola* species. Given the demonstrated importance of light intensity for swimbladder inflation and fish survival, and the fact that quality of light may differ between natural ocean and artificial tank environments, further studies should focus on understanding the effects of light periodicity (photoperiod) and light source, including wavelength and spectrum, on performance of YTK larvae.

Important target biological indicators were partially achieved in this project in selected larval rearing tanks/systems, including; jaw malformation <14% to ≥10 mm, survival 17% to the end of the larval tank phase, and >97% swimbladder inflation in commercially produced 5 g fingerlings in 2010.

High malformation levels remain the biggest single problem for CST in the production of YTK fingerlings. CST is committed to ongoing research, particularly in the new R&D system in Arno Bay, to trial factors with scientific rigour to better understand and solve the jaw malformation issue.

LIST OF OUTPUTS PRODUCED

Eleven monthly reports on the R&D progress; five oral or poster presentations to national and international conferences; one newsletter article; review of Standard Operating Procedures and revised SOPs for CST hatcheries to support YTK and SBT hatchery production; two published papers; new R&D system commissioned in Arno Bay; several new skilled staff recruited to CST; eight experiments and four proof-of-concept trials with YTK.

KEYWORDS: Yellowtail Kingfish, aquaculture, fingerling production, survival, swimbladder, jaw malformation

Acknowledgements

The Yellowtail Kingfish Larval Research Implementation Group (LRIG) is thanked for their commitment to developing the project, critical experiment design and support of communication with the industry partner, Clean Seas Tuna (CST). The dedicated group met nine times during the course of the project, mostly by teleconference and twice in person in Arno Bay, and their input to the R&D effort was greatly appreciated. Dr Stewart Fielder led the PSFI fish hatchery team in NSW in YTK larval experimentation. Wayne Hutchinson and Dr Bennan Chen of SARDI provided invaluable support to research activity in Arno Bay, assisting with system operations, live feeds experiment equipment, larval husbandry and monitoring. From IMAS in Tasmania, Assoc Prof Stephen Battaglene provided valuable advice on swimbladder inflation, water treatment and experiment design, and Ms Melanie Evans provided enthusiastic technical support in live feeds, larval rearing and malformation assessments in Arno Bay. Assoc Prof Jian Qin supervised PhD candidates in YTK research, and provided advice to the LRIG. Dr Gavin Partridge and Mr Andrew Tindale of Challenger IT are thanked for their review of CST hatchery Standard Operating Procedures and design and implementation of some proof-of-concept trials during YTK production. Dr Partridge and Dr Fielder also supported subsequent experiments by PhD candidate Lindsay Woolley in WA and NSW, respectively.

The LRIG thanks Dr Craig Foster and all members of RMAG for facilitating project development and direction. Dr Len Stephens and Dr Graham Mair of the Seafood CRC provided insightful assistance during project development and submission and demonstrated active interest in the project over the year.

The CST management team, in particular Clifford Ashby, Hagen Stehr, Morten Deichmann and Mike Thomson were supportive of the research approach and rapid expansion of the hatchery R&D team in Arno Bay. We thank the CST Board for their support and interest as the project progressed. Thanks to all of the CST administrative support team in Arno Bay and Port Lincoln who capably handled the additional workload associated with the project in HR and accounts.

The research team at CST; Nicolas Mace, Alex Czypionka, David Poppi, and Atefeh Ghaltii are thanked for their many hours of dedicated hard work in the hatchery. You all played a vital role in getting the SBT Hatchery operational, with a commercial-scale run of YTK, and establishing a firm foundation for R&D in Arno Bay in the future. The new staff in the SBT Hatchery, Marcell Boaventura and Antonio Lillo brought some important skills and professional experience to Arno Bay. Antonio hit-the-ground-running in the SBT Hatchery for the YTK production run – awesome job. The extended CST Yellowtail Kingfish hatchery production teams from Arno Bay and Port Augusta, led by Jamie Crawford, Travis Dymmott, Mark Quinn and Konrad Czypionka, are thanked for working with the R&D team in pilot scale trials and sample collection.

John Carragher (Logifish Consulting) provided assistance editing CST sections of the Final Report.

Definitions

AB:	Arno Bay, South Australia. The location of one of Clean Seas Tuna's YTK hatcheries, the SBT Broodstock Facility and SBT Hatchery.
ACAAR:	Australian Centre for Applied Aquaculture Research (Challenger IT, Western Australia).
Broodstock:	Adult male and female YTK that are held in captivity and used as the source of eggs and sperm for the hatchery production of fish. The CST YTK broodstock are a combination of wild caught animals and fish reared by the company in the hatchery and then on-grown in seacages. They undertake natural spawning behaviour in the tanks and the resulting fertilised eggs float to the water surface and are collected in mesh screen bags from the surface overflow water.
CST:	Clean Seas Tuna Ltd.
DPH:	Age of fish larvae in days post-hatch.
Enrichment:	Enrichment is the process whereby live feeds, rotifers and *Artemia*, are fed diets to improve (or modify) their nutrient content prior to adding to culture tanks to feed fish larvae. Enrichment products are usually high in marine lipids, especially highly unsaturated fatty acids (HUFAs), protein, vitamins and minerals. Enrichments are blended in water and added to tanks holding the live food for variable times (e.g. 2 h to 24 h), depending on the live feed species, recommendations of the enrichment product manufacturers, and the desired content of various nutrients.
Fingerling:	A fish that has passed through the larval phase such that it has been fully weaned off live foods and is eating a formulated diet. Most organs are developed and the fish has more control over its position in the water column.
Formulated diet:	A fish that has passed through the larval phase such that it has been fully weaned off live foods and is eating a formulated diet. Most organs and fins are developed and the fish has more control over its position in the water column.
Hatchery run:	Broodstock YTK are conditioned and induced to spawn for a few days. Each spawning event can produce 20-100 million fertilized eggs. These numbers are sufficient to fill the capacity of the larval rearing and live food systems in the hatchery.
Hatchery season / Hatchery year:	The YTK hatcheries usually operate from August to October to undertake sufficient (usually 2 or 3) hatchery runs to produce the target number of quality fingerlings that can be stocked into seacages from September to December.
Hatchery:	A facility where fertilized eggs are reared through egg incubation, larval rearing and nursery/fingerling stages.
Larvae:	A newly hatched YTK fish that is ingesting live prey as food. Not all organs will have developed, and they usually have little control over their position in the water column.

Live feeds:	In the case of YTK this is a combination of microalgae, rotifers and *Artemia* nauplii, with the regime dependent upon the age and development stage of the fish.
N-rich:	Concentrated algae paste for live feed, usually rotifer, enrichment from Reed Mariculture.
O.range:	Formulated weaning diet for marine finfish larvae from INVE.
Malformation:	Developing larvae can be affected by endogenous and exogenous factors such that the spine, bones in the jaw and head, and/or swimbladder may not form properly. This can give rise to problems with the fish as it grows.
PA:	Port Augusta, South Australia. The location of one of Clean Seas Tuna's YTK hatcheries.
Proton/NRD:	Formulated weaning diet for marine finfish larvae from INVE.
PSFI:	Port Stephens Fisheries Institute, NSW Fisheries,
QC:	Quality control protocols in place at Clean Seas Tuna, with a prescribed procedure for the routine assessment of quality of batches of YTK juveniles prior to transfer to seacages. This involves assessment of swimbladder inflation, jaw malformation, variation in fish weight, screening for fish health.
RSD:	Residual density of live prey in larval tanks (rotifers or *Artemia*), determined by counting prey in known volume subsamples of water from the tanks.
Sanolife MIC-F:	Probiotic from INVE in dry powder form. Mixture of *Bacillus* sp.
SBT:	Southern Bluefin Tuna, *Thunnus maccoyii* (Castelnau, 1872). The Southern Bluefin Tuna is a fast-swimming pelagic species that occurs around the globe in southern temperate waters. The species can grow up to 2.25 m (fork length).
SOP:	Standard Operating Procedures describe operational methods in CST hatchery activities. Documents written by hatchery staff, used for training new staff and day-to-day operations, and reviewed regularly.
S.parkle:	Rotifer culture diet from INVE. A yeast-based product provided in dry powder form.
S.presso:	Rotifer and *Artemia* enrichment diet from INVE. A fish oil based liquid emulsion.
SR:	Survival rate of fish larvae or fingerlings. This can be survival from stocking yolksac larvae in larval tanks to the transfer of metamorphosed fish to the nursery (at around 22 DPH), or within the nursery (from approx. 22 DPH to 60 DPH), or across a whole Run, from stocking all larval tanks to the number of fingerlings transferred to seacages.
WW:	Wet weight, usually of YTK larvae or fingerlings. This is commonly measured as a bulk weight of fish in seawater, and the fish weight is determined by subtracting the weight of the water. By measuring the wet weight of a known number of fish, the number of fish from the whole population can be estimated.

YTK:	Yellowtail Kingfish, *Seriola lalandi* Valenciennes in Cuvier & Valenciennes, 1833. The Yellowtail Kingfish occurs in tropical and temperate waters of the southern hemisphere and the northern Pacific. In Australia, it is recorded from North Reef, Queensland (23°11'S) to Trigg Island, Western Australia (31°52'S), and as far south as Tasmania. The common name for this species comes from the conspicuous yellow caudal fin. The Yellowtail Kingfish can grow up to 2.5 m in length and can weigh up to 70 kg.

1 Introduction and Background

1.1 Need

Clean Seas Tuna is the largest commercial hatchery producer of Yellowtail Kingfish fingerlings in Australia. Following three years of research (2007-2010) into larval rearing issues, the company still experienced variability in results between the two hatcheries, Port Augusta and Arno Bay, between runs within hatcheries, and between individually stocked larval tanks (even those stocked from the same egg batch). 2009 was particularly challenging compared to the previous two years with an increase in skeletal malformations (jaw deformity from 20-25% to > 50%), decrease in survival (from >10% to 8%), and decrease in swimbladder inflation (from 99% to 80%). This 1 year project built on and applied, at a commercial scale, the results of the previous projects (SfCRC 2007/718 and 2009/749). The project was designed to support improved Standard Operating Procedures and test promising alternative rearing conditions, identified in previous research (especially temperature and artificial light), at a medium-scale in Arno Bay and Proof-of-Concept (commercial-scale) in both hatcheries. Funding was requested to invest in skilled personnel, upgrade systems, support key national collaboration, and provide operational costs at partner organisations.

CST is moving to the capacity for a 5,000 ton per annum sustainable YTK business by 2020. To underpin this growth in the YTK industry it is critical that the hatchery production efficiency and quality of fingerlings produced are improved. In 2012, health issues in the growout phase have slowed industry growth and are addressed in other research.

This project directed investment in skilled personnel and resources to increase our understanding of critical factors that can be manipulated for improved yield of YTK juveniles and lower production costs by refined culture conditions to achieve reliably higher survival, higher swimbladder inflation rate and lower incidence of skeletal deformities.

The project fits within the Seafood CRC outputs and milestones, as follows, toward the outcome of a 'substantial increase in the production and profitability of selected wild-harvest and aquaculture species'.

1.1 (Output) Technically verified new aquaculture production systems on a commercial scale

1.1.2 (Milestone) Key researchable constraints identified and characterised in at least two new production systems

1.1.3 (Milestone) Key researchable constraints successfully addressed in at least two new production systems

Three significant issues were identified in YTK larval rearing over the previous 3 years of research (2007-2010) that presented bottlenecks to hatchery production efficiency. These include:

1. low survival (potentially linked to high microbial load in live feeds and larval cultures, sinking of older larvae, variable temperature which is constrained by 'outside' rearing conditions under ambient natural sunlight)
2. low swimbladder inflation rates (associated with changed husbandry conditions; upwelling, mister fans, skimming efficiency, algae pastes, larval health/nutrition)
3. high rates of jaw deformity (potentially linked to quality and quantity of enriched rotifer and *Artemia* diets, temperature)

This project addressed all three issues, with a focus on applying results from previous research at medium and commercial-scales (light intensity and quality, and temperature, live feed regimes) and investigating larval nutrition and photoperiod at a small-scale.

1.2 Objectives

1 To identify key factors which can be manipulated in the hatchery to increase Yellowtail Kingfish survival and swimbladder inflation rate (>97% in 5g fingerlings in 2010), and reduce malformations

2 To identify key factors which can be manipulated to increase production of high quality rotifers

3 To rapidly apply research findings to production scale systems for Yellowtail Kingfish at Clean Seas Tuna

1.3 Report Format

This report is presented in standalone sections which include methods, results and discussion content for each of the research partner components. This is followed by an overall conclusion and final report sections that outline the application of the 'whole of project' research activity.

The CST R&D team activity was largely in support of SARDI experiments with YTK in this project. Associated activity in live feeds and commissioning of the SBT Hatchery with a YTK commercial-scale production trial, is reported in the SBT project Final Report 2010/750. The R&D team also facilitated and supported the review of CST SOPs. As a result, there is no separate chapter of CST R&D research in this report.

The report contains summary information about experiments conducted by Flinders PhD candidate, Zhenhua Ma, at SARDI, which were directly funded by this project. The full reports from this work are included in Zhenhua's PhD project reports to SfCRC (2009/700). In addition, PhD candidates Lindsey Woolley (Flinders) and Pollyanna Hilder (UTas) have both conducted experiments with YTK that are complementary to this project, and their work is reported separately to the SfCRC in their project reports (2009/733 and 2009/760, respectively).

2 Yellowtail Kingfish commercial trials - CST Production

Section written by and in collaboration with Konrad Czypionka, Travis Dymmott, Jamie Crawford, Adam Miller, Ryan Harrison, Clare Flanagan and Morten Deichmann

Introduction

This section of the report describes Yellowtail Kingfish (YTK) commercial production run outcomes and results of proof-of-concept scale trials (tank size and operations equivalent to commercial production).

In 2010 CST did not have extra R&D staff employed to gather YTK production data as was the case in 2009, this has meant that data collection has been less intensive and concentrated on actual production data relevant for day to day operations. Instead the R&D man power was channelled into a dedicated R&D hatchery with its own management and staffing. This would make it possible to conduct tests with scientific rigour that produces clear outcomes, which could be adopted in commercial production.

The YTK production result in 2009 was disappointing with a large amount of fish going to sea which did not meet the grade A quality control (QC) criteria with regard to malformations and swimbladder inflation. On the basis of this, the 2010 season saw a cautious approach towards new methods and a general tightening of protocols and procedures (SOPs) during an internal review.

This saw items in the 2009 protocol which were suspected to have a negative effect on egg quality or larval survival were removed from the 2010 protocol.

In the 2010 season, the project also included an external review and larval rearing support from ACAAR (WA) for one larval run in Arno Bay.

2010 saw two trials conducted in production at the Arno bay hatchery:

- Running 2 inside tanks in Arno Bay on artificial light
- Trialling the so-called ‘meal’ and ‘hybrid’ feeding concepts for the rotifer phase

As part of the internal review we adhered to a dry out procedure in Arno Bay, which we were unable to do in 2009 due to the presence of SBT juveniles.
Furthermore, all rotifer production cultures were restarted from stock cultures held in live algae, as bacterial contamination/bacterial overload of the production cultures could have contributed to the poor 2009 result.

The 2010 season saw both positive and negative outcomes as we cleared out many problems from 2009. However two issues were still not resolved, one being the variability in production output in terms of larval survival rates but especially the high malformation level is crippling outputs.

Variability is evident by the two very different results in Arno Bay (AB). The report will show that some of the trials we did in run 1 in AB had a negative impact and most definitely contributed to the poor overall results of that run.

However the most important issue for CST remains the high level of malformation because we still do not understand the triggers.

Methods

Broodstock, egg production and incubation

The general spawning protocols, collection of eggs and harvesting of larvae for stocking of tanks did not change in 2010, and are described in previous Seafood CRC reports. One change involved the ozonation of eggs which was trialled in 2008 and adopted in production for 2009. On review, we decided against ozonation in 2010 based on veterinarian advice being; if there is no suspicion of a virus then ozonation of eggs, as all handling, may pose a risk to the egg and the early larvae. Seeing our problems in 2009 we did opt out of several changes previously adopted in 2009 for the 2010 season, including ozonation.

Tank Setup

Larval tank set-up:

- Natural light conditions*
- Flow through systems
- UV sterilised micro-filtered seawater
- AB 7 x 8 m^3 tanks and 2 x 8 m3 tanks (*inside)
- PA 7 x 5 m^3 tanks and one 9.6 (10) m^3 tank
- No water upwelling only aeration

*2 tanks in the nursery in AB were set-up as part of the ACAAR presence on site during the external SOP review. These 2 tanks were N3 and N5, positioned inside in the nursery area and each was artificially lit by 2 fluorescent tubes and 2 metal halide bulbs (Philips 400w) above each tank. This gave a light intensity range of 1,100 lux at the rim of the tank (minimum) to 6,800 lux just under the halide lights (maximum). In comparison the L1-L7 tanks in natural sunlight had a light intensity range of 1,800 – 2,600 lux (8 am) to 9,000 – 11,000 lux (5 pm) to a maximum of approx. 23,000 lux at mid-day on full-sun days. This inside tanks with artificial light approach had been attempted before in 2009 however swimbladder inflation was disappointing.

The rearing under artificial light allows for a rearing unit to be placed in an isolated building with set light regime, eliminating the variability in temperature and light levels seen in more exposed locations like a “sun room”. Further it allows working hours to better suit staff as there is no need to begin very early in the morning and work very late following the natural day length.

Live Feeds

Live feed set-up:

- Green water: *Nannochloropsis* paste (Reed)
    - Probiotics in tanks in Port Augusta (PA)/No probiotics in AB
    - Manual addition PA and AB1 (Arno Bay Run 1)
    - Dosing pumps AB2 (based on ACAAR recommendation)
- Rotifers at 2 days post-hatch (DPH)
    - Both hatcheries used recirculation systems with yeast and commercial diet (S.parkle INVE)
    - Enrichment with INVE S.parkle, INVE S.presso, probiotic INVE Sanolife MIC-F
    - Density – maximum 15 rotifers (r)/ml*
- *Artemia* start 10DPH

    - 24 h hatch and 24 h enrichment protocol, enrichment with DC DHA Selco, INVE S.presso
- Weaning onset at about 25DPH
    - INVE O.range and Proton/NRD

* Several tanks in the 1st run in AB were set-up testing feeding concepts with ACAARs day to day supervision. Three concepts were run: CST rotifer density regime, a meal concept and a hybrid concept. The approach of these feeding strategies was:

CST density: Kept rotifers represent at all times in a density which stimulates maximum uptake, but can result in uneaten and rotifers of poor nutritional value to remain in the tank which are also available as prey for the larvae. The method is easy to manage.

Meal: Only added enough rotifers for one meal, thus only feeding freshly enriched rotifers. However the feeding densities were low and overall intake could suffer if not controlled extremely well because the populations total ingestion and digestion needs to be followed closely.

Hybrid: Was an intermediate of the two using the density model at onset of feeding accepting that larvae are not good predators and density does stimulate feeding, however as the larvae grow and become better at swimming, targeting and capturing prey, the feeding regime reverts to the meal concept. Again control is important.

A more detailed description of the methods for this trial is found in the ACAAR Review (Appendix 3).

Larval rearing

Larval rearing protocol was not altered significantly for the 2010 run, but as mentioned above, concepts tested in 2009 were eliminated and protocols tightened. This included not using formalin and decreasing the maximum rotifer feed density to 15 r/ml.

On hardware, the use of fan misters to cool the larval area was abandoned and only aeration would be used in the tanks to provide turbulence.

We made two case studies under ACAAR supervision as mentioned under the live feed section above:

- Inside (artificial light) and outside (natural light) for larval rearing
- Meal, Hybrid vs. Density rotifer feeding

Samples for larval assessment for malformation were conducted as in 2009 by the R&D team on site.

Transfer to the nursery was by wet weight where possible, providing an estimate of the number transferred and survival from each larval tank.

Nursery

No changes were introduced to the nursery operations.

Malformations were culled by hand sorting the juveniles and fish were sampled for swimbladder inflation by autopsy. Fish were anaesthetised and floated in high salinity

seawater, as per the hatchery SOPs, when needed to remove fish with non-inflated swimbladders.

Seacage grow-out staff conducted health and QC checks prior to sea transfer.

Bacteriology

Due to lack of staff and the low managerial benefit of the results, this operation omitted in 2010.

Probiotics were used in the tanks and enrichment of rotifers in PA but not in AB, where the probiotics were only used in the rotifer enrichment.

Formalin treatment of larval tanks was omitted as its impact on larval quality was not clear.

Results and Discussion

General hatchery performance

Both hatcheries had two runs to complete the target for 2010 (Table 1).

Table 1. Summary of Yellowtail Kingfish production runs in 2010 at CST Arno Bay and Port Augusta hatcheries. (Abbreviations: WW = wet weight, non-inflated = fish with non-inflated swimbladder, malform = malformed and culled fish, To sea = number of juveniles transferred to seacages, QC = quality control)

Arno Bay 2010

Year	run	larvae	WW	larval	non-inflated	malform	To sea (QC)
		stocked	to nurs	survival %	%	%	
	1	6,220,000	394,100	6	0	49	148,373
	2	7,120,000	676,488	10	0	36	365,850
Total	2	13,340,000	1,070,588	8	0	43	514,223

Port Augusta 2010

	run	larvae	WW	% Larval	non-inflated	malform	Total
Run		stocked	to nurs	survival	%	%	to sea
	1	3,965,000	437,482	11	2	43	194,745
	2	4,120,000	418,161	10	0	23	223,870
Total	2	8,085,000	855,643	11	0	36	418,615

Note: Larval survival rate was calculated from wet weight (WW) transfer number. The malformation and non-inflation rates were calculated from the counted numbers (number non-inflated, number malformation, and number to sea).

Table 2. Comparison of Yellowtail Kingfish production runs from 2007 through to 2010 at CST Arno Bay and Port Augusta hatcheries.

		2007				2008		
		Larval (2+3)	Nursery	CST culled		Larval	Nursery	CST culled
Arno Bay	Run 1	20	28	26 (21)		25	15	20
	Run 2	8	19	13 (11)		32	12	15
	Run 3	5	12	14 (12)		30	25	24
	Overall	**11**	**20**	**16**		**29**	**18**	**19**
Port Augus	Run 1	30	49	27 (23)		35	42	42
	Run 2	22	29	17 (13)		19	28	24
	Overall	**26**	**39**	**23**		**27**	**35**	**31**
		2009				**2010**		
		Larval	**Nursery**	**CST culled**		**Larval**	**Nursery**	**CST culled**
Arno Bay	Run 1	26	35	50		39	nd	49
	Run 2	44	46	58		37	nd	36
	Run 3	nd	34	41		na	na	na
	Overall	**35**	**39**	**53**		**38**		**43**
Port Augus	Run 1	32	25	39		41	nd	43
	Run 2	33**	nd	36		18	nd	28
	Overall	**32****	**25****	**38**		**29**	**nd**	**36**

Note: Larval and Nursery figures are early malformation checks done with the IMAS method using a microscope during larval transfer to nursery and after weaning, while "CST culled" is the commercial removal for malformed fish as a percentage of fish transferred to sea.

A comparison between the last four years shows that the production parameters with regard to swimbladder inflation were back to normal in 2010, but that malformation was still at record high levels (Table 2). AB had improved over 2009 but PA had not, and prior to 2009 the overall CST levels were much lower as seen in the year to year comparison. This could however also be due to the very strict QC criteria in 2009, 2010 and 2011.

Malformation levels continue to be the main problem.

The output from both hatcheries was approximately 930,000 juveniles to sea, with AB run 1 amongst the poorest outputs seen from CST, while run 2 was amongst the highest. It was in AB run 1 that the ACAAR feeding trials were done. Unfortunately the tanks that were on the hybrid or meal concepts were compromised in growth and/or survival and/or larval quality, while run two was done with the CST feeding SOP. The PA runs were on average with previous runs.

SOP Review

ACAAR's review of SOPs lead to several changes and upgrades either by their recommendation or by discussion. The SOPs have been reviewed again since as part of a plan to keep them updated.

Some of the adopted changes after recommendations from ACAAR:

- Skimming procedure:
    - Concentrate skimming to 2 – 5 DPH instead of 3 – 9 DPH. Skimming does continue until 7-8 DPH

    - Streamline larval tank set-up to improve ease of skimming and skimming effectiveness
- Algae and Rotifer stock procedure: Created a live algae stock culture SOP to use in conjunction with the rotifer stock culture SOP.
- Algae addition: Dose algae paste from pre-dawn in AB (PA is not doing this and any benefit of this to production is not clear)
- *Artemia*: SOP on the use of the *Artemia* product Sep-Art.
- Larval rearing: Prevent removing shade cloth above larval tanks during daylight hours
- Bacteriology: Updated old SOP
- Broodstock (YTK): Removing a rogue procedure in egg quantification.
- General:
    - We have expanded on all SOPs, adding more detail where necessary
    - We have formatted all SOPs into a consistent layout and text
    - Updating examples

Other changes in discussion with ACAAR and in line with the internal 2009 review in CST in June 2009 for the 2010 protocol:

- Lowering of live feed density back down to 15 r/ml from the high density
- Remove water upwellers from larval tanks
- Remove demisting fans (interfere with SWB inflation)

We still need to do more and the SOP section is an area of constant improvement and updating.

Table 3. List of current CST hatchery Standard Operating Procedures.

Number	Title	Version	Version Issued
CS-201	Safe Practices Chlorine	1	29/12/2010
CS-202	Chlorination Rates	1	29/12/2010
CS-203	Wearing of Gum Boots	2	17/18/10
CS-204	Dumping of Waste – Hatchery	2	30/06/2009
CS-205	Good Hygiene & Biosecurity	3	8/03/2006
CS-206	Scales Calibration	1	29/12/2010
CS-210	Daily Procedures for YTK Broodstock	1	25/10/2011
CS-211	Photoperiod and light regime for YTK	1	25/10/2011
CS-212	Water Quality Testing	1	25/10/2011
CS-213	YTK Egg Collection	2	25/10/2011
CS-214	Egg Ozonation	2	25/10/2011
CS-215	Egg packing for transfer	1	25/10/2011
CS-216	Incubation for YTK Eggs	2	25/10/2011
CS-217	Harvest of YTK Eggs	1	25/10/2011
CS-218	Transferring larvae to larval tanks	1	25/10/2011
CS-219	Formalin treatments for YTK	1	25/10/2011
CS-230	Batch tank Rotifers	1	29/12/2010
CS-231	Recirculation System	1	29/12/2010
CS-232	Rotifer Enrichment	1	29/12/2010
CS-233	Rotifer Feed Strategy	1	29/12/2010
CS-234	Rotifer Calculation	1	29/12/2010
CS-250	Decapsulation of Artemia	1	29/12/2010
CS-251	Hatching the Artemia Cyst	1	29/12/2010
CS-252	Harvest of the Separt Nauplii	1	29/12/2010
CS-253	Artemia Enrichment	1	29/12/2010
CS-254	Artemia Tables	1	29/12/2010
CS-255	Artemia Calculations	1	29/12/2010
CS-270	Larvae Stocking	1	29/12/2010
CS-271	Feeding and Maintaining Larval Tanks	1	29/12/2010
CS-272	Swim Bladder Inflation	1	29/12/2010
CS-273	Moving Larvae	1	29/12/2010
CS-274 AB	Larval Rearing Summary - Arno Bay	1	30/12/2010
CS-274 PA	Larval Rearing Summary - Port Augusta	1	30/12/2010
CS-058	Floating Procedure - Kingfish	1	17/08/2010
CS-290	Nursery Daily Duties	1	29/12/2010
CS-291	Weaning and Feeding	1	29/12/2010
CS-292	Weaning Manual	1	29/12/2010
CS-293	Automatic Grading	1	29/12/2010
CS-294	Using raceway	1	29/10/2010
CS-295	Counting Fish Manually	1	29/12/2010
CS-296	Counting Fish Automatically	1	29/12/2010
CS-297	Hand sorting	1	29/10/2010
CS-298	Hand sorting Guide	4	29/12/2010
CS-299	VAKI Counter	1	29/12/2010
CS-300	VAKI Counter Calibration	1	29/10/2010
CS-301	Transfer from Hatchery to Grow out	1	29/12/2010
CS-302	Nursery Calculations	1	29/12/2010

Egg production and egg quality

Table 4. The data collected from a total of 103 egg batches from all CST Yellowtail Kingfish broodstock populations (B1, B2 and B3) in 2010.

	Date		Viability		Egg Diam (micron)	
	Start	End	min	max	min	max
B1	11-Aug	13-Oct	85%	97%	1280	1363
B2	30-Jul	12-Oct	78%	96%	1233	1359
B3	5-Aug	12-Oct	80%	95%	1270	1341

Most YTK egg batches were above 85% viability (Table 4) and data showed no apparent differences when compared to previous years.

Table 5. Details of YTK egg batches used to stock into larval tanks in 2010.

Egg stocking 2010

Run 1 Port Augusta					
Date	B tank	Viability %	Size (my)	Hatch Rate %	Destination
6-Aug	B2	85%	N/A	85%	L1, L2, L3
11-Aug	B2	88%	N/A	81%	L4, L5, L6
15-Aug	B2	89%	N/A	95%	L7, L8
		87%		87%	

Run 2 Port Augusta					
Date	B tank	Viability %	Size (my)	Hatch Rate %	Destination
18-Sep	B1	87%	1320	81%	L1, L2, L3
24-Sep	B3	88%	1333	79%	L4
24-Sep	B1	94%	1348	78%	L5, L6
26-Sep	B1	93%	1335	57%	L7
30-Sep	B2	90%	1343	54%	L8
				69%	

Egg stocking 2010

Run 1 Arno Bay					
Date	B tank	Viability %	Size (my)	Hatch Rate %	Destination
24-Aug	B1	85%	1333	89%	L1, L2, N3, N5
27-Aug	B1	86%	1358	42%	L3
28-Aug	B1	86%	1363	87%	L4, L5
2-Sep	B1	93%	1323	99%	L6, L7
				82%	

Run 2 Arno Bay					
Date	B tank	Viability %	Size (my)	Hatch Rate %	Destination
2-Oct	B3	86%	1305	96%	L1, L2, L3 (50%)
2-Oct	B1	92%	1344	93%	L3(50%), L4
7-Oct	B1	94%	1326	100%	L2(75%)
8-Oct	B1	93%	1349	84%	L2(25%), L5
10-Oct	B1	92%	1343	94%	L6, L7
17-Oct	B1	95%	1355	100%	N3, N5
				94%	

Note: There were three instances of poor hatch out, not related to egg quality, but operational failures. In PA the aeration on the eggs failed leading to sinking of eggs, clogging of the outlet screen and loss of eggs, while in AB a hole in the screen meant eggs were lost during incubation.

CST has a strict production target and production timeline, meaning that eggs were used as they were available and in the quantities needed for stocking the incubators and larval tanks efficiently. While we did try and use all three broodstock populations in order to get wider genetic diversity in our genetics program, it is clear from the data that tank (population) B1 contributed more to the fingerling production, than B2 or B3.

Live feed

Live feed data is presented here.

<u>Rotifers</u>

The feed out regime was 5 r/ml at 2-3 DPH, followed by a daily increase until 5 DPH were it plateaued at 15 r/ml in all tanks in PA and in the AB 'Density' tanks. By 13 DPH the rotifers are normally decreased until none are fed by 17-18 DPH, but can be extended.

Port Augusta Run 1

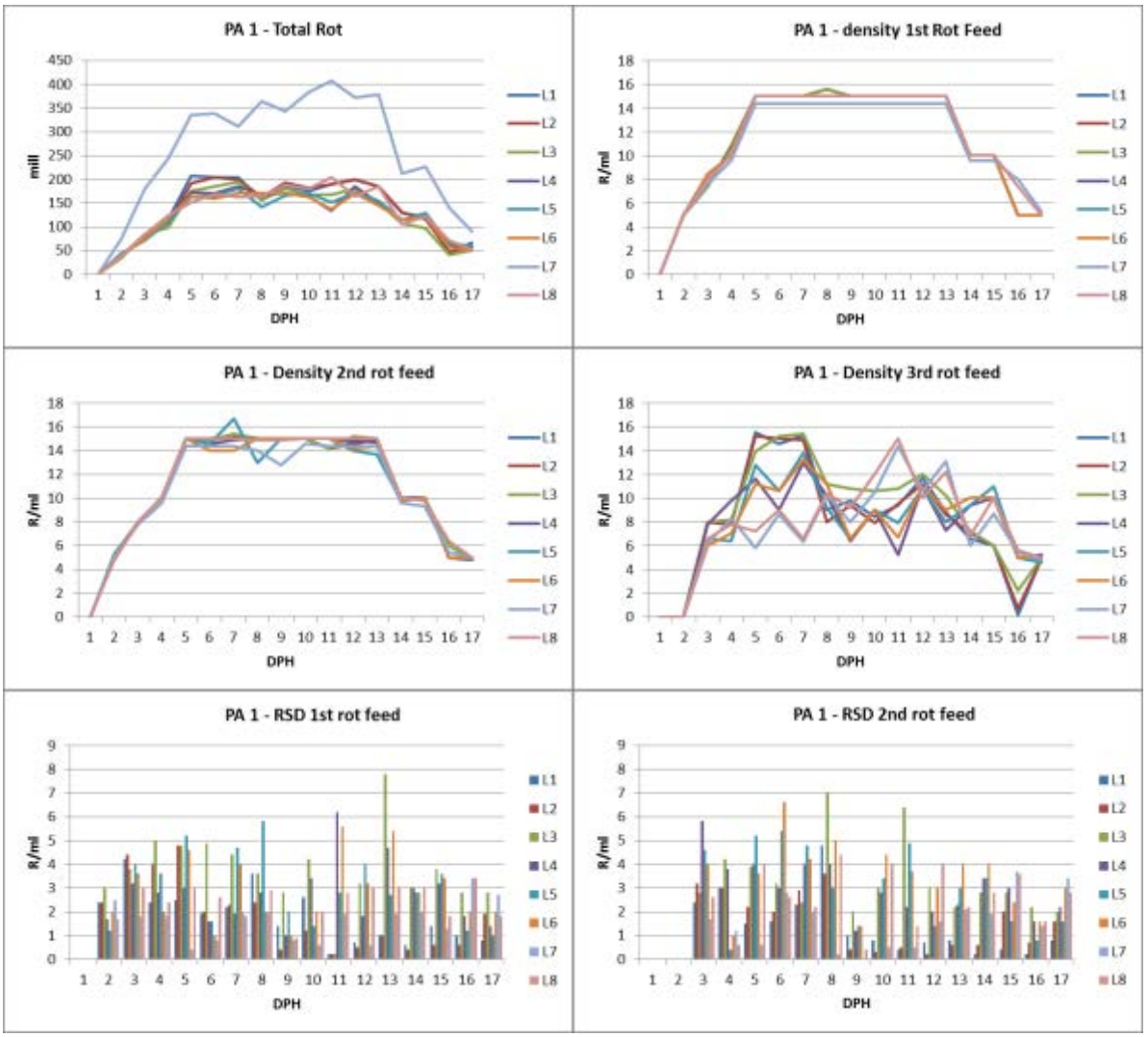

Figure 1. The feed out amounts of rotifers added to Yellowtail Kingfish larval tanks at

Port Augusta in Run 1 2010.

Port Augusta Run 2

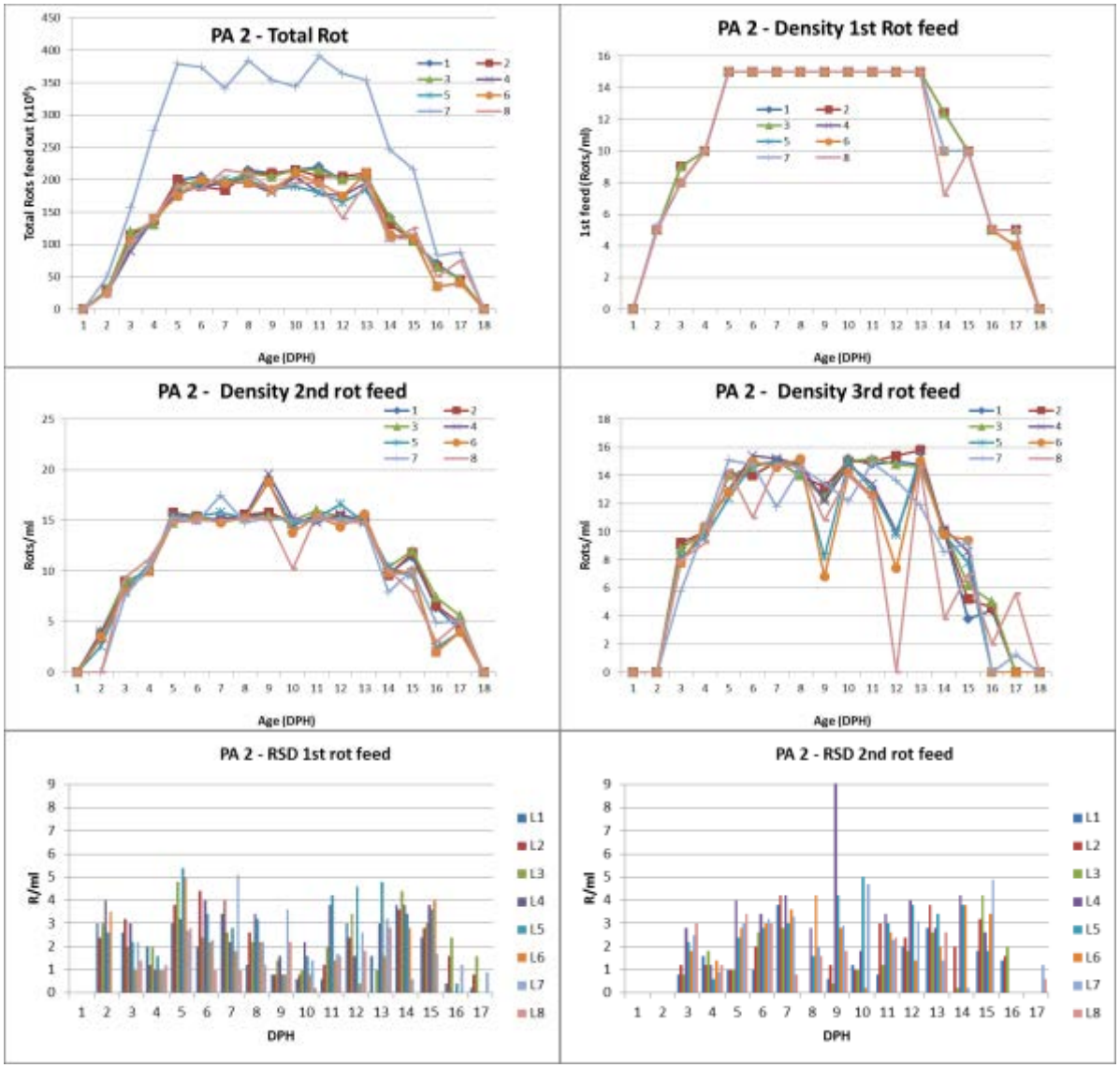

Figure 2. The feed out amounts of rotifers added to Yellowtail Kingfish larval tanks at Port Augusta in Run 2 2010.

The results of rotifer feed out at Port Augusta (Figs 1 and 2) show that all tanks were fed similar amount of rotifers and that the last feed was used as a top up in the late afternoon, a few hours before sun set. Residuals (RSD) after the first and 2nd feed were high in the first 7-8 days but then began to drop to very low levels. The swings in rotifer densities in the 3rd feed were due to rotifer availability and to avoiding too large a feed too late in the day. However at this point *Artemia* was being co-fed and water exchange increased, leading to higher wash-outs.

Arno Bay Run 1

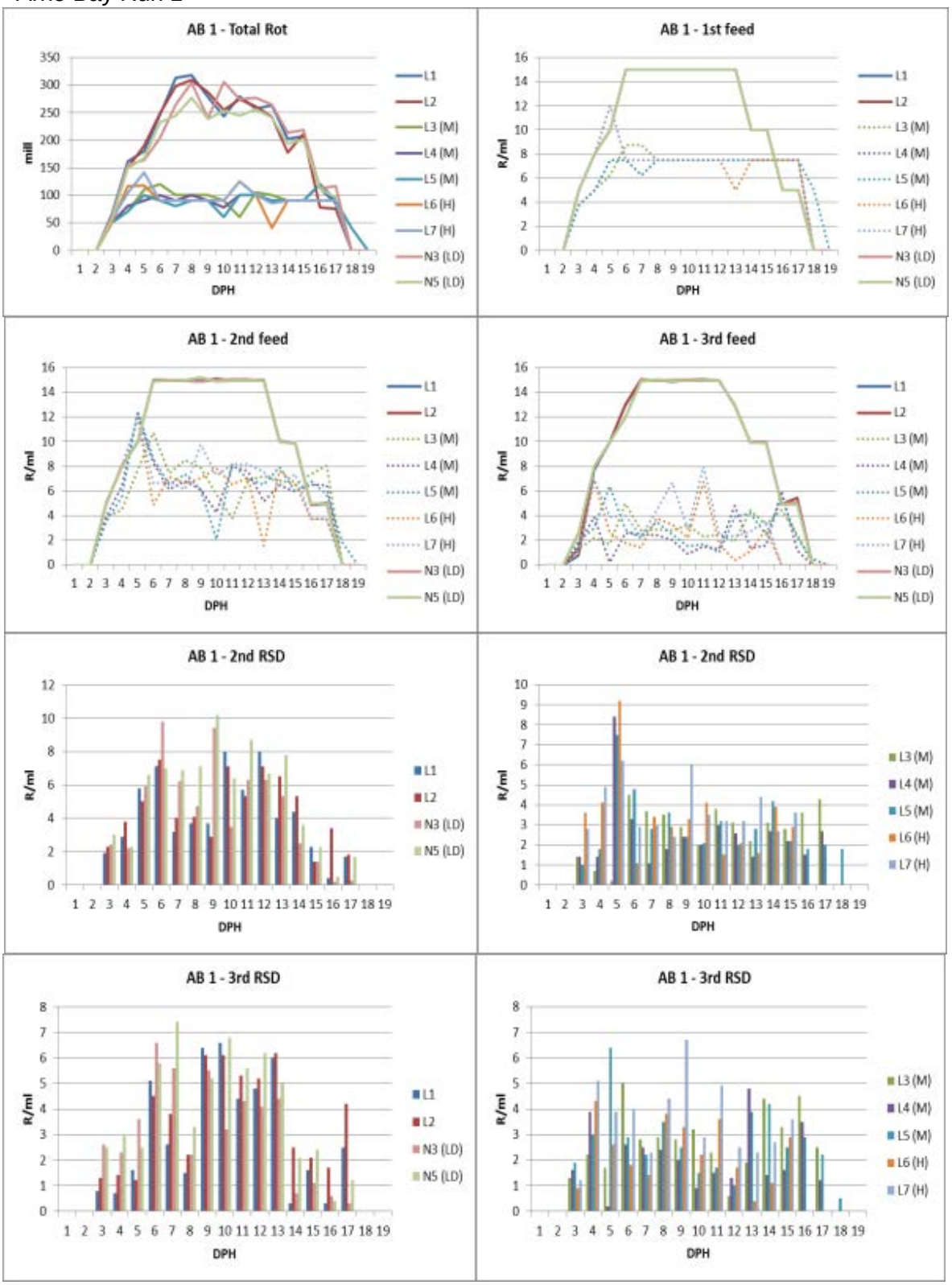

Figure 3. The feed out amounts of rotifers added to Yellowtail Kingfish larval tanks at Arno Bay in Run 1 2010.

The data from Arno Bay run 1 reflects the three different methods used (M = meal; H = hybrid; LD = inside tanks with low larval stocking density & density feeding strategy; the standard 'density' feeding strategy tanks have no letter) as the density tanks were feed to higher levels than the meal or hybrid tanks (dotted lines) (Fig 3). Residuals were highest in the density tanks, and lowest in the M and H tanks both for the midday RSD (2nd RSD) and the early afternoon RSD (3rd RSD).

Arno Bay Run 2

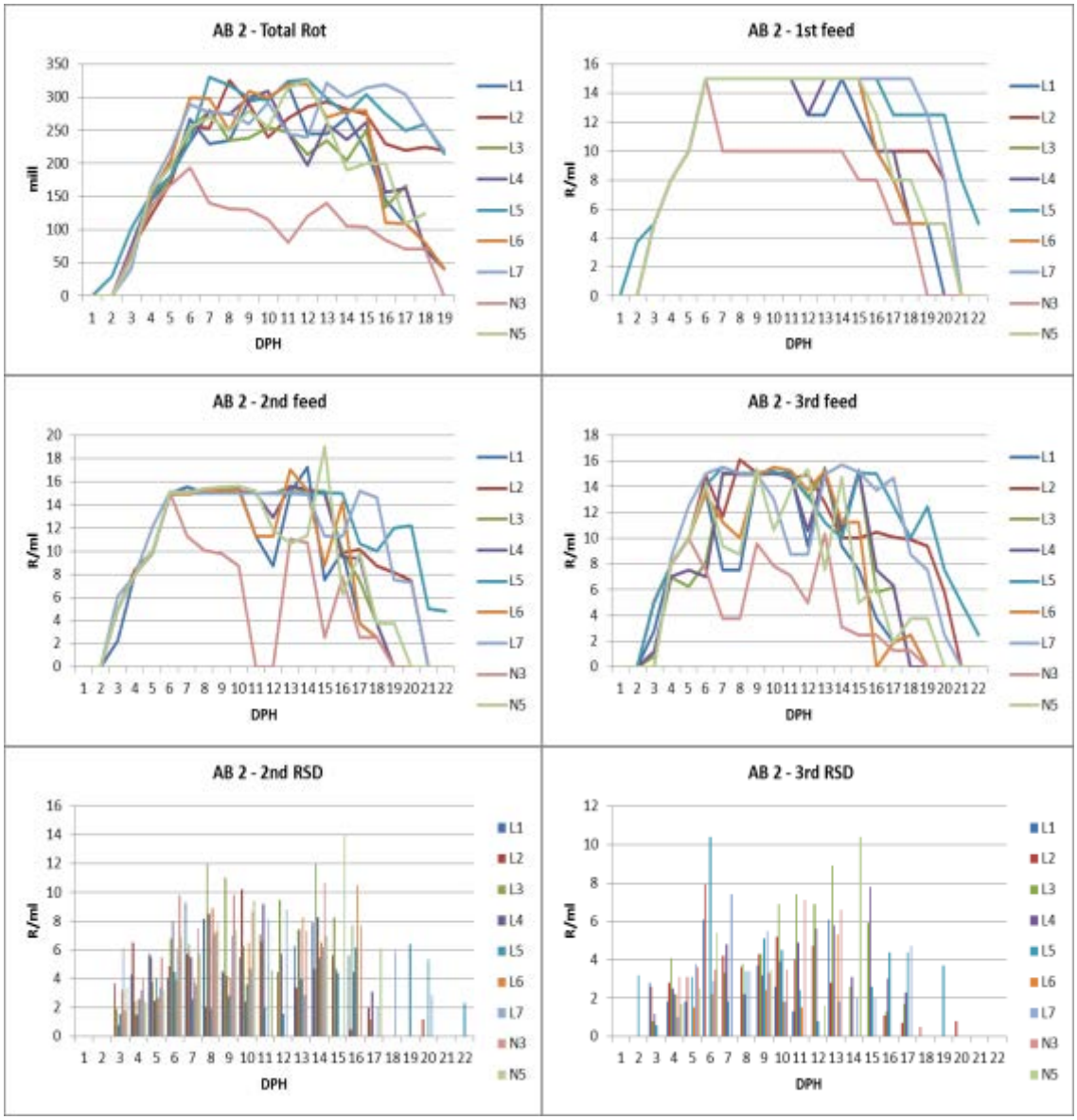

Figure 4. The feed out amounts of rotifers added to Yellowtail Kingfish larval tanks at Arno Bay in Run 2 2010.

The results from run 2 show that the AB rotifer system had problems producing enough rotifers to stay on feed out protocol with 9 larval tanks running (Fig 4). The 2nd and especially the 3rd feed reflect this. Furthermore, some tanks were held on rotifers for longer because the fish were deemed small. In the case of L7 this was mainly due to the higher survival. The N3 tank basically was fed any rotifers available as the numbers were very low.

Residuals were surprisingly low in the first 4-5 days which could indicate more feed could have been presented (more often). There is also a greater variability in RSD compared to PA, which could be due to the fact that the tank hydrology (mixing) is different in the AB tanks.

Artemia

The *Artemia* feeding regime in 2010 continued to follow the 'feed concept' approach where *Artemia* was fed as needed and as often as needed. This means the operator uses the amount fed out, the amount of *Artemia* fed the day before and left in the tank from feed to feed during the day, and observations of fish behaviour, to determine and administer the correct amount of *Artemia*. This is a balancing act between actual need on the day and predicted need because the *Artemia* is produced with 48 hours delay.

Port Augusta

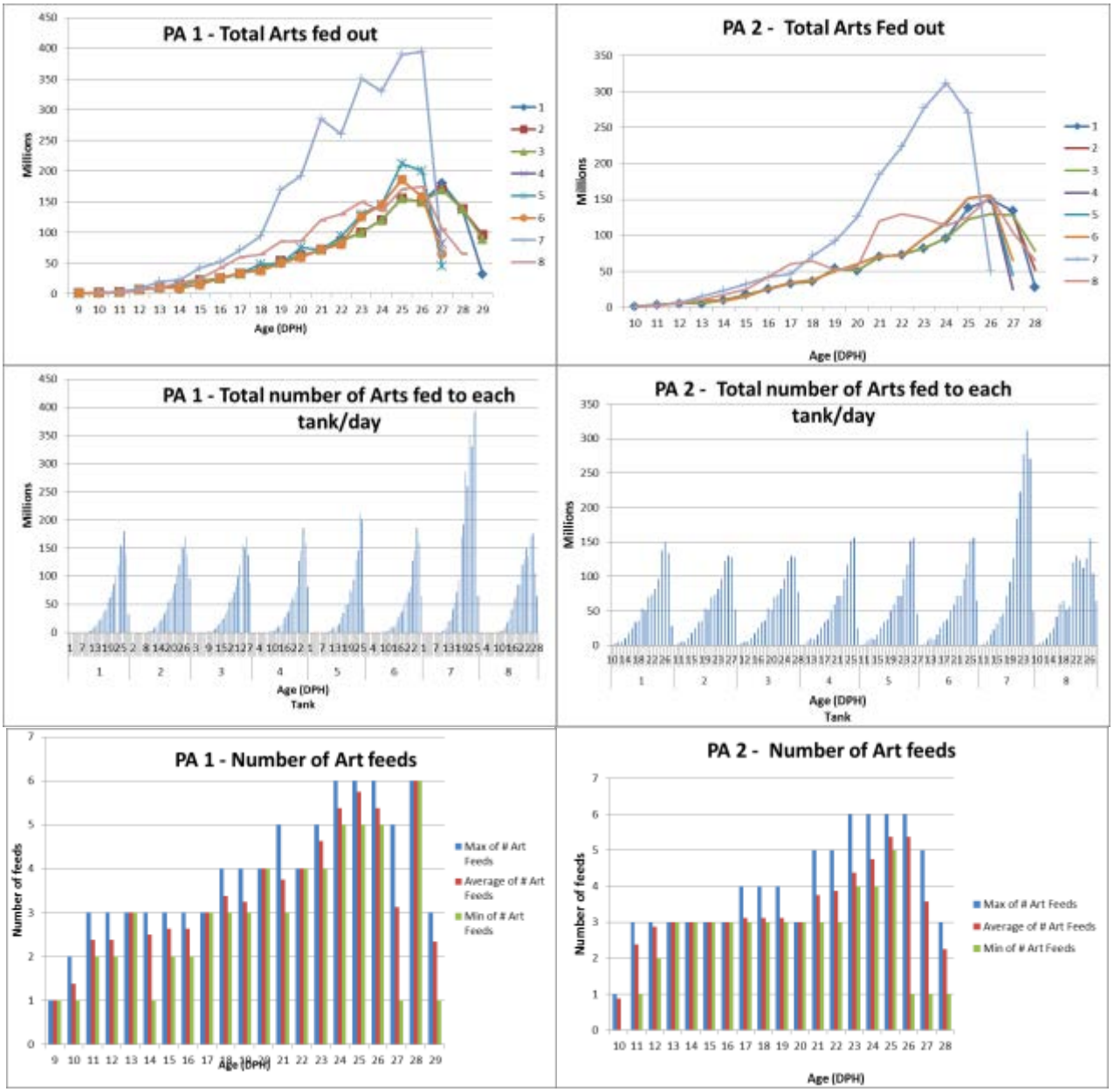

Figure 5. The feed out amounts of *Artemia* added to Yellowtail Kingfish larval tanks at Port Augusta in 2010.

The Port Augusta feed out shows a very similar input of *Artemia* to all tanks, again apart from L7, which is the larger tank with a higher number of fish (Fig 5). This tank

also supports a very good survival rate. The low output tanks (like L5 in PA run 2) did not necessarily receive less *Artemia* because the meal concept was not that precise, there was washout but mainly because a tank with a poor survival rate (normally established before day 10-15 DPH) can have bigger fish, which require relatively more *Artemia* than a tank with more smaller fish.

The meal approach is an intensive one, which is obvious by looking at the number of feed outs. Basically, three feeds is enough to have some *Artemia* present in the tanks until 20 DPH, hereafter it becomes increasingly difficult to feed enough *Artemia* and feed rates increase up to six times per day. This does open up the need for discussions on early weaning, where we so far have not been successful.

Arno Bay

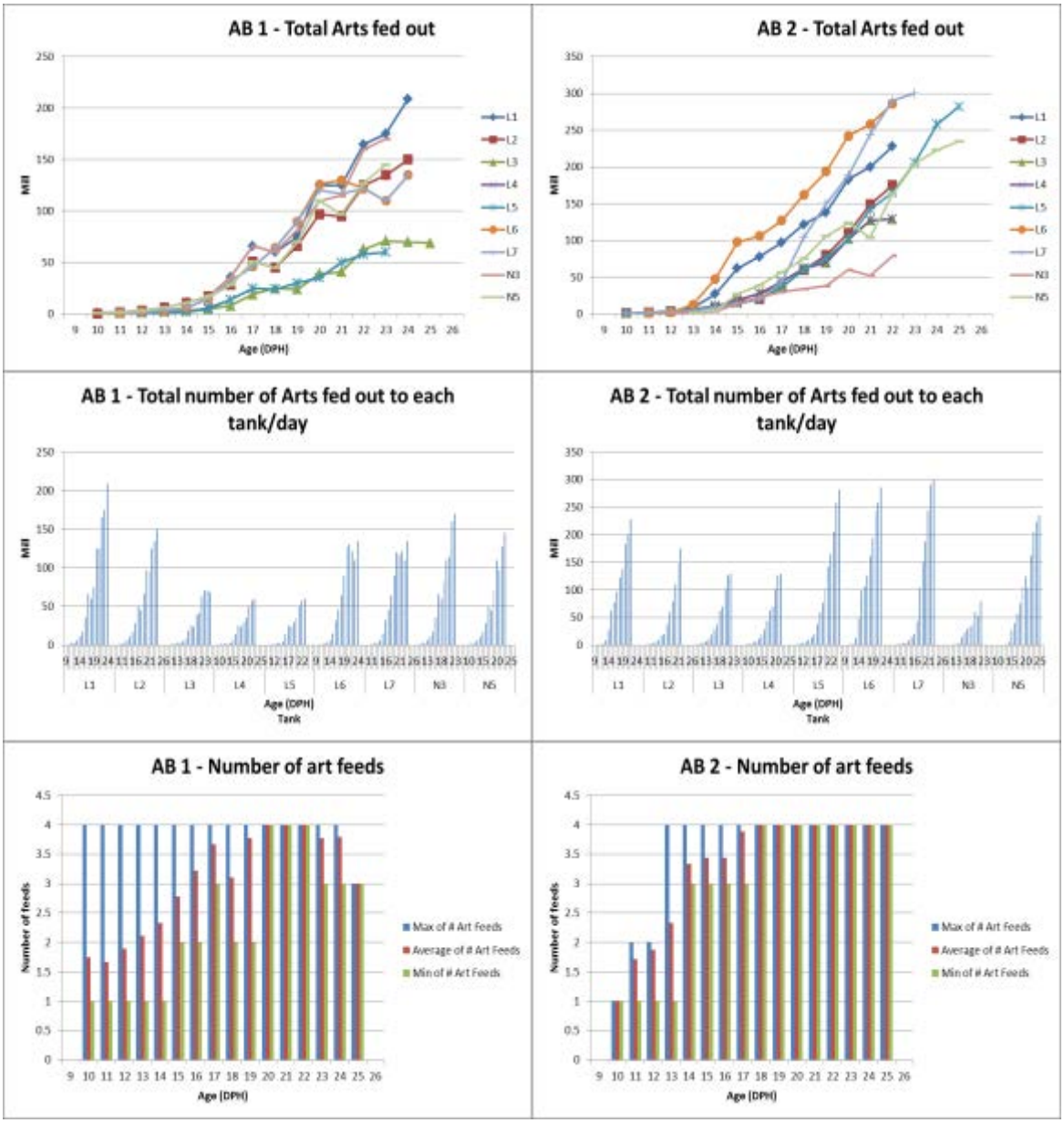

Figure 6. The feed out amounts of *Artemia* added to Yellowtail Kingfish larval tanks at Arno Bay in 2010.

The Arno Bay feed out shows a very similar input of *Artemia* to most tanks apart from tanks with few fish or very small fish (Fig 6). In run 1 L3 and L4 received less *Artemia* because the fish were small, while N3 in run 2 crashed and had an exceptionally low number of fish.

AB, like PA starts out with fewer feeds to start with, however if a tank picks up the consumption of *Artemia* immediately so will the feed out rate.

Larval results

Arno Bay Run 1

The transfer from the larval tanks is done using wet weight (WW) estimations by average weights (the so called WW nursery number) as it allows us to gauge individual larval tank survival rate. The accuracy of the calculated numbers from WW decreases with a large size variation of the larvae or if some fish are too small and weak to be weighed.

Table 6. Survival rate of YTK in individual larval tanks in Arno Bay Run 1 2010.

Arno Bay 2010						
AB	**Run 1**	**WW**	**SR**			
Tanks	**Stocked**	**Fish**	**(%)**	***Feeding***	***Light***	
L1	720000	60250	8%	*Density*	*Natural*	
L2	720000	32840	5%	*Density*	*Natural*	
L3	630000	22428	4%	*Meal*	*Natural*	
L4	700000	75000	11%	*Meal*	*Natural*	
L5	700000	60000	9%	*Meal*	*Natural*	
L6	790000	27581	3%	*Hybrid*	*Natural*	
L7	800000	20868	3%	*Hybrid*	*Natural*	
N3	580000	50455	9%	*Density*	*Artificial*	
N5	580000	44678	8%	*Density*	*Artificial*	
TOTAL	6220000	394100	6%			
** L4 and L5 includes 52000 (L4) and 30000 (L5) which could not be weighed*						

Note: Larval survival for AB run 1 L4 and 5 (meal concept) is not a true measure of the production succes as those tanks had the highest number of weak and small fish which could not be weighed.

Artificial light (ACAAR) N3 and N5
The results showed that growth and survival were similar to outside (natural light) tanks and that a commercially acceptable level of swimbladder inflation can be achieved under artificial light (Table 6, Fig 7), however the inside tanks were not stocked at the equivalent high density used in production. We would have expected a higher survival rate in tanks stocked at lower density, nevertheless we were encouraged by the findings.

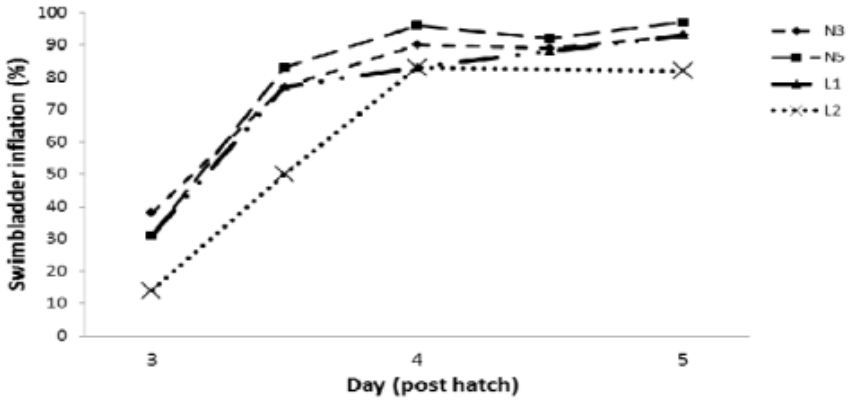

Figure 7. Change in percentage of Yellowtail Kingfish larvae with inflated swimbladders with age. N3 and N5 were indoor tanks under artificial light. L1 and L2 were outdoor tanks (graph courtesy of L. Woolley).

Meal (L3-L4-L5)/Hybrid (L6-L7) concept (ACAAR)
With regard to the meal concept, and the hybrid concept it is clear that the meal concept (not used in CST since 2007) is difficult to manage, which was the main reason to go to the density model in 2008. The survival outcome (Table 6) looks good, however the outcome in actual healthy transferable larvae to the nursery was much lower as noted before. The fish number estimates of 52,000 (out of 75,000) in L4 and 30,000 (out of 60,000) in L5 have significant error attached to them, but we believe these small fish died in weaning within a few days. The growth data also shows that the meal larvae did not grow well while the hybrid fed larvae grew as well as density fed larvae (Figs 8 and 9).

From these results we cannot say the hybrid version supports a better result that the density method. In fact, the survival rates of the hybrid tanks were lower than the density tanks.

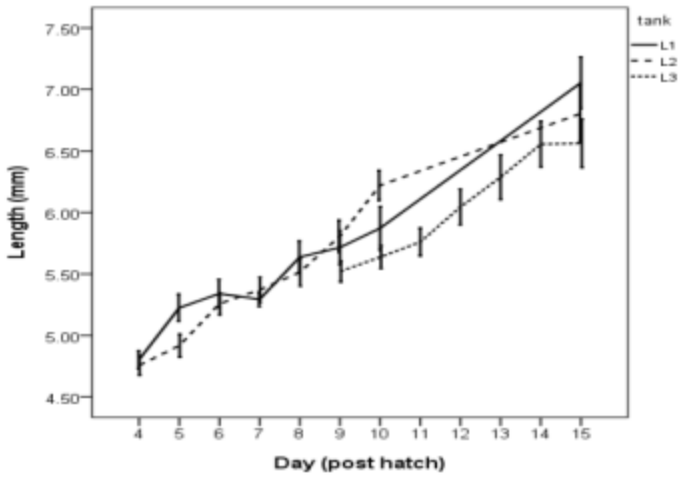

Figure 8. A comparison of growth rates of Yellowtail Kingfish larvae fed either by the density or meal methods (graph courtesy of L. Woolley).

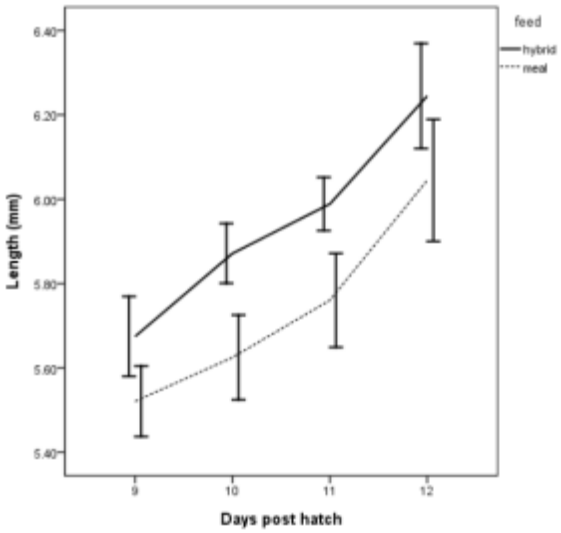

Figure 9. A comparison of growth rates of Yellowtail Kingfish larvae fed either by the meal method or a hybrid of the meal and density method (graph courtesy of L. Woolley).

AB Run 2 and PA runs

Comparing Inside vs. Outside tanks Run 2 AB

After the positive result in Run 1 using two inside tanks (nursery area), we restocked the same two tanks (N3 and N5) to repeat the process and gather more information on inside larval rearing vs outdoor larval rearing. In the second round of stocking, we aimed to keep all controllable larval rearing protocols and parameters the same between the two rearing systems to help us to draw valid conclusions rather than having multiple variables as seen in Run 1.

Both of the inside and outside tanks were fed to a density concept, with the same temperature regime, same larvae stocking density, same algae addition, and same tank set-up and water exchange rates. The remaining difference was light source, and thus inherently the light intensity, light type and light distribution, since the inside tanks were in artificial light. Another difference was day length as the nursery was in use for Run 1 fingerlings.

Table 7. Comparison of production performance from two tanks inside (artificial light) and two tanks outside (natural light) in Arno Bay Run 2 2010.

Position	Run	Larval stocking density	rotifer feeding strategy	Av. age at transfer (days)	Av. wet weight at transfer (g)	malformation assessment (%)	survival in larval tanks (%)
outside	2	100/litre	Density*	23.6	0.076	38	9.34
inside	2	100/litre	Density*	24.5	0.064	25	7.68

* Text explains 'Density' feeding is adding live feed at regular intervals during the day to maintain a nominated prey density appropriate to larval age.

Before looking at the empirical data, it is worth noting that the fish showed behaviour differences between the inside and outside tanks in this Run 2 stocking. The inside larvae created a 'donut' shaped aggregation around the centre standpipe, whereas

the larvae in the outside system were evenly scattered toward and at the water surface. The inside fish appeared less active than the outside fish, and would hold their position a few inches below the surface, while the outside fish occupied more of the tank depth. The inside fish were slower to inflate their swimbladders, and in general were developing slower than the outside fish. Their average weight and mean length (not shown) were lower in spite of being transferred out of the larval tanks at an older stage (Table 7).

We believe this difference was due to lower feed intake and a shorter feeding window, due to the light intensity and the shorter day length inside. The conditions were, 6,800 lux (maximum inside) and 9,000 – 11,000 lux (average outside), 10.5 hours light (inside) and 12 hrs light (outside).

The data indicate that the early malformation assessment of the indoor larvae in Run 2 is lower than the outdoor system – similar to that seen in Run 1. Unfortunately there is no malformation data for N5, so the malformation data for the inside system in Table 7 is based on the results only obtained from N3 (which had significantly lower survival and hence larger fish than seen in N5).

Could this slower development in fact help to lower the rate of malformation through improved skeletal and /or tissue development? Is a lower light intensity beneficial? Very few fish were seen walling in the inside tanks, but the level of walling appeared similar to the outside system. Using the inside tanks under artificial lighting, we could manipulate the dusk light to turn on at a convenient time of 7:30 am, rather than at 5:30 am under natural lighting in the outdoor system. Having this control allowed algae to be added in complete darkness to the indoor tanks, which has the ability to reduce walling behaviour. Although both systems followed the same temperature curve of 21.5 °C increasing to 24.5 °C two days after stocking, the temperature stability seen in the indoor system was better (lower daily variation) when compared to the outdoor system, and this too may have had a positive effect on the malformation rate.

Overall jaw malformation rates – all Runs 2010

As in previous years there was considerable variability in jaw malformation between larval tanks within and between Runs (Table 8). The lowest prevalence of commercially significant deformities was 8% in L7 in PA Run 2, whilst the highest was 63% in L5 in PA Run 1. In Arno Bay, the variability was from 18% in L7 in Run 2 to 55% in L1 in Run 2. For some stocked egg batches (Table 5), the jaw malformation rate was similar between tanks, such as L6 and L7 in AB Run 1, with 47 and 48%, respectively. In contrast, for other stocked batches, the results were highly variable, such as L4 (19%) and L5 (34%) in AB Run 1. This supports results from previous years which indicate that the conditions within individual larval tanks have a greater influence on jaw malformation than the egg batch (with associated genetic and egg quality parameters).

Table 8. Jaw malformation assessment for every larval tank in CST 2010 Yellowtail Kingfish Runs in Arno Bay and Port Augusta.

2010								
Hatchery Run	Tank Number	Malformation sample date	Age	Number sampled	Total length (mm)		Commercially significant malformations	Proportion (2+3)%
					mean	± SD	(%)	
AB Run 1								
	N3	19/09/2010	24	102	11.0	1.7	28.4	28
	N5	19/09/2010	24	102	11.2	2.0	26.5	25
	L1	20/09/2010	25	101	12.7	3.1	50.5	50
	L2	20/09/2010	25	100	14.3	2.7	51	41
	L3	23/09/2010	25	100	9.8	2.2	48	48
	L4	23/09/2010	24	100	7.7	1.5	19	20
	L5	23/09/2010	24	100	8.6	1.9	34	34
	L6a	29/09/2010		100	12.8	2.6	47	47
	L7a	29/09/2010		100	16.3	2.2	48	48
	Overall			**905**			**39**	**38**
AB Run 2								
	L1	8/11/2010		100	10.7	1.6	55	55
	L2	1/11/2010		100	12.2	2.0	21	21
	L3	27/10/2010		99	12.8	2.1	40	40
	L4	27/10/2010		97	11.1	2.5	37	38
	L5	5/11/2010		100	13.7	2.5	53	53
	L6b	8/11/2010		100	10.9	1.7	43	43
	L7b	5/11/2010		100	11.1	1.7	18	18
	N3	8/11/2010		100	11.4	2.3	25	25
	Overall			**796**			**37**	**37**
AB run 1								
SBT hatchery	3	10/12/2010	20	100	10.8	2.0	29	29
	4	10/12/2010	20	100	12.0	1.7	25	25
	7	10/12/2010	20	100	11.5	2.0	24	24
	8	10/12/2010	20	100	10.1	1.9	32	32
	12	10/12/2010	20	100	10.9	1.9	37	37
	15	10/12/2010	20	100	11.4	1.7	24	25
	Overall			**600**			**29**	**29**
Arno Bay overall average 2010							**35**	**34**
PA run 1								
	L1	6/09/2010	29	100	14.4	2.6	36	35.0
	L2	6/09/2010	29	101	14.2	2.6	49.5	40.6
	L3	6/09/2010	29	99	15.7	3.2	43.4	41.4
	L4	9/09/2010	27	100	15.0	2.7	50	45.0
	L5	9/09/2010	27	101	13.8	3.0	63.4	55.4
	L6	9/09/2010	27	98	15.8	3.2	53.1	53.1
	L7	13/09/2010	27	102	14.1	2.4	16.7	16.7
	L8	13/09/2010	27	98	14.6	3.1	12.2	12.2
	Overall			**799**			**41**	**37**
PA run 2								
	L4	23/10/2010	27	100	13.7	1.9	25	24
	L5	23/10/2010	27	100	16.3	2.4	19	18
	L6	23/10/2010	27	100	13.3	2.1	18	18
	L7	24/10/2010	26	98	13.8	2.1	8	8
	L8	30/10/2010	28	99	14.1	2.2	19	19
	Overall			**497**			**18**	**17**
Port Augusta overall average 2010							**29**	**27**

Note – “commercially significant” could be any malformation with severity sufficient to be culled (and will include spinal and opercular deformities). Proportion (2 + 3)% relates to severe jaw deformity and in almost all cases these would be culled.

Table 9. Survival rate of YTK in individual larval tanks in Arno Bay Run 2 and Port Augusta Runs 1 and 2, 2010.

Arno Bay 2010

AB Tanks	Run 2 Stocked	WW Fish	SR (%)
L1	800000	94698	12%
L2	800000	63752	8%
L3	820000	63471	8%
L4	790000	62107	8%
L5	780000	41851	5%
L6	800000	99260	12%
L7	750000	128186	17%
N3	790000	25067	3%
N5	790000	98094	12%
TOTAL	7120000	676488	10%

** L3 and L4 includes 5000 (L3) and 30000 (L4) which could not be weighed*

Port Augusta 2010

PA Tanks	Broodstock Tank	Run 1 Stocked	WW Fish	SR (%)
L1	BS2	450000	56276	13%
L2	BS2	450000	52340	12%
L3	BS2	450000	44083	10%
L4	BS2	408000	28193	7%
L5	BS2	408000	44445	11%
L6	BS2	408000	24139	6%
L7	BS2	917000	144778	16%
L8	BS2	474000	43228	9%
TOTAL		3965000	437482	11%

PA Tanks	Broodstock Tank	Run 2 Stocked	WW Fish	SR (%)
L1	BS1	431000	59055	14%
L2	BS1	431000	45994	11%
L3	BS1	431000	45518	11%
L4	BS3	463000	44397	10%
L5	BS1	500000	26022	5%
L6	BS1	500000	44480	9%
L7	BS1/BS3	864000	86106	10%
L8	BS2	500000	66589	13%
TOTAL		4120000	418161	10%

The data from AB run 2 and PA, all on density feeding regime did not promote a change in the approach to feeding (Table 9). All subsequent runs have similar or better survival rates than the concepts tested in run 1 in AB. CST would need a trial done in the R&D facility to finally decide if hybrid promotes a better QC yield (survival and malformations).

Further, the ACAAR recommendation of using algae pumps was not employed in PA, showing that the dosing pumps, while making the process easier and smoother, is not all out important to the survival rates.

All in all, the 1st run in AB was below expected survival rates, while the remaining runs landed on an average of approximately 10%. While CST would like to see the survival rates lifted to 15%, history tells us that apart from a few runs, 10% average over a season is what can be expected.

Abiotic parameters

The main issue continues to be the temperature stability when using outside tanks (Table 10). We attempt to compensate manually by setting the boiler higher or lower, but on cold nights or hot sunny days we can lose the control.

Our target would be to have less than 1 °C variation on a set point.

Table 10. An overview of environmental parameters in CST Yellowtail Kingfish larval rearing runs in 2010 showing the maximum and minimum values of: temperature (˚C), dissolved oxygen (mg/l) and pH at both hatcheries.

parameter		AB	PA
temp	min	21.0	19.1
	max	25.1	25.5
DO mg/L	min	4.9	5.5
	max	7.7	9.1
pH	min	7.75	7.67
	max	7.87	8.18

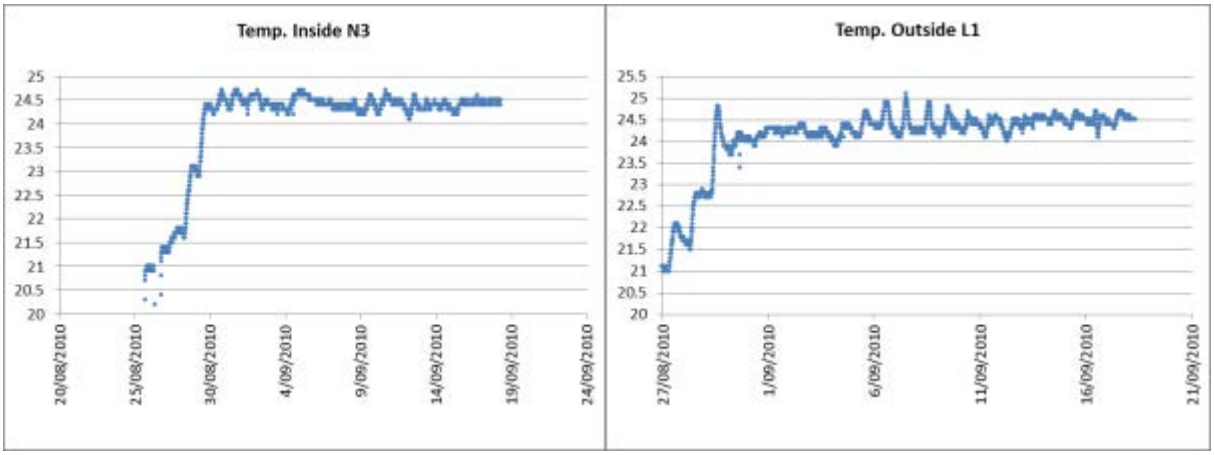

Figure 10. Temperature profile in Yellowtail Kingfish larval rearing tanks from an inside and an outside tank in Arno Bay run 1 2010.

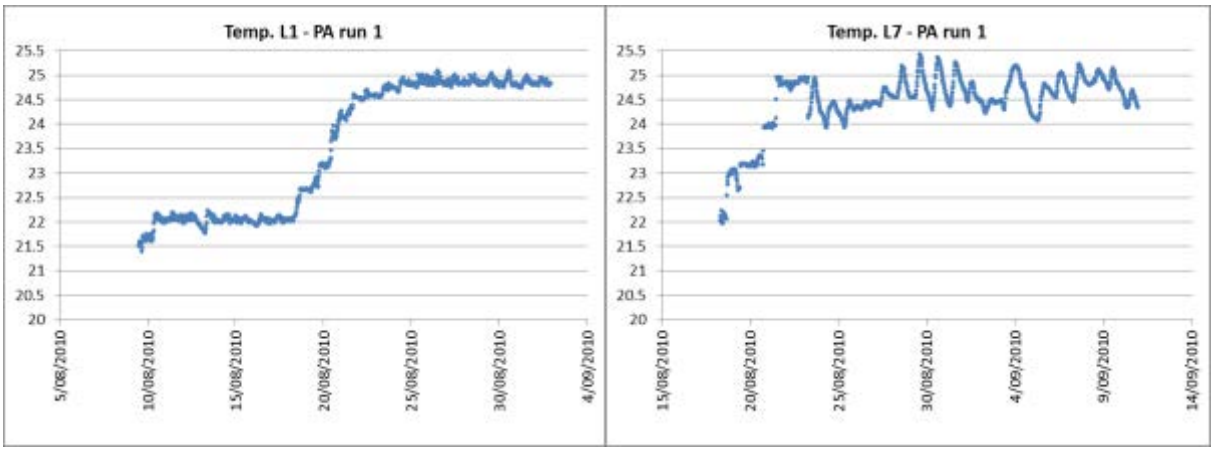

Figure 11. Temperature profile in Yellowtail Kingfish larval rearing tanks from an inside and an outside tank in Port Augusta run 1 2010.

The temperature results confirm that the inside tanks are very stable in temperature, while the outside tanks are less stable (Figs 10 and 11). The AB and the PA figures for L1 do however show that it is possible to keep a tight temperature regime and our target of ±1 °C variation. It also shows that in PA the temperature was kept lower for longer until all tanks were stocked (L1 was stocked first and L7 last). Speculations into the temperature as a potential culprit for high malformations rates could be investigated by looking at individual PA production tanks over the years. The larval output from the PA L7 tank in spite of the bigger temperature variation was very good.

Nursery

During nursery operations we try to estimate the weaning losses, as fish removed by daily cleaning operations in the tank, and any fish that have been accidentally killed (if any) floated or culled under the malformation QC procedures. Adding these numbers up gives us the so-called counted number of fish into the nursery. We use this counted number for the nursery performance with regard to weaning success. The malformation and floating or accidental rates are calculated from the fish number transferred to sea including all losses except weaning.

Table 11. The discrepancy between the Yellowtail Kingfish numbers in the nursery estimated from wet weight (WW) at transfer from larval tanks and the counted number.

Nursery	WW	Counted	
Site/Run	N stocked	N stocked	Diff
AB 1	394100	371199	106%
AB 2	676487	714012	95%
PA 1	437482	397477	110%
PA 2	418161	344051	122%

Table 11 shows that the WW is normally higher than the counted number.

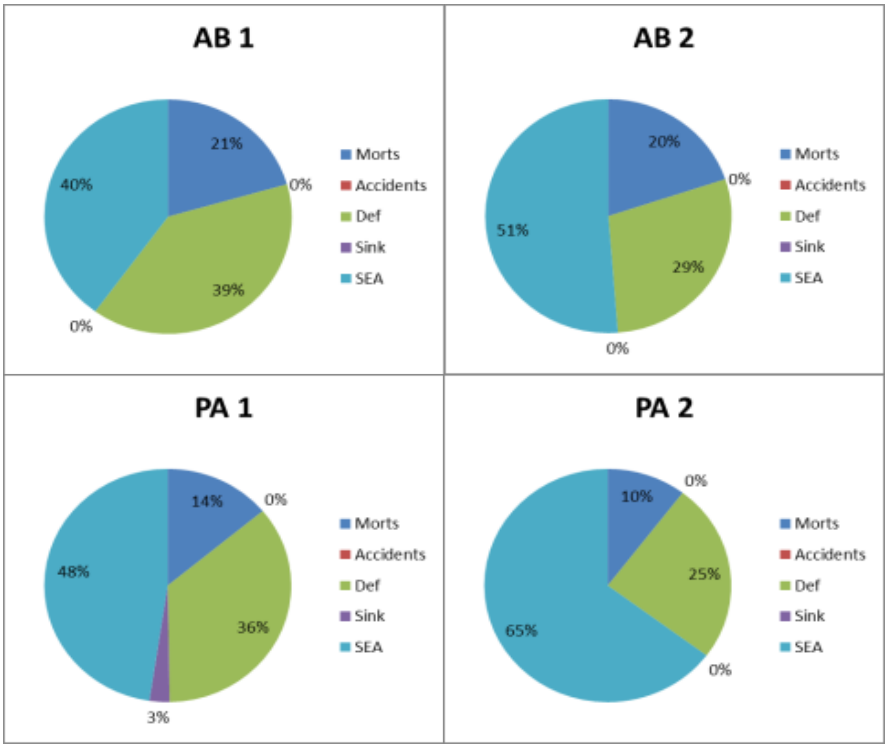

Figure 12. Yellowtail Kingfish production and source of losses during nursery operations from CST hatchery runs in Arno Bay and Port Augusta in 2010. Note that these proportions are based on the counted number, so all fish were accounted for in nursery operations; proportion of mortalities (Morts), proportion of accidental mortalities (Accidents), proportion of fish with non-inflated swimbladders or 'sinkers' (Sink), proportion of malformed fish (Def), and proportion of fish transferred to seacages (SEA).

In the nursery production statistics, the high deformity culls are apparent, but the low or non-existence of sinkers (non-inflated fish) shows that the larval rearing went well on that aspect in 2010 (Fig 12). It is noticeable that PA had a higher weaning success than AB, respectively 90 and 80% (based on averages of 10 and 20% mortalities in the nursery, Fig 12), which is a very good result from the PA nursery which traditionally struggles with weaning due to the large tanks. The poorer result in AB, which normally is 10% or less, is not fully understood. However AB run 1 had a large amount of weak and small fish, most with hyper-inflation, all of which could not wean. In run 2, the AB output into the nursery was more than 700,000 larvae, and in large runs the average fish size is normally smaller making weaning more difficult.

Conclusion

CST had a good YTK production season in 2010, with one of the highest outputs from a run in AB and all fingerlings adhered to the QC. The swimbladder issues seen in 2009 were rectified and many protocols were tightened, updated and improved.

However, it is still the high malformation levels which are the biggest single problem CST faces in the production of YTK fingerlings. If CST had nursery runs in 2010 with say 10% malformation instead of the 25-40%, the output, all things equal (like larval survival rates and weaning losses), would have been 1.5 million instead of 900,000

or 60% higher output. This would represent a big decrease in the production cost for CST hatcheries; it would have allowed CST to put more fish earlier to sea and gain from early growth. Further it would allow CST to optimise other areas where improvements are sought, like early larval survival, early weaning, etc.

The past years' changes in the production have not brought a solution on the issue of malformation closer. CST believes that moving into a designated on site R&D facility, with its own team and R&D program, is the way forward. By trialling factors under scientific rigour, real differences should stand out and become clearer in order to better understand and solve the malformation issue.

The collaboration with ACAAR was very fruitful and their review of the SOPs very helpful and did lead to a few new ones and an overall streamlining of the existing ones. It also lead to an overhaul of all SOPs which was needed. Their insight and experience with swimbladder inflation in Yellowtail Kingfish certainly helped the process and supported the production teams. The inside system under artificial light produced acceptable swimbladder inflation and was relatively successful. However, their advocating of the meal and hybrid rotifer feeding concept over the density concept was not demonstrated as an advantage in the proof-of-concept trial.

Ultimately CST is looking at developing protocols and techniques allowing for production indoors under artificial light and with malformation rates at 5% or less in order to do away with hand sorting all together.

3 Effect of rotifer enrichment on YTK larvae - SARDI

Section written by Wayne Hutchinson and Bennan Chen

Effect of commercial rotifer enrichment products on the performance of Yellowtail Kingfish larvae

INTRODUCTION

The success of marine fish larval rearing is influenced by the nutritional quality of diets provided during first feeding. Marine fish larvae have an essential requirement for long-chain highly unsaturated fatty acids (HUFAs), specifically docosapentaenoic acid (DPA, 22:5n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (AA, 20:4n-6) (Watanabe et al, 1983a; Estudillo-del Castillo et al., 2009). These fatty acids are major components of phospholipids that are structural and physiological components of cell membranes (Park et al., 2006). In particular DHA is the major component of the central nervous system of vertebrates (Masuda, 2004) and is regarded as superior to EPA as an essential fatty acid for Red Sea bream, *Pagrus major*; striped jack, *Psuedocaranx dentex*; striped knifejaw, *Oplegnathus fasiatus* and Japanese flounder, *Paralichthys olivaceus* (Kanazawa, 1993; Watanabe, 1993). DHA is required for proper neural development of the retina and brain, and plays an essential role in metamorphosis, pigmentation, and stress resistance of marine fish larvae (Izquierdo, 1996; Sargent et al., 1999). Accumulation of DHA in the brain during development has been shown for several species of marine fish (Mourente, 2004). In developing larvae, neural tissue, especially the eyes, represent a large proportion of the total body mass requiring a high proportion of the DHA for their normal development and function (Rainuzzo et al., 1997; Mourente, 2004). Furuita et al. (1996) reported that elevation of DHA and EPA level in the live food improved survival and growth of yellowtail *Seriola quinqueradiata*. Ishizaki et al (2001) demonstrated that *S. quinqueradiata* larvae fed *Artemia* enriched with DHA had improved schooling behaviour attributed to a larger volume of tectum opticus and cerebellum regions in the brain that are involved with processing of visual information; compared to larvae fed prey enriched with EPA or oleic acid. Adequate provision of HUFAs, especially DHA, is critical for normal development of the neural system during the early stages of larval development and deprivation of DHA may be particularly deleterious to fast growing pelagic species such as *Seriola* and tuna species (Mourente, 2004).

Lipids are the main energy source of developing larvae (Villeneuve et al., 2005). First feeding larval fish digest and metabolise phospholipids and use fatty acids from phospholipids for energy (Sargent et al., 1997). Eggs of marine fish generally contain high amounts of essential fatty acids that are used by larvae as they develop. The level of DHA especially declines toward metamorphosis (Masuda, 2004) while the levels of EPA (Kanazawa, 1993) and arachidonic acid remain the same and saturated (e.g. palmitic acid) and monounsaturated (e.g. oleic acid) fatty acids are

catabolised (Izquierdo, 1996). Fish larvae have a limited capacity to synthesis HUFAs that is not sufficient to sustain the needs of high cell development occurring during larval growth and must acquire these needs from their prey (Coutteau et al., 1997; Estudillo-del Castillo et al., 2009). Phospholipids rather than triacylglycerols, are the preferred source for supplying HUFA to fish larvae (Sargent et al., 1997). In the wild, marine fish larvae ingest prey that have high levels of phospholipids and the ratio of DHA:EPA in the phospholipid of these diets is commonly 2:1 (Sargent et al., 1997). Hilton et al (2008) concluded that among the neutral lipids, wax esters and triacylglycerol are import sources of energy for Yellowtail Kingfish, *Seriola lalandi*, larvae during the early stage of development from hatching until 7 days post hatching (DPH). From 10 DPH the level of protein and all classes of lipid except wax esters increased in larvae as their improved sensory and swimming ability allowed greater feeding intensity. It is suggested that providing dietary lipid at first feeding may be important for survival of Yellowtail Kingfish larvae (Hilton et al., 2008).

The rotifer, *Brachionus plicatilis* (large strain, L) or *Brachionus rotundiformis* (small strain, S) are the predominant live prey organisms cultured in commercial hatcheries to provide the first live feed requirements for intensively cultured marine fish larvae. Rotifers are not part of the natural diet of many marine fish and they are used in aquaculture because they are well suited to mass production and provide a prey of the appropriate size that offer visual stimuli to induce feeding by marine fish larvae (Cavalin and Weirich, 2009). Rotifers that are mass cultured are nutritionally inadequate unless they have been enriched with commercial products that contain HUFAs and other nutrients required for development and survival of marine fish larvae (Sorgeloos et al., 1988). Studies have shown that the concentration of n-3 HUFA in rotifers is proportional to the content of n-3 HUFA in the enrichment formulation (Watanabe et al., 1983b; Izquierdo et al., 1989).

There are several commercial products available that are formulated to enhance the lipid, protein and vitamin content of rotifers. A number of studies showed that feeding larvae with rotifers enriched with enrichment products improved the growth and survival for larvae of species such as Red sea bream, *Pagrus major* (Izquierdo et al., 1989; Takeuchi et al., 1990), dolphin fish, *Coryphaena hippurus* (Ostrowski and Divakaran, 1990), sea bream, *Sparus auratus* (Koven et al., 1990), red drum, *Sciaenops ocellatus* (Craig et al., 1994), yellowtail flounder, *Limanda ferruginea* (Copeman et al., 2002), haddock, *Melanogrammus aglefinus* (Castell al., 2003), Atlantic cod, *Gadus morhua* (Cutts et al., 2006; Park et al., 2006; Garcia et al., 2008), and Florida pompano, *Trachinotus carolinus* (Cavalin and Weirich, 2009). This study was conducted to investigate the effect of selected commercial rotifer enrichment products on the growth, survival, rate of swimbladder inflation and incidence of jaw deformity of Yellowtail Kingfish larvae.

MATERIALS AND METHODS

Egg incubation and larval stocking

Yellowtail Kingfish eggs were collected in April 2011 from broodstock maintained under controlled photoperiod and water temperature conditions at the Arno Bay hatchery of Clean Seas Tuna Limited, South Australia. Eggs were transferred and hatched in 500 L fibreglass incubators at water temperature controlled at about 21°C. At approximately 2 hours after the completion of hatching, larvae were transferred using 1 to 5 L containers into rearing tanks that were stocked at a density of 40 larvae L^{-1}.

Experimental system

The experiment was conducted using a system comprised of 3 recirculating water treatment systems that each provided water to 4 conical bottom fibreglass larval rearing tanks (i.e. total 12 x 3,000 L tanks). All seawater was ozonated, foam fractionated and UV disinfected before being used to fill or provide exchange water to the 3 recirculating systems. Each recirculating water treatment system provided mechanical filtration (Hydrotech Model 501) to 40 µm, biological filtration, foam fractionation, and UV disinfection. Seawater was delivered to the bottom of each tank (i.e. up-flow) and discharged through a screen (375 µm or 500 µm, 150 mm diameter x 1000 mm long) mounted at the side wall of each larval rearing tank. The walls and bottom of each tank were green and 2 air stones were placed in the centre of each tank to create additional aeration and movement of live foods and larvae. During the experiment, water temperature was maintained at 22.98 ± 0.05 °C, DO from 6.1 to 7.2 mg l^{-1}, pH from 7.97 to 8.06, salinity was 36.5 g l^{-1} and the photoperiod was 14 h light:10 h dark. A 75 w halogen light and 40 w fluorescent light were mounted above each tank providing light intensity that averaged 1,925 ± 293 lux at the water surface at the centre for all tanks and 1,086 ± 510 lux at the edges of all tanks.

Experimental design and larval rearing

Rotifers were enriched using 4 different commercial enrichment products: Ori-Green (Skretting, Sjøhagen 154016, Stavanger, Norway), N-Rich® PL Plus (Reed Mariculture Inc., 871 East Hamilton Ave, Suite D, Campbell, CA 95008), S.presso® and S.pirit® (INVE Aquaculture, Hoogveld 93, B-9200, Dendermonde, Belgium) (Table 1). Rotifers were enriched in separate 500 L conical bottom tanks in the desired volume of water. Each tank was supplied with an immersion heater, aeration and oxygenation. From approximately 4:00 pm each day, the required amount of rotifers was harvested from culture tanks and each enrichment product was prepared following the manufacturers recommendations (Table 2). Each product was transferred into a 20 L bucket adjacent to the designated tank and 1 to 2 L bottles of frozen water were added to reduce water temperature prior to addition of the contents of the bucket at the desired time for the first dose (T1) of the enrichment (Table 1). If required, a second dose (T2) of enrichment was prepared and stored in a separate bucket. Each bucket contained a small submersible pump controlled via an electronic timer set to turn on at the desired time (T1 or T2) to add each pre-

prepared dose of the enrichment. The product N-Rich® PL Plus was added continuously for 6 hours using a peristaltic pump controlled by an electronic timer. After rotifers had been added to each enrichment tank a background feed of either Culture Selco 3000® (INVE Aquaculture) or Nanno 3600™ (Reed Mariculture Inc) was added to sustain rotifers in each tank until the start of the enrichment period. The probiotic MIC-F (INVE Aquaculture) was also added during rotifer enrichment for all products.

Table 1. Available information on the composition of the rotifer enrichment products investigated for Yellowtail Kingfish larval rearing.

Component	Enrichment Product			
	S.presso®	N-Rich® PL Plus	Ori-Green	S.pirit®
Crude protein	3%	66%	40%	
Crude lipid	32%		35%	
DHA		30 mg g^{-1}		
EPA		13 mg g^{-1}		
Total n3 HUFAs	150 mg g^{-1}	50 mg g^{-1}	105 mg g^{-1}	
DHA/EPA	9.0	2.31	4.0	

Table 2. Operational procedures followed to prepare commercial rotifer enrichment products evaluated for Yellowtail Kingfish larval rearing.

Product	Form	Dose (g/m3)	Dose (g/100L)	Enrichment duration (hr)	Tank preparation (3:00 - 5:00 pm)	First dose (T1, g/100L)	Second dose (T2, g/100L)	T1	T2	Delivery method
S.presso	Liquid	350	35	10	CS 3000 background at 100g/m3	35	35	10:00pm	1:00am	Delivered by two pumps controlled by separate electronic timers. First dose adds MIC-F (0.5g/L
S.pirit	Powder	200	20	10	CS 3000 background at 100g/m3	20	20	10:00pm	1:00am	Delivered by two pumps controlled by separate electronic timers. First dose adds MIC-F
Ori-Green	Powder	250	25	4	Nanno paste 0.5ml/million	25	-	3:00am		Delivered by a pump controlled by an electronic timer. Dose adds MIC-F
		(ml/L/hr)	**(ml/100**							
Reed N-Rich PL	Liquid	0.2	120	6	Nanno paste 0.5ml/million	-	-	1:00am		Delivered continuosly from start time by a persistaltic pump controlled by an electronic timer. Dose adds MIC-F
CS 3000 = Culture Selco 3000			Nanno paste = Nannochloropsis 3600 (Reedmariculture)				MIC-F = MIC-F microbial water conditioner (Sanolife, INVE Aquaculture)			

Each morning all enriched rotifers from each tank were harvested, thoroughly rinsed and counted to determine total number for each enrichment product. Rotifers were then divided into a number of 20 L buckets that were transferred into a cold storage water bath until use. The amount of rotifers to achieve the desired feed density were fed to Yellowtail Kingfish larvae in 3 tanks that had been randomly assigned (n = 3 replicates, 1 replicate per water treatment system). Rotifer feeding commenced at approximately 1500 h on 2 DPH. From 3 DPH rotifers were added 3 times daily at 0800, 1200 and 1630 h to maintain targeted food densities with amounts added determined from residual counts taken before each feeding time. Concentrated microalgae, Nanno 3600™ was continuously added to all larval rearing tanks using a

dosing pump to maintain cell density at about 50,000 cells ml^{-1}. The experiment was terminated on 9 DPH as swimbladder inflation rate had not exceeded a 70% criteria set for R&D trials.

Larval sampling and measurement

Larvae were randomly sampled from each tank in the morning before the morning feed on 3, 4, 6 and 9 DPH. Standard length was measured, as a mean of 20 larvae per tank to the nearest 0.05 mm when samples were taken on 3, 4 and 6 DPH, whilst 50 larvae were taken on the final day, i.e. 9 DPH. Swimbladder inflation (%) was assessed under dissect microscope on 20 fish taken on 3, 4, 5, 6 and 7 DPH, and 50 fish on 9 DPH from each tank in the morning before lights switched on.

On the final day of the experiment, Aqui-S® (Aqui-S New Zealand Ltd, Lower Hutt, New Zealand) was added to each tank at 28.6 mg L^{-1} to anaesthetise larvae before sampling and harvesting to avoid flaring of the opercula. To determine final survival, all remaining larvae were harvested from each tank into a 20 L bucket and 2 x 1-2L sub-samples counted to estimate the final number. A sample of 20 larvae from each tank was measured under a stereo microscope.

Jaw malformation assessment

A sample of and 50 larvae (including 20 measured) were examined for the incidence and degree of jaw deformity. Jaw malformation was assessed for each larvae and was rated on a scale of 0 to 3 according to the jaw malformation index (Cobcroft et al., 2004) modified for Yellowtail Kingfish larvae. A score of 0 indicated normal jaw formation while a score of 0.5 indicated a very minor malformation that was unlikely to impair larval performance and that would not be considered a malformation from a commercial perspective. Larvae were defined as malformed when the jaw score was 1 (minor), 2 or 3 (major) which would be considered a malformation of commercial significance resulting in fingerlings being culled following quality control protocols conducted in the nursery prior to transfer of fingerlings to sea cages.

Statistical analysis

Statistical analyses were performed with RASW Statistics 18 (PASW Statistics, Rel. 18.0.2. 2009 Chicago: SPSS INC.). One-way ANOVA was used to test effects of rotifer enrichment with different products on larval fish survival, growth, swimbladder inflation, jaw malformation and fatty acids contents. All results were presented in mean ± SD and the level of significant difference was set at $P < 0.05$ unless otherwise stated.

RESULTS

Survival

The highest survival rate at 9 DPH was for larvae fed rotifers enriched with S.presso® (37.6 ± 4.9%) but there was no significant difference (*P* >0.05) between the survival of larvae fed rotifers enriched with any of the different commercial enrichment products (Fig. 1).

Growth

The highest mean growth at 9 DPH was recorded for larvae fed rotifers enriched with Ori-Green and the lowest growth was recorded for larvae fed rotifers enriched with N-Rich® PL Plus although there was no significant difference (*P* >0.05) in growth of larvae fed rotifers enriched with any of the different enrichment products (Fig. 2).

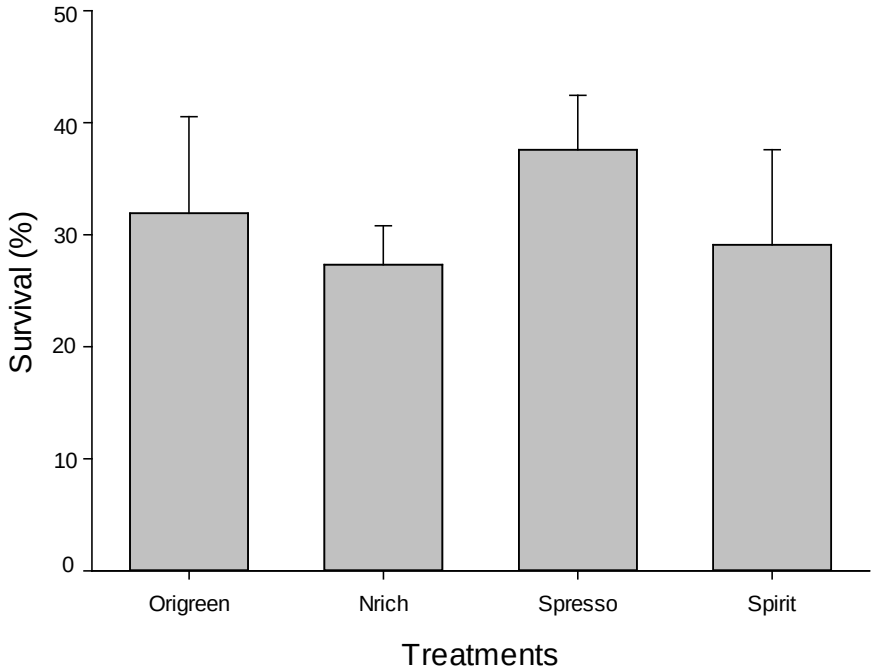

Fig. 1. Comparison of Yellowtail Kingfish larval survival rate (mean ± SD) among treatments fed on rotifers enriched using different commercial products.

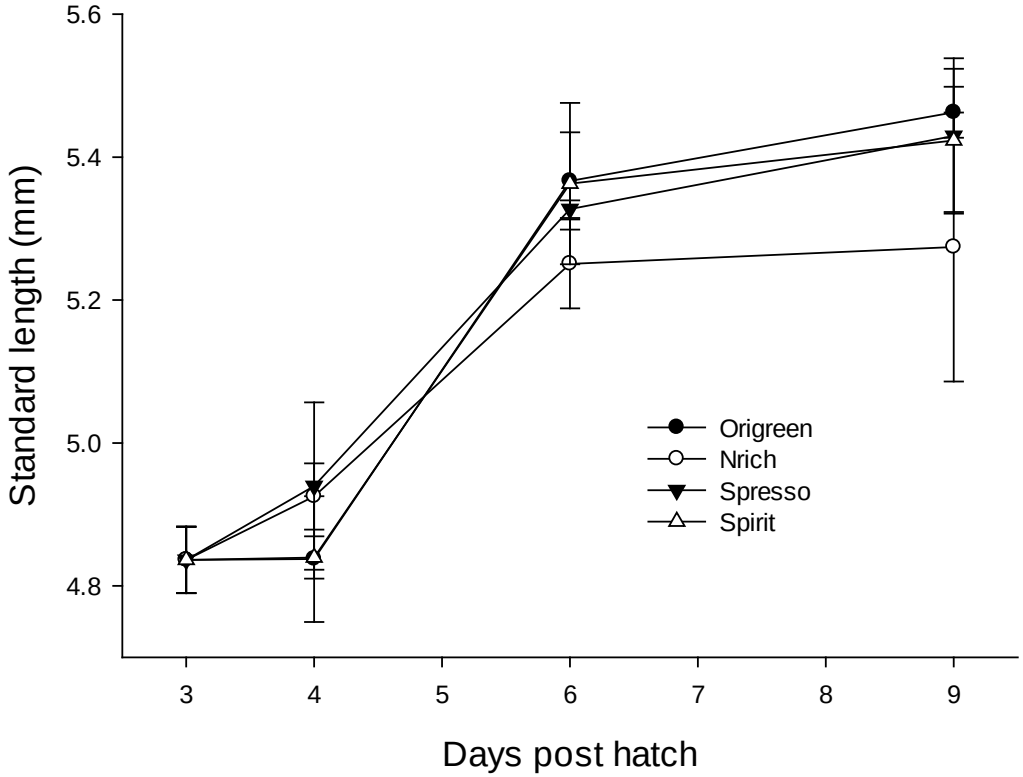

Fig. 2. Comparison of Yellowtail Kingfish larval growth in standard length (mean ± SD) among treatments fed on rotifers enriched using different commercial products.

Swimbladder inflation

Yellowtail Kingfish larvae started swimbladder inflation on 3 DPH and approximately 60% of larvae in all treatments had inflated their swimbladders on 4 DPH. From 5 DPH the swimbladder inflation rates either remained similar or declined. At the end of the experiment (9 DPH) there was a significantly lower (P <0.05) swimbladder inflation rate (34.7 ± 8.1%) was recorded for larvae fed rotifers enriched with N-Rich® PL Plus. At this time the highest swimbladder inflation rate was recorded for larvae fed rotifers enrich with S.pirit® (68.7 ± 10.1%) and S.presso®, (65.3 ± 9.0%) which were not significantly higher the rate achieved by larvae fed rotifers enriched with Origreen (60.0 ± 17.4%, Fig. 3).

Jaw deformity

The lowest incidence of jaw malformation was observed for larvae fed rotifers enriched with S.presso® (8.0 ± 6.9%) and the highest incidence was observed for larvae fed rotifers enriched with S.pirit® (15.3 ± 6.4%). However, there was no significant difference (P >0.05) in the incidence of jaw malformation (% of larvae, mean ± SD) of Yellowtail Kingfish larvae fed on rotifers enriched using the different products.

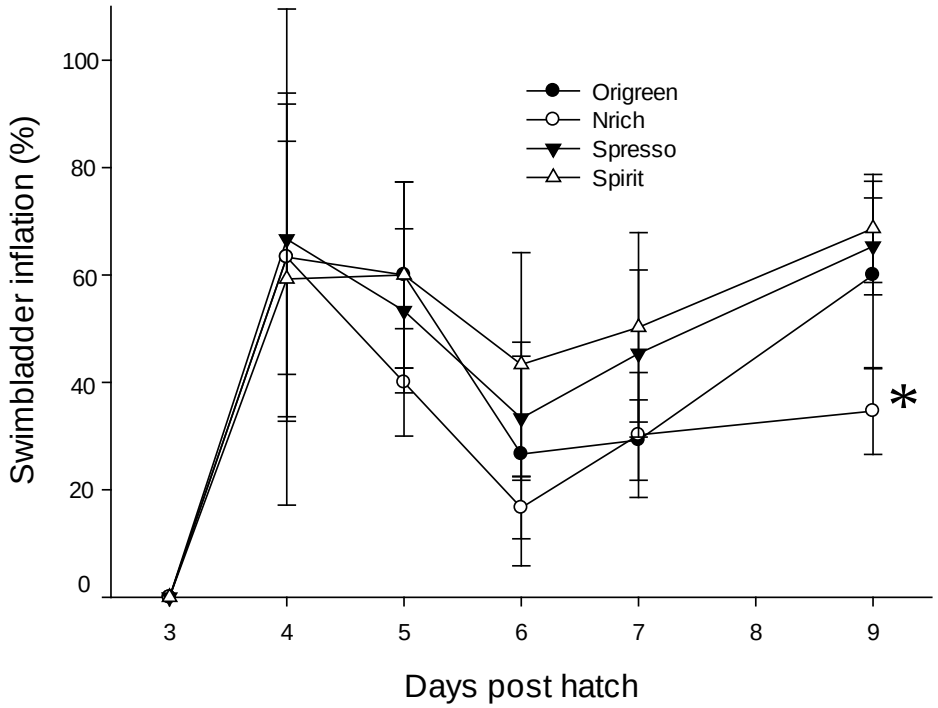

Fig. 3. Comparison of the swimbladder inflation rate (%, mean ± SD) for Yellowtail Kingfish larvae fed on rotifers enriched using different commercial products.

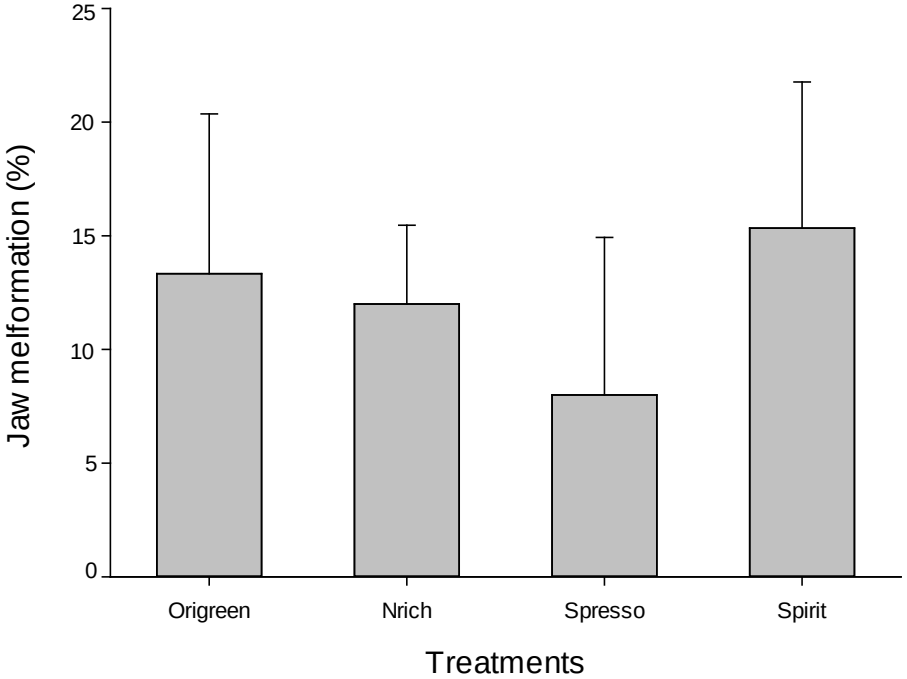

Fig. 4. Comparison of the incidence of commercially significant jaw malformation (jaw score of 1, 2 or 3, % larvae, mean ± SD) for Yellowtail Kingfish larvae fed on rotifers enriched using different commercial products.

DISCUSSION

The quality of Yellowtail Kingfish juveniles is of high priority for commercial production and the influence of nutrition of larvae during early stages of development is of concern to hatchery operators. Growth and survival of marine fish larvae are not necessarily associated with normal development and quality of juveniles. Rather their morphological and physiological characteristics, combined with their behaviour, will define the quality of juveniles (Fushimi, 2001).

The trial investigated the use of different commercial products used to nutritionally enrich rotifers and covered the early stage of Yellowtail Kingfish larval rearing including the onset of first feeding and significant developmental events including initial swimbladder inflation. Hilton et al. (2008) experienced high mortality of Yellowtail Kingfish larvae between 5-7 DPH and suggested that lipid provisioning could be an important factor for survival around the first feeding period for this species. Although not significantly different from other treatments, the best survival in this trial was obtain for Yellowtail Kingfish larvae fed on rotifers enriched with S.presso® (37.6 ± 8.5%) and Ori-Green (31.9 ± 8.6%). These two products contained the highest amounts of crude lipid, 32% (150 mg g^{-1} DHA) and 35% (105 mg g^{-1} DHA) respectively, amongst all of the enrichment products used. It is accepted that dietary lipid and phospholipids can promote growth, survival, vitality and reduce the level of malformation of marine fish larvae (Cahu et al., 2003; Tocher et al., 2008). Furita et al. (1996) demonstrated that yellowtail, *Seriola quinqueradiata*, larvae show improved growth, survival and vitality when fed with *Artemia* enriched with 3.9% n3-HUFA compared to larvae fed with *Artemia* enriched with 0.6% n3-HUFA. There are instances were studies have shown no significant improvement in survival for feeding larvae with enriched rotifers. Koven et al. (1990) found no significant improvement in survival for gilthead sea bream, *Sparus auratus*, fed roifers enriched with increasing levels of n3-HUFA. Castell et al. (2003) reported no improvement in survival at 41 DPH for larval haddock, *Melanogrammus aeglefinus*, fed emulsion enriched rotifers compared to larvae fed rotifers reared on mixed microalgae. Bransden et al. (2005) reported no significant difference in growth of striped trumpeter, *Latris lineata* larvae was recorded at 18 DPH following feeding seven emulsions formulated to have increasing concentrations of DHA between 1.9 mg g^{-1} and 16.4 mg g^{-1}.

Similar growth *was* obtain for Yellowtail Kingfish larvae fed on rotifers enriched with Or-Green, S.presso® *a*nd S.pirit®. Lower but not significantly different growth was recorded for larvae fed on rotifers enriched with N-Rich® PL Plus compared to all other treatments. Feeding marine fish larvae with n-3 HUFA enriched rotifers generally improves growth. An increase in n-3 HUFA significantly increased larval growth of red sea bream, *Pagrus major* (Izquierdo et al., 1989), gilthead sea bream, *S. auratus* (Koven et al., 1990, Mourente et al., 1993), red drum, *Sciaenops ocellatus* (Craig et al., 1994), yellowtail flounder, *Limanda ferruginea* (Copeman et al., 2002), Atlantic cod, *Gadus morhua* (Cutts et al., 2006; Garcia et al., 2008). Studies on other species have not shown an improvement in growth due to feeding larvae with n3-HUFA enriched rotifers. No significant difference in growth of striped trumpeter larvae was recorded at 18 DPH following feeding seven emulsions formulated to have increasing concentrations of DHA (Bransden et al., 2005).

The only significant difference determined was the lower proportion of larvae that displayed swimbladder inflation by 9 DPH when fed rotifers enriched with N-Rich® PL Plus. No significant impact on swimbladder inflation was found for sea bream larvae fed with rotifers enriched with increasing levels of n3-HUFA (Koven et al., 1990). The most significant factor that affects the ability of larvae to initially inflated the swimbladder is access to air at the water surface (Chatain and Ounais-Guschemann, 1990) although other abiotic factors (i.e. temperature, salinity, light) have been may influence the success of this event (Koven et al., 1990). During the trial it was observed that, compared with tanks of larvae fed with rotifer enriched with other products, the water surface of tanks fed with N-Rich® PL Plus were difficult to keep clean using a combination of surface skimmers and surface cleaning with paper towel. This is more likely to have reduced the rate of swimbladder inflation for larvae from this treatment rather than a nutritional effect due to the enrichment treatment.

In this trial, the lowest incidence of jaw malformation was obtain for Yellowtail Kingfish larvae fed on rotifers enriched with S.presso® although no significant difference in the incidence of jaw deformity could be attributed to any of the rotifer enrichment products. Fish tissues contain relatively higher amounts of HUFA than those of mammals and the content of HUFA in bones of some marine fish can be as high as 24 – 90% w/w lipid (Phleger, 1991). The levels and form of dietary lipid affect the growth and the rate of malformation of marine fish larvae although high levels of malformations can occur with high levels of HUFA supplied as dietary phospholipid at first feeding (Villeneuve et al., 2005). It is suggested that HUFA's can affect expression of genes that are involved in skeletal development during ontogenesis (Cahu et al., 2003). HUFA's are also precursors to the eicosanoids, prostaglandins and leukotrienes that have diverse actions including involvement in bone metabolism. Prostoglandins are a principal mediator of bone cell functions and phospholipids facilitate cartilage mineralisation (Lall and Lewis-M^{c}Crea, 2007). These functions support that possibility that the type and amount of dietary lipid may affect the incidence of jaw deformity in Yellowtail Kingfish larvae.

CONCLUSION

Apart from the low level of swimbladder inflation for Yellowtail Kingfish larvae cultured using N-Rich® PL Plus, no major differences in performance were observed between the rotifer enrichment products tested. The trial was terminated at 9 DPH due to the less than acceptable level of swimbladder inflation recorded at this time. It is expected that more definitive results may have been achieved if larvae progressed until the end of the rotifer feeding phase (12 – 15 DPH). A number of modifications have been proposed for the larval rearing system used for this trial with the expectation that improved levels of swimbladder inflation can be reached to allow trials to be completed. The incidence of jaw deformity in Yellowtail Kingfish juveniles is of most concern for hatchery operators. The skull, jaw of fish larvae are complex in derivation and structure and develop rapidly in advance of swimming structures and the axial skeleton (Kjørsvik et al., 2004). This suggests that the factor/s that influence the incidence of jaw deformity observed in juvenile Yellowtail Kingfish may be acting upon larvae during the early stages of development. Consequently further investigations should concentrate on those abiotic and biotic factors known to induce jaw deformity during the early stages of marine fish larval development.

4 Effect of light intensity on YTK larvae - SARDI

Section written by Wayne Hutchinson and Bennan Chen

Effect of the level of illumination on the performance of Yellowtail Kingfish larvae

INTRODUCTION

Marine finfish larvae rely on visual stimuli to hunt and capture their prey and rely on suitable light intensity and photoperiod during their early stages of development to ensure high survival and growth (Blaxter, 1968). Survival of larvae following the onset of first feeding can decline dramatically if not all of the environmental requirements are not adequately met. Additionally, failure of larvae to ingest sufficient nutrients or energy during the onset of exogenous feeding can reduce survival and the normal development organs and structures important for normal growth and development (Yufera and Darias, 2007).

Light conditions during the early development of fish larvae should be taken into account for the optimization of rearing protocols in the hatcheries because light is a key environmental factor that synchronizes all life stages of fish, from embryo development to sexual maturation (Villamizar et al., 2011). Most marine finfish larvae are primarily dependent on vision for feeding and require a light intensity threshold for first feeding (Blaxter, 1986). In the eyes of fish the retinal pigment epithelium extends or contracts to regulate the amount of light reaching the retina photoreceptors (rods and cones) to optimise light capture for vision (Kjørsvik et al., 2004; Keith et al., 2006). First feeding larvae of most marine fish have retinas comprised solely of cone photoreceptors that provide acute visual resolution and colour contrast under high light intensities (i.e. photopic conditions). In addition, initially the lens may be located on the retina so the field of focus of early stage larvae may be short and fixed so they are not able to focus so high prey densities are needed increase the chance of prey entering their field of focus (Kjørsvik et al., 2004). Because cones require high light intensities, the cone only retina of larvae limits their visual function to near surface waters where prey capture is assisted by high light levels (Pankhurst and Hilder, 1998). Marine fish larvae require higher levels of light for feeding than at later stages of development. As larvae develop prior to metamorphosis rod neurogenesis occurs and rod photoreceptors begin to be recruited into the retina to allow larvae to see at lower light levels (i.e. scotopic sensitivity). Fish focus by moving the lens of the eye using small muscles (retractor lentis) that attach during metamorphosis providing an extended field of focus (i.e. to and a wider field of view and includes the ability to detect prey at greater distances. The increased visual acuity of developing larvae allows prey density and levels of light intensity to be deceased without affecting the rate of capture (Kjørsvik et al., 2004).

Light is one of the environmental parameters that can be managed within marine finfish hatcheries by selecting the type of light, height lights are fixed above tanks and the depth and colour of larval rearing tanks. Addition of microalgae during larval rearing can also influence the distribution of light within tanks. Previous experience with Yellowtail Kingfish has shown that larval rearing conducted under natural illumination provided superior growth and swimbladder inflation than that observed for larvae cultured using a new R&D system located indoors using moderate light intensity (i.e. 1,925 ± 293 lux at the water surface at the centre for all tanks and 1,086 ± 510 lux at the edges of all tanks). Consequently a trial was conducted to investigate if improved performance of Yellowtail Kingfish larvae could be achieved using high levels of illumination during the early stages of culture.

MATERIAL AND METHODS

Egg incubation and larval stocking

Yellowtail kingfish eggs were collected in April 2011 from broodstock maintained under controlled photoperiod and water temperature conditions at the Arno Bay hatchery of Clean Seas Tuna Limited, South Australia. Eggs were transferred and hatched in 500 l fibreglass incubators at water temperature controlled at about 21°C. Larvae were stocked into larval rearing tanks at 28 larvae l^{-1} approximately 2 hours after completion of hatching.

Experimental system and larval rearing

The experiment used 9 tanks rather than 12 x 3,000 L conical bottom larval rearing tanks with seawater provided from 3 recirculating water treatment systems (i.e. 3 tanks per system). Combinations of fluorescent and halogen lights (400 W, Philips) were positioned above the tanks to supply illuminance at the water surface at the centre of each tank of 1,000 lux (1,073 ± 90 lux), 7,000 lux (7,150 ± 650 lux) or 12,000 lux (12,200 ± 608 lux) with 3 replicates of each treatment. Illuminance was measured using a light meter (MC-88, TPS Pty Ltd, Springwood, Queensland, Australia). Water temperature was 23.1 ± 0.1°C, dissolved oxygen ranged from 6.1 to 7.7 mg L^{-1}, pH was between 7.87 to and 8.13, salinity was 36.5 g l^{-1} and the photoperiod was 14 h light: 10 h dark. Larval tanks were siphoned daily to remove dead larvae and other debris. Two surface skimmers were installed on each larval rearing tank to remove surface films. To maximise swimbladder inflation between 3 – 7 DPH the water surface of each tank surfaces was also cleaned using paper towel at approximately hourly intervals from after the first feed until one hour before the lights were turned off.

Rotifers were enriched using S.presso® (INVE Aquaculture, Hoogveld 93, B-9200, Dendermonde, Belgium) following the manufacturers recommendations and were fed to larvae once on 2 DPH at 1500 h, then 3 times daily for the remainder of the trial at 0800 h, 1200 h and 16:30 h to maintain targeted food densities. The amount of rotifers added at each feed was determined from residual rotifer counts taken from each tank before each feeding time. Concentrated microalgae Nanno 3600TM

(*Nannochloropsis sp*., Reed Mariculture Inc., 871 East Hamilton Ave, Suite D, Campbell, CA95008 USA) was added to larval rearing tanks continuously by a multi-head dosing pump (ISMATEC ISM444, IDEX Health & Science SA, Feldeggstrasse 6, CH-8152 Glattbrugg, Switzerland) to maintain the density of microalgae at about 50,000 cells mL^{-1}. The experiment was terminated on 9 DPH.

Larval sampling and measurement

Larvae were randomly sampled daily from each tank in the morning before the morning feed from 1 DPH to 9 DPH. The standard length of 20 larvae from each tank was measured to the nearest 0.05 mm under a dissecting microscope (Canon, SZ61) whilst 50 larvae were taken at 9 DPH. Swimbladder inflation (%) of each larvae was also assessed was assessed daily from 3 DPH.

On the final day of the experiment, Aqui-S® (Aqui-S New Zealand Ltd, Lower Hutt, New Zealand) was added to each tank at 28.6 mg l^{-1} to anaesthetise larvae before sampling and harvesting to avoid fish flaring the operculum. To determine final survival rate, all remaining larvae were harvested from each tank and counted by volumetric method. Meanwhile, 50 fish taken for measuring standard length were also used for jaw malformation assessment.

Statistical analysis

Statistical analyses were performed with RASW Statistics 18 (PASW Statistics, Rel. 18.0.2. 2009 Chicago: SPSS INC.). One-way ANOVA was used to test effects of different light intensities on larval fish survival, growth, swimbladder inflation and jaw malformation. All results were presented in mean ± SD and the level of significant difference was set at $P < 0.05$ unless otherwise stated.

RESULTS

Survival

Survival of Yellowtail Kingfish larvae was significantly lower in the 1,000 lux treatment (12.3 ± 4.7%) compared to larvae cultured at illumination levels of 7,000 or 12,000 lux. There was no significant difference in larval survival between the 7,000 lux (45.6 ± 5.6%) and 12,000 lux (51.8 ± 2.6%) treatments (Fig. 1).

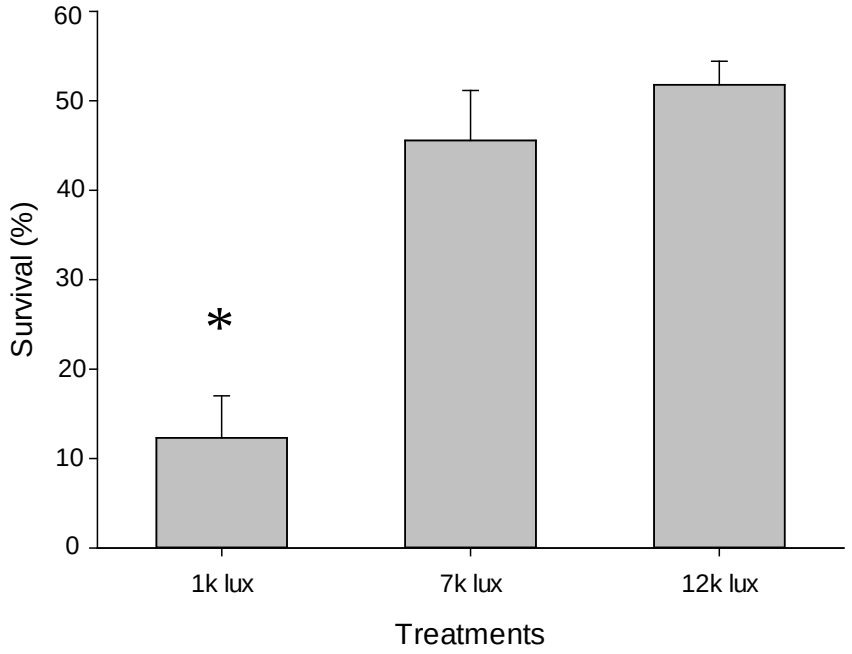

Fig. 1. Comparison of Yellowtail Kingfish larval survival rate (mean ± SD) among treatments exposure to different levels of light intensity. ***** denotes a significant difference ($P < 0.05$) from the other treatments.

Growth

Although larvae in the 1,000 lux treatment tended to be smaller at 9 DPH, there was no significant difference ($P > 0.05$) in the size of larvae among any of the treatments (Fig. 2).

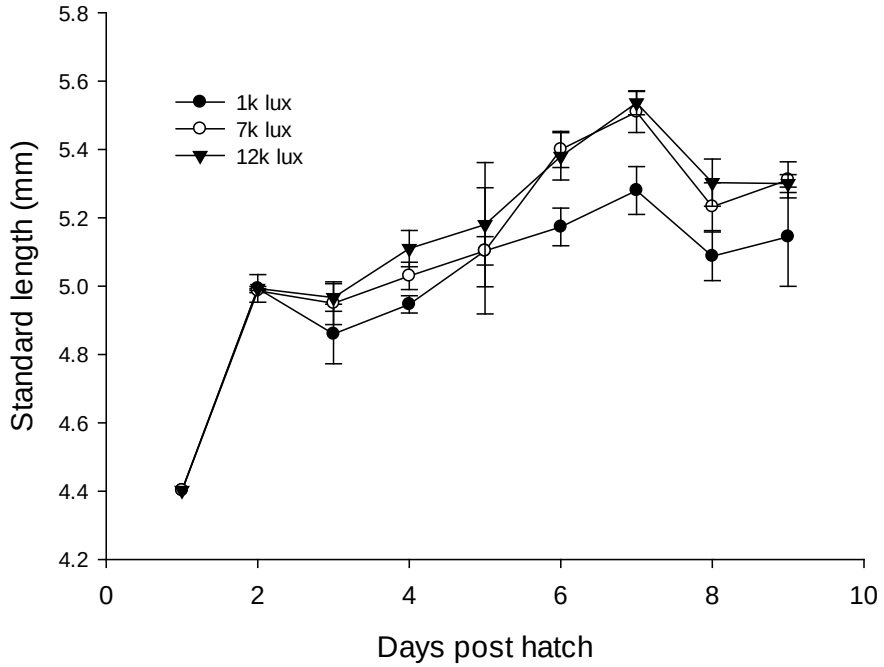

Fig. 2. Comparison of Yellowtail Kingfish larval growth in standard length (mean ± SD) among treatments exposure to different levels of light intensity.

Swimbladder inflation

In this trial, Yellowtail Kingfish larvae started to inflate their swimbladder from 3 DPH. The percentage of larvae with inflated swimbladders in the 1,000 lux treatment was 22.9 ± 9.4 % at 9 DPH, which was significantly ($P < 0.05$) lower than the swimbladder inflation for larvae cultured using 7,000 lux (72.7 ± 14.2%) or 12,000 lux (60.0 ± 10.0%) (Fig. 3).

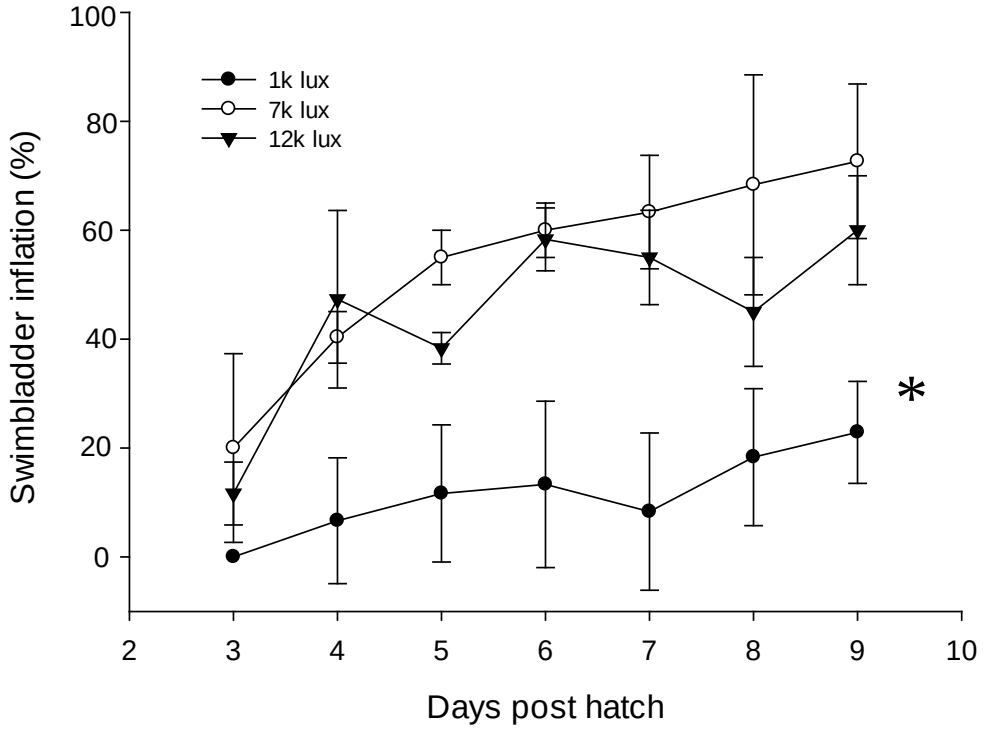

Fig. 3. Comparison of Yellowtail Kingfish swimbladder inflation (mean ± SD) among treatments exposure to different levels of light intensity. ***** denotes a significant difference ($P < 0.05$) from the other treatments.

Jaw deformity

The incidence of commercial level jaw deformities was significantly higher for larvae cultured using an illumination level of 12,000 lux (26.7 ± 5.0%) compared with larvae cultured using an illumination level of 1,000 lux (13.2 ± 3.8%). The incidence of commercial level jaw deformities was not significantly different ($P > 0.05$) for larvae cultured using an illumination level of 7,000 lux (16.7 ± 5.0%) when compared to larvae cultured at illumination levels of 1,000 lux or 12,000 lux (Fig. 4 and Fig. 5).

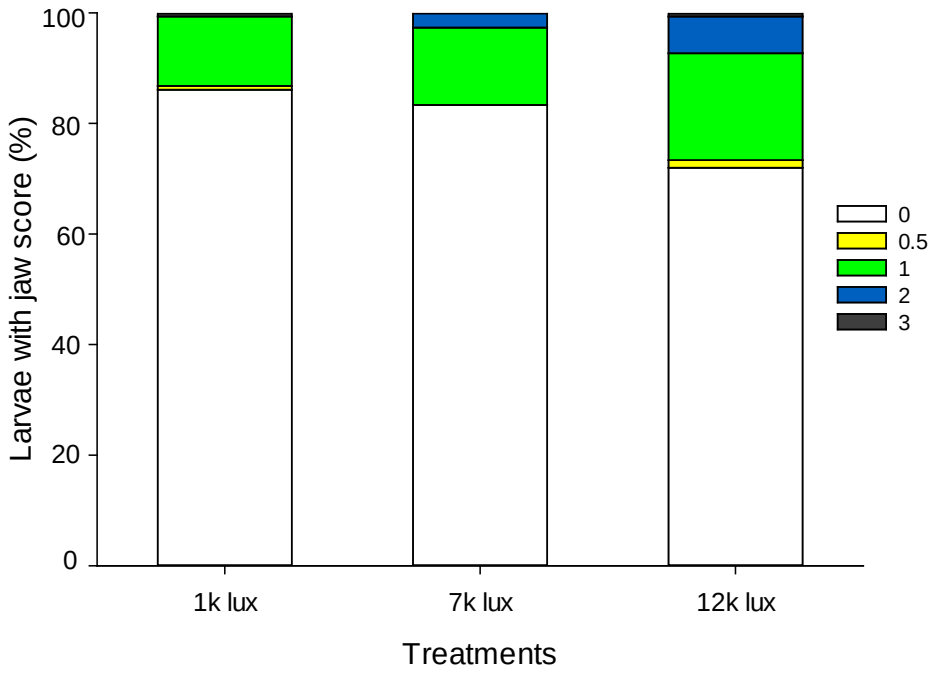

Fig. 4. Comparison of Yellowtail Kingfish jaw severity (proportion of fish %, mean ± SD) among treatments exposure to different levels of light intensity.

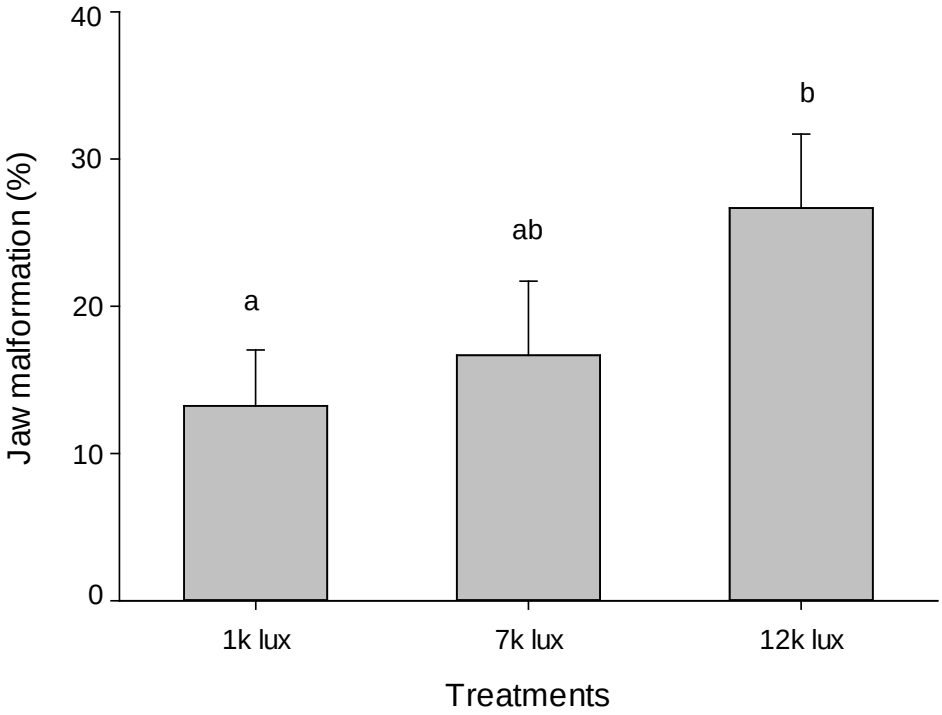

Fig. 5. Comparison of Yellowtail Kingfish jaw malformation (proportion of fish with jaw score of 1, 2 and 3, mean ± SD) among treatments exposure to different levels of light intensity. Treatments that share a common superscript are not significantly different ($P > 0.05$).

DISCUSSION

The visual system of larval marine fish will perform best under the lighting conditions particular to the ecological niche of each species (Downing and Litvak, 2001). Yellowtail kingfish are pelagic predators and it is likely that they are adapted to life in oceanic waters that typically have high clarity that are exposed to intense sunlight. Juveniles of amberjack, *S. dumerili*, between 32-210 mm standard length (Wells and Rooker, 2004) and yellowtail, *S. quinqueradiata*, between 10-150 mm fork length (Uehara et al., 2006) are associate with floating *Sargassum sp.* mats. For both species the abundance of juveniles associated with offshore (15 – 70 nautical miles) mats was greater than for inshore (< 15 nautical miles) mats. These observations suggest that *Seriola* sp. larvae are oceanic and inhabit surface waters exposed to high levels of illumination.

Previous experience with Yellowtail Kingfish has shown that natural illumination provided superior growth and swimbladder inflation than that observed for larvae cultured using a new R&D system located indoors using moderate light illumination (i.e. 1,925 ± 293 lux at the water surface at the centre for all tanks and 1,086 ± 510 lux at the edges of all tanks) provided by artificial lighting. Apart from the intensity of the natural illumination, the spectral quality of sunlight may also be beneficial as teleosts retinas have visual pigments that respond optimally at specific wavelengths to enable detection of prey and provide maximal contrast sensitivity (Lythgoe, 1979; Downing and Litvak, 2001).

The results of this trial support the notion that improved survival of *S.lalandi* larvae can be achieved using high levels of illumination during the early stages of rearing. Furuita et al. (1996) conducted larval rearing of yellowtail, *S. quinqueradiata*, using illumination levels of either 1,300 or 7,000lux. Carton (2005) reported significantly higher feeding incidence (% larvae feeding) and feeding intensity (rotifers larvae^{-1} h^{-1}) for first feeding *S. lalandi* larvae (4 DPH) cultured in clearwater under light intensities of 8 and 17 µmol s^{-1}m^{-2} than under lower light intensities of 1, 0.1 or 0 µmol s^{-1} m^{-2}. Larvae of the same age cultured in greenwater (*Chaetocerros meulleri,* 80,000 cell mL^{-1}) showed similar feeding incidence under light intensities of 1, 8 or 17 µmol s^{-1} m^{-2} although feeding intensity (rotifers larvae^{-1} h^{-1}) was significantly higher under light intensities of 8 or 17 µmol s^{-1} m^{-2} than under light intensities of 1, 0.1 or 0 µmol s^{-1} m^{-2}. The light intensities used represent levels of illumination of approximately 5, 50, 400 and 850 lux respectively (Table 1). From 6-7 DPH Yellowtail Kingfish larvae in both clear and green water showed considerably higher feeding intensity that was greater at light intensities of 8 and 17 µmol s^{-1} m^{-2}, than at 0.1 or 1 µmol s^{-1} m^{2}. Recently Stuart and Drawbridge (2010) used 24 h lighting at levels of illumination between 5,000 and 13,000 lux to culture *S. lalandi* in greenwater until 16 DPH. These authors subsequently compared larval culture of *S. lalandi*, at illumination levels of 360, 1,675 and 14,850 lux with added microalgae (300-500,000 cells mL^{-1}, Nanno 3600^{TM}, *Nannochloropsis sp*., Reed Mariculture Inc), or in clear water. The fastest growth and highest survival was recorded for larvae cultured at the highest level of illumination with green water (Stuart and Drawbridge, 2011). These results are supported by the results of this trial that demonstrated that survival was significantly

higher for larvae cultured at 7,000 or 12,000 lux compared to larvae cultured at 1,000 lux.

Table 1. Approximate conversion between illuminance units (lux) to irradiance intensity units (µmol $s^{-1}m^{-2}$, photon flux density) of different light sources.

	Light Source					
	Daylight	Metal Halide	Sodium (HP)	Mercury	White Fluorescent	Incandescent
To convert	Multiply by					
lux to µmol s^{-1} m^{-2}	0.018	0.014	0.014	0.014	0.012	0.020
	Divide by					
µmol s^{-1} m^{-2} to lux	0.018	0.014	0.014	0.014	0.012	0.020

Adapted from Biggs, W. 1991.

Generally upper levels of intensity are required to optimise growth although excessive light intensity is considered to be stressful or even lethal to fish larvae (Boeuf and Le Bail, 1999). Abnormal behaviour including “nose-walling” and poor feeding performance has been reported for striped trumpeter, *Latris line*ata, larvae at exposed to high light intensities (Pankhurst and Hilder, 1998). Juvenile Atlantic cod, *Gadus morhua*; Atlantic salmon, *Salmo salar*; and European sea bass, *Dicentrachus labrax,* showed varying degrees of retinal damage when exposed continuously (i.e. 24 h day^{-1}) to extreme light intensities between 34,830- 259,540 lux (Vera and Migaud, 2009). The results from this trial suggest that levels of illumination in excess of 7,000 lux may not provide further benefits for performance of *S. lalandi* larvae and may be detrimental as indicated by the significantly higher incidence of jaw deformity at 12,000 lux compared to larvae cultured at 1,000 lux.

In this trial the incidence of swimbladder inflation of Yellowtail Kingfish larvae was significantly higher at illumination levels greater than 7,000 lux than at 1,000 lux. A similar result was also report by Stuart and Drawbridge, (2011) who showed that the swimbladder inflation rate for *S. lalandi* larvae was 68.6 ± 3.1% at a high illumination level (14,859 lux) compared to 26.3 ± 13.1% or 16.3 ± 9.6 % at lower illumination levels of 1,675 or 360 lux respectively. Yellowtail kingfish inflate the swimbladder during the light period (Battaglene and Cobcroft, 2008). At first feeding Yellowtail Kingfish larvae are strongly phototactic moving to the surface when lights are turned on. These observation and the results reported suggest that the positive phototaxis displayed by first feeding larvae may assist swimbladder inflation for Yellowtail Kingfish.

At first feeding the eye of marine fish larvae do not have illumination adaption ability and functioning more as a positioning sensor in contrast to eyes of more advanced larvae that can adapt to variations in illumination through a greater range of pigments, photoreceptor migration and lens movement (Blaxter and Staines, 1970; Huse, 1994, Kjørsvik et al., 2004). These results suggest that high levels of light intensity are beneficial for first feeding Yellowtail Kingfish larvae that may have limited visual acuity at this stage. Although this trial has shown that first feeding Yellowtail Kingfish performed better at an illumination level of 7,000 lux or greater, it is likely that there may be an optimal level that provides benefits of feeding and

swimbladder inflation while minimising adverse effects observed for the incidence of jaw deformity.

CONCLUSION

Based on the results from this study, it is recommended that an illumination level of 7000 lux is appropriate for larval rearing of Yellowtail Kingfish as this level of illumination improved survival and swimbladder inflation and did not significantly increase the incidence of jaw deformity in comparison to larvae cultured at 1,000 lux. Increasing the illumination level to 12,000 lux provided no significant improvements for any of the parameters measured when compared to larvae cultured at 7,000 lux. Further research should investigate a range of levels of illumination between 1,000 lux and 7,000 lux as this trial omitted a range of moderate levels of illumination that may have provided improved performance of larvae.

Further trials should be conducted to investigate the interaction between the level of illumination and the density of microalgae used to maintain “green water” conditions during the early stages of larval rearing. The influence of tank colour should also be investigated with any further trials including measurement of light within the water column rather than at the surface as this will better reflect the conditions to which larvae are exposed and allow better quantification of the combined effects of light intensity, microalgal density and tank colour. A study of the development of vision in Yellowtail Kingfish larvae (refer to Pollyanna Hilder’s PhD Project 2009/760) would provide guidance of the likely lighting conditions appropriate for different stages of larval development.

5 Effects of live feed enrichment, light and oxygen on YTK larvae – PSFI and Flinders

Swimbladder inflation, growth, survival and deformity of yellowtail kingfish *Seriola lalandi* larvae under different temperature, live feed enrichment, light, and oxygen conditions

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Abstract

This study investigated the effects of temperature, live feed enrichment, light intensity and oxygen concentration on initial swimbladder inflation, growth, survival and deformity of yellowtail kingfish larvae. Poor survival can be associated with low inflation of the swimbladder for many marine finfish species and failure of swimbladder inflation may be attributed to an inappropriate range of abiotic and biotic rearing conditions. Four experiments were done to determine the effects of temperature(low, 21.5 °C and high, 24.5 °C), live feed enrichments (S.presso and Algamac), light intensity (low, 700 lux and high, 32,000 lux), and dissolved oxygen concentration (ambient and supersaturated). Fish growth was improved at the higher temperature and at the higher light intensity. Similarly, survival was significantly influenced by light intensity, with the highest survival (11.0 ± 2.3%) at the high intensity (32,000 lux). However, dissolved oxygen at supersaturated levels did not improve either swimbladder inflation or fish growth and survival compared with oxygen at ambient levels. Larval survival was significantly improved when fed rotifers and *Artemia* enriched with S.presso compared with Algamac. There were no effects of any treatment on the level of larval jaw deformity. Approximately 60-80% of larvae in each experiment had normal jaws while the remainder had low level jaw malformations. The percentage of larvae with excessively deformed jaws and thus requiring culling from the population was <5% in 3 of the 4 experiments. We recommend an optimal YTK larval rearing regime to include temperature of 24.5^{o}C, rotifer enrichment with Inve S.presso, light intensity of 32,000 lux and saturated dissolved oxygen.

Introduction

The supply of high quality fingerlings is essential to the success of finfish aquaculture while fish survival during larviculture is a major bottleneck limiting the production of fingerlings. Larval fish growth, development and mortality are influenced by a wide range of environmental factors such as temperature, light and dissolved oxygen and

these factors may also influence swimbladder inflation (Hadley, Rust, Van Eenennaam & Doroshov, 1987; Battaglene & Talbot, 1990; Trotter, Battaglene & Pankhurst, 2003a). Swimbladder inflation (SBI) is a crucial developmental milestone in the early larval developmental stages of most teleosts (Woolley & Qin, 2010; Woolley, Partridge & Qin, 2012).

The environmental factors affecting the mechanism of initial SBI have been studied in various fish species. However there is controversy on which conditions promote successful SBI and the requirement of environmental conditions for SBI appear to be species specific (Martin-Robichaud & Peterson, 1998). Previous studies have shown some abiotic factors that promote high inflation rates often correlate to high fish growth and survival rates (Hadley *et al.*, 1987). Fielder, Bardsley, Allan & Pankhurst (2005) found that temperature variations from 18 to 24 °C did not affect SBI in Australian snapper, *Pagrus auratus* (Bloch & Schneider), but SBI and fish survival were low when temperatures were below 15 °C or above 27 °C.

Water temperature significantly affects development, growth and survival of fish larvae (Blaxter, 1991) and is a critical factor for hatchery production. Successful initial SBI can be hindered by slight changes in rearing temperatures (Sanabria, Diamant & Zilberg, 2009). In the early larval stage, the timing of swimbladder inflation is advanced at higher temperatures. Martin-Robichaud and Peterson (1998) found the timing of initial inflation in striped bass, *Morone saxatilis* (Walbaum) is primarily temperature -dependent. A mistiming of the development of the primordial swimbladder at suboptimal rearing temperatures may delay the timing of initial inflation and consequently this uncoupling of developmental sequences could reduce the rate of swimbladder inflation (Marty, Hinton & Summerfelt, 1995). Initial SBI in striped trumpeter, *Latris lineata* (Bloch and Schneider) was around 70% when larvae were reared in temperatures of 14-17 °C but the inflation significantly decreased in temperatures >17 °C and <14 °C (Trotter *et al.*, 2003a). The low initial inflation rates at lower and higher rearing temperatures are possibly related to the poor development of the primordial swimbladder. Although previous investigations have included the optimal rearing temperature for *Seriola* spp. (Moran, Gara & Wells, 2007; Hirata, Hamasaki, Imai, Teruya, Iwasaki, Hamada & Mushiake, 2009) none of these studies have scrutinised how temperature can affect SBI, which leaves the question open if the fish survival was due to low SBI at inappropriate temperatures.

Light is another crucial physical parameter affecting growth and survival of larvae. Most marine fish larvae are visual feeders and therefore require light to search and capture their prey (Fielder, Bardsley, Allan & Pankhurst, 2002). Yellowtail kingfish *Seriola lalandi* (Valenciennes) are positively phototactic rising to the surface of rearing tanks at the light period (Carton, 2005). Larvae reared under natural light show typical diel rhythm activity (Carton, 2005) and changes in light intensity can impact feeding and survival of larvae during early development. Although light intensity can affect fish growth, its impact on SBI is largely species specific. In many species light acts as a cue for fish to swim to the surface and gulp air (Woolley & Qin, 2010). Swimbladder inflation of Californian yellowtail *Seriola lalandi* significantly improved when larvae were reared at a light intensity of ~14 500 lux (Stuart & Drawbridge, 2011). In contrast, Australian bass *Macquaria novemaculeata*

(Steindacher) require low light and darkness to reach maximum rates of swimbladder inflation (Battaglene & Talbot, 1990; Trotter *et al.*, 2003a). Most intensive culture systems are located indoors where fluorescent and halogen tubes are used as the light source (Villamizar, Garcia-Alazar & Sanchez-Vazquez, 2009). The levels of light intensity provided by the indoor facility are much less than those of the natural light in which larvae are exposed to in the ocean. Furthermore, the light properties between the artificial and natural sources are quite different especially when light penetrates the water column (Loew & Wahl, 2008; Villamizar, Blanco-Vives, Miguad, Davie, Carboni & Sanchez-Vazquez, 2011).

The level of ambient dissolved oxygen can affect oxygen consumption and metabolic activity as the oxygen demand of fish larvae increases rapidly just prior to hatching and during larval development (Miyashita, Hattori, Sawadw, Ishibashi, Nakatsukasa, Okada, Murata & Kumai, 1999; Dong, Qin & Zhang, 2011). Wexler, Margulies & Scholey (2011) found that increasing dissolved oxygen concentration improved feeding incidence and survival in yellowfin tuna, *Thunnus albacares* (Bonnaterre), but there is little knowledge on the impact of dissolved oxygen on the SBI. The level of dissolved oxygen not only affects fish metabolism, but also fish behaviour (Dong *et al.*, 2011). Therefore, it is possible that the level of dissolved oxygen may influence the swimming behaviour of larvae to gulp air from water surface. Yellowtail kingfish (YTK) is a high-energy-demand species with an aerobic metabolic scope similar to that of highly active teleost such as tuna and salmon (Clark & Seymour, 2006). Pirozzi & Booth (2009) reported that juvenile YTK have a high oxygen demand and that culture systems require high levels of dissolved oxygen. Many studies have focused on the limiting effect of oxygen and hypoxia on developing larvae (Sawada, Hattori, Iteya, Takagi, Ura, Seoka, Kato, Kurata, Mitatake, Katayama & Kumai, 2006) but the effect of high dissolved oxygen concentrations during early larval development has not been investigated for YTK.

Variable survival, growth and quality of hatchery-reared marine fish larvae are common even when optimal abiotic conditions are provided (Howell et al., 1998). Identification of optimal nutrition for marine fish larvae has been fundamental to development of rearing methods and much focus has been placed on the role of lipids for larvae (Watanabe, 1993; Battaglene and Brown, 2006). In particular, development of methods for enriching the live feeds, rotifers and *Artemia*, has received much research attention. In hard to rear species including striped trumpeter (*Latris lineata*) problems with early-stage swimbladder inflation and larval jaw and spinal malformations were reduced as live feed enrichment techniques were refined. Several different commercial live feed enrichment products are currently being used by YTK hatcheries however there is no understanding of the optimal product for YTK production.

Yellowtail kingfish is a well-established culture species in Australia where industry production is dominated by one South Australian company, Clean Seas Tuna Ltd. However, variation of fish survival and high incidence of larvae malformation in the hatchery period is an obstacle for viable YTK production. The rate of SBI is closely related to fish survival (Doroshov, Cornacchia & Hogan, 1981), but the environmental parameters regulating SBI in YTK larvae are largely unknown. Similarly, the abiotic

environmental and biotic factors including live feed nutrition affecting malformation of YTK larvae are unknown. Quality of YTK larvae, especially jaw malformation, is a major problem in hatcheries resulting in reduced survival and growth due to the inability of larvae to feed adequately, and potentially the need to discard (cull) excessively deformed fish because they cannot be sold (Cobcroft et al., 2003). In some cases, up to 50% of a hatchery population of YTK larvae has been culled due to excessive jaw deformity. In this study, we designed a series of experiments in an attempt to understand if temperature, live feed enrichment, light intensity and oxygen concentration affect the rate of SBI, larval performance and quality of YTK larvae. The identification of optimal environmental and live feeding conditions for swimbladder inflation will have direct implication to improve YTK survival and quality in hatcheries.

Materials and Methods

All experiments were done at the NSW Department of Primary Industries Port Stephens Fisheries Institute hatchery (PSFI) in New South Wales, Australia. Larvae were cultured in 2000 L conical-bottom tanks fitted with an upwelling manifold positioned at the base of the tank. Water flowed out of the tank through a 500um mesh-covered standpipe into a 200 L sump, where the water was returned to the tank via the manifold at 24 L min^{-1}. In addition, 100% of the tank volume was exchanged daily with disinfected seawater (32‰). A photoperiod of 12 h light (0900 h to 2100 h) and 12 h dark (Fielder & Heasman, 2011) was used in all experiments. For each successive experiment, the rearing parameter tested in previous experiments which produced best results for YTK performance was adopted as best-practice.

Larval rearing protocol

Fertilized eggs were sourced from captive YTK broodstock (Cleans Seas Tuna Limited, Experiments 1 and 4; PSFI, Experiments 2 and 3; Fielder & Heasman, 2011) and hatched in 450-1000 L incubators. After hatching, the larvae were randomly stocked into experiment tanks. *Tetraselmis* and *Nannochloropsis* spp. or *Nannochloropsis* sp. alone (Instant Algae, Reed Mariculture Inc, USA) were added to the tanks to create a greenwater background and maintained at an algal density of 5 × 10^4-1 × 10^6 cells mL^{-1}. Fish larvae were fed rotifers *Brachionus plicatilis* at a target density of 10 rotifers mL^{-1} from 2 days post hatch (dph) enriched with Algamac 3050 (Aquafauna Biomarine, CA; Experiment 1) and S.presso (INVE Aquaculture, Belgium; Experiments 2, 3 and 4) according to manufacturer's instructions. From 12 dph larvae were fed *Artemia* enriched with S.presso (INVE Aquaculture, Belgium) according to manufacturer's instructions. Surface skimmers were used during all experiments to remove oil debris from the water surface.

Experiment 1: Effect of temperature

A preliminary trial conducted on a small-scale (200 L tanks) showed that a rearing temperature of 21.5 °C was optimal for the survival of YTK larvae compared with the conventional practice of rearing larvae at 24.5 °C in the South Australian Research

and Development Institute YTK hatchery. Our experiment compared the effect of these two temperatures on SBI, fish growth, survival and malformation of YTK larvae in larger 2 000 L tanks (Table 1). Fertilized eggs were hatched in a 450 L incubator at 21.5 °C. Larvae were randomly stocked into six tanks at 24 larvae L^{-1}. Fluorescent lights were fitted above each tank to give a light intensity of ~700 lux at the centre of each tank. The tanks were randomly assigned with the treatment of two temperatures (21.5 °C or 24.5 °C; n=3 tanks). Larvae were acclimatized from 21.5 to 24.5 °C during the first 2 dph. The trial was terminated when larvae reached a mean standard length of 10 mm for each treatment, (i.e., 20 dph for the high and 27 dph for the low temperature treatments) when metamorphosis had occurred. Larvae at the higher temperature of 24.5 °C grew more quickly than those at 21.0^{o}C and there was no significant effect on survival thus this temperature was used in the succeeding experiments.

Experiment 2: Effect of live feed enrichment

This experiment aimed to determine the effects of live feed enrichment on SBI, growth, survival and malformation of YTK larvae (Table 1). Fertilized eggs were hatched in a 450 L incubator at 21.5 °C and larvae then randomly stocked into six 2000 L tanks at 3.4 larvae L^{-1}. Fluorescent lights were fitted above each tank to give a light intensity of ~700 lux at the centre of each tank. The tanks were randomly assigned with the treatment of two live feed enrichment regimes (Algamac 3050 and S.presso). Larvae were acclimatized from 21.5 to 24.5 °C during the first 2 dph. The trial was terminated at 20 dph when larvae reached a mean standard length of approximately 10 mm and the number of larvae from each tank was counted to determine survival rates.

Experiment 3: Effect of light intensity

The aim of this experiment was to determine the effect of light intensity with an artificial lighting source (Table 1). Three tanks were each randomly assigned one of two light intensities, either ~700 lux (~10 µmol $s^{-1}m^{-2}$) or ~32,000 lux (~450 µmol $s^{-1}m^{-2}$). The reason for using 700 lux as the low light intensity was to further explore the low end of light intensity at a level reported in a previous study on yellowtail kingfish larvae (Fielder & Heasman, 2011). The reason for setting the high light intensity at 32,000 lux was because it was close to the lowest intensity obtained under natural conditions. The other reason to choose such a level was a compromise between water temperature and light intensity as a higher intensity would cause an increase in water surface temperature. Halogen lights were fitted above each tank and the light intensity for each tank was established by adding or removing layers of shade cloth below the light until the desired intensity was reached. Fertilized eggs hatched in a 450 L incubator at 21.5 °C and larvae then randomly stocked into six 2000 L tanks at 3.6 larvae L^{-1}. The trial was terminated at 18 dph and the survival rates were determined at harvesting. Larvae reared under the high intensity of 32,000 lux had significantly higher survival compared to those reared under the low intensity of 700 lux. Therefore, the higher light intensity was used in the following Experiment 4.

Table 1: Experiment design, swimbladder inflation rates (SBI), final standard length, wet and dry weight, survival and number of culled *Seriola lalandi* larvae reared under different abiotic and biotic factors (values are mean ± SD). For each parameter, data with the same superscripts are not significantly different ($P > 0.05$).

Fixed Factors	Factor level	Treatment Factor	Treatment levels	Source of larvae	Number replicate tanks (*n*)	Tank size (*L*)	Stocking density (larvae/L)	Duration (days)	Swibladder inflation (6 DAH;%)	Final SL (mm)	Final wet weight (mg)	Final dry weight (mg)	Survival (%)	Culled (score 2+3, %)
Temperature	*variable*	**Temperature**	**21.5°C**	CST	3	2000	20	27[a]	100[a]	10.8±1.4[a]	38.1±14.2[a]	7.4±2.7[a]	6.2±2.6[a]	13.7±4.5[a]
Live feed enrichment	Algamac		**24.5°C**		3	2000	20	20[b]	100[a]	11.2±2.4[a]	34.2±18.4[a]	6.6±3.7[a]	8.1±3.1[a]	7.3±2.5[a]
Light intensity	700lux													
Dissolved oxygen	supersaturated													
Temperature	24.5°C	**Live feed enrichment**	**Algamac**	PSFI	3	2000	3.4	20	53.3±32.1[a]	13.3±1.2[a]	65.1±10.5[a]	12.8±1.8[a]	2.5±2.3[a]	2.5±2.1[a]
Live feed enrichment	*variable*		**Spresso**		3	2000	3.4	20	46.7±30.6[a]	11.8±1.7[b]	31.6±11.4[b]	6.1±2.4[b]	11.7±5.6[b]	4.3±3.5[a]
Light intensity	700lux													
Dissolved oxygen	supersaturated													
Temperature	24.5°C	**Light intensity**	**700 lux**	PSFI	3	2000	3.6	18	93.3±11.5[a]	9.8±0.2[a]	29.3±5.1[a]	5.1±.2[a]	4.9±3.6[a]	2.3±1.2[a]
Live feed enrichment	Spresso		**35,000 lux**		3	2000	3.6	18	96.7±2.9[a]	11.2±0.5[b]	36.5±7.0[a]	6.1±.7[a]	11.0±2.3[b]	2.3±1.5[a]
Light intensity	*variable*													
Dissolved oxygen	supersaturated													
Temperature	24.5°C	**Dissolved oxygen**	**ambient**	CST	3	2000	16	12	90±8.7[a]	6.8±0.3[a]	5.7±1.2[a]	1.1±.2[a]	18.5±6.4[a]	1.7±1.5[a]
Live feed enrichment	Spresso		**150% saturation**		3	2000	16	12	80±5.0[a]	6.4±0.6[a]	4.6±1.6[a]	0.9±0.3[a]	25.1±8.5[a]	0.7±1.2[a]
Light intensity	35,000 lux													

Note: Differences in parameter were deciders for inclusion of treatment factor in subsequent experiments.

Experiment 4: Effect of oxygen supersaturation

This experiment tested the effect of dissolved oxygen on SBI, growth, survival and malformation of YTK larvae (Table 1). Larvae were randomly stocked into six 2 000 L tanks at 16 larvae L^{-1}. Halogen lights were fitted above each tank to give a light intensity of ~32,000 lux (~450 µmol $s^{-1}m^{-2}$) at the centre of each tank. The tanks were randomly assigned one of two dissolved oxygen levels, either an ambient (7.1 ± 0.2 mg L^{-1}, 102 ± 2%) or a supersaturated (10.5 ± 0.4 mg L^{-1}, 151 ± 6%) dissolved oxygen level. Supersaturated oxygen levels were maintained by supplying compressed oxygen gas through air stones into each treatment tank. Oxygen levels were monitored using a multi- water quality probe (Horiba U-10, Horiba Ltd., Japan). The trial was terminated on 12 dph and the number of larvae from each tank was counted to estimate survival.

Larval sampling protocol

In each experiment, 20 larvae from each tank were sampled daily at 1200 h. Larvae were anesthetized in 0.2 g L^{-1} AQUI-S® (contains 540 g L^{-1} isoeugenol, AQUI-S New Zealand Ltd.) prior to measuring fish standard length (SL, measured from the tip from lower jaw to the end of the notochord, mm) and the dimensions of the yolk sac, oil globule and SBI rates represented by the percentage of the larvae with successful SBI to the total larvae sampled. At the termination a further 50 larvae were sampled from each tank to determine body weight. Larvae were rinsed in deionized water and weighed on pre-weighed glass slides. The slides were then dried in an oven at 80 °C for 24 h and reweighed to provide estimates of the dry weight. In addition, 100 larvae were randomly sampled from each tank, fixed in formalin for 7 days and transferred to 70% alcohol. These larvae were than assessed under dissecting microscope for jaw malformation using an index developed by Cobcroft et al. (2003) and adapted for commercial application by Clean Seas Tuna. The index used is commercial in confidence, but briefly the larvae were scored depending on the degree of jaw deformity (0 - no deformity; 0.5 – lower jaws shorter or bent down right,; 1 – lower jaws bent down left, longer or shorter lower jaws; 2 – longer lower jaw, fusion of one maxilla; 3 – longer lower jaw and maxilla fusion both sides. Fish with jaw deformity scores of 2 and 3 are unsuitable for ongrowing and the larvae are discarded from commercial production.

Statistical analyses

Results were analysed by one-way ANOVA to determine statistical differences between treatments parameters for SBI, swimbladder volume, growth, survival and jaw deformity. Data were arcsine transformed were needed. The relationship between swimbladder volume and larval length was determined by Spearman's correlation coefficient. Values were presented as mean ± SD and significance was set at $P < 0.05$.

Results

Experiment 1: Effect of temperature

Temperature did not affect swimbladder inflation (Table 1) which reached 100% by 4 dph and 5dph in the 24.5 °C and 21.5 °C treatments, respectively. There was a strong positive correlation between swimbladder volume and larval growth, measured in standard length for both the high (R^2 = 0.871) and low (R^2 = 0.864) temperature treatments (Fig. 1). Fish growth was significantly different between treatments (P = 0.023). At 20 dph, larvae reared at 24.5 °C were 10.6 ± 1.0 mm compared with larvae reared at the lower temperature (8.0 ± 0.8 mm). Larvae reared at 21.5 °C required a further 7 days to reach a mean length of 10 mm by 27 dph. Survival at harvest was not different between rearing temperatures (P = 0.46) (Table 1). Temperature did not significantly affect the degree of larval jaw deformity, although there was a trend in data suggesting fewer YTK larvae were deformed when cultured at 24.5^{o}C (Fig. 2). There was no difference between temperature treatments in the number of larvae with excessive jaw deformity requiring culling for commercial production (range 7-14%) (Table 1)

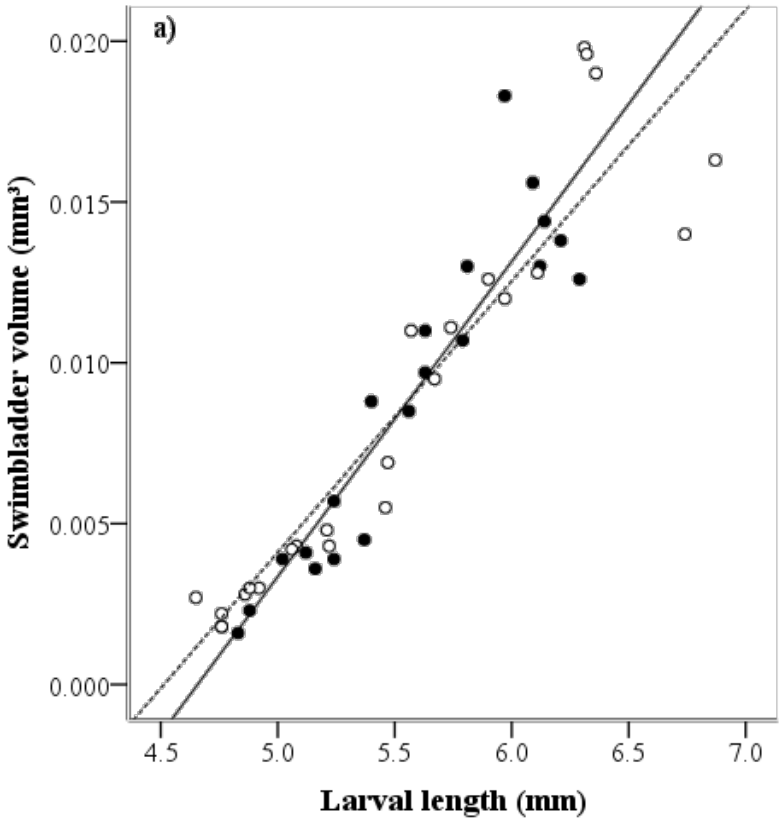

Figure 1. Correlation between swimbladder volume and standard length of *Seriola lalandi* larvae reared at 21.5 °C (—●—, R^2 = 0.864) and 24.5 °C (···○···, R^2 = 0.871).

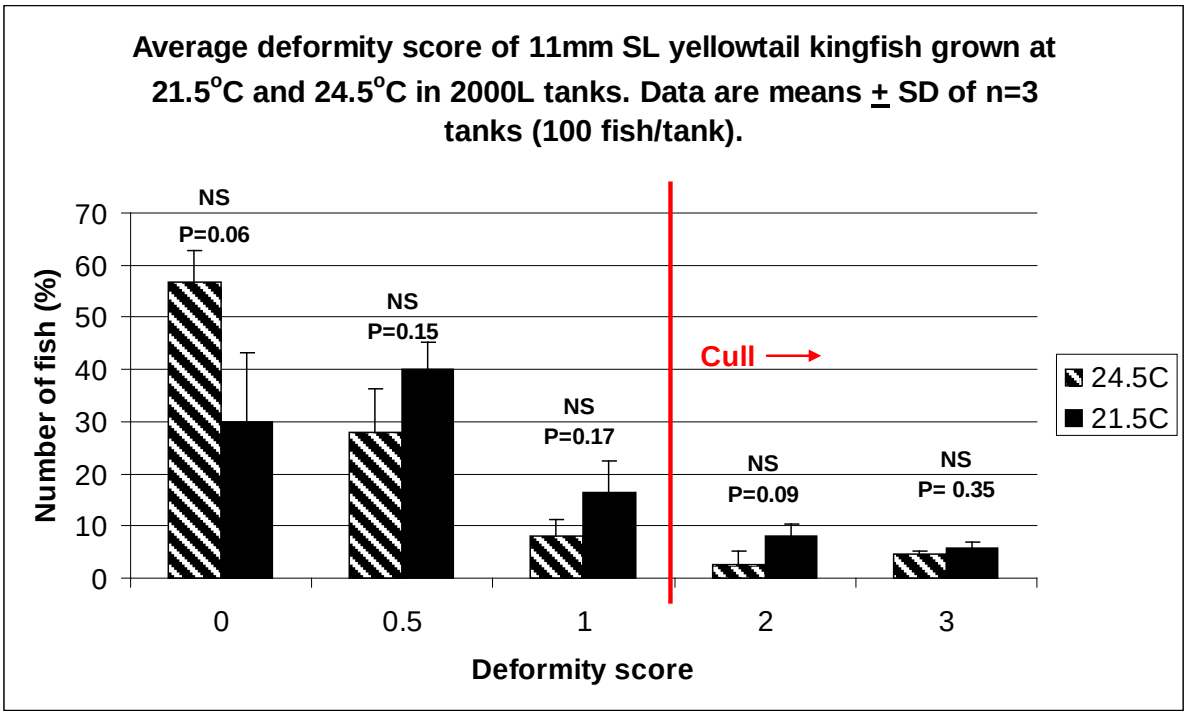

Figure 2. Average jaw deformity score of Yellowtail Kingfish (11 mm) at different rearing temperatures.

Experiment 2: Effect of live feed enrichment

Swimbladder inflation was not affected by live feed enrichment; however YTK larvae fed Algamac-enriched rotifers and *Artemia* were significantly bigger at 20 dph than those larvae fed S.presso-enriched live feeds (Fig 3; Table 1) Growth of YTL larvae for both treatments was similar during the rotifer feeding phase, but once *Artemia* feeding commenced at 12 dph, larvae fed Algamac-enriched *Artemia* grew more quickly than those larvae fed S.presso-enriched *Artemia*. In contrast, survival of YTK larvae fed S.presso-enriched live feeds was significantly higher than larvae fed Algamac-enriched live feeds (Table 1). There was no effect of live feed enrichment on the level of larval jaw deformity, which was low. Approximately 20% of the population of both live feed enrichment treatments had some degree of jaw deformity, with <5% having excessive jaw deformity and necessitating culling for ongrowing (Fig. 4).

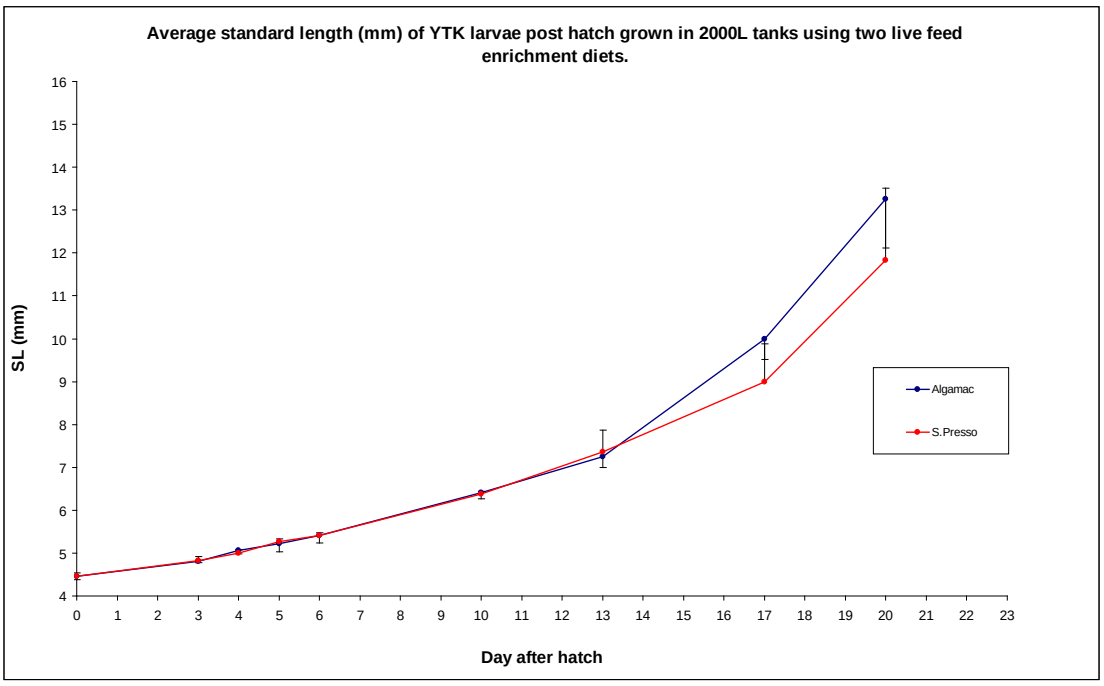

Figure 3. Growth of Yellowtail Kingfish larvae fed rotifers enriched with different commercial products. Larval size in standard length (SL, mm).

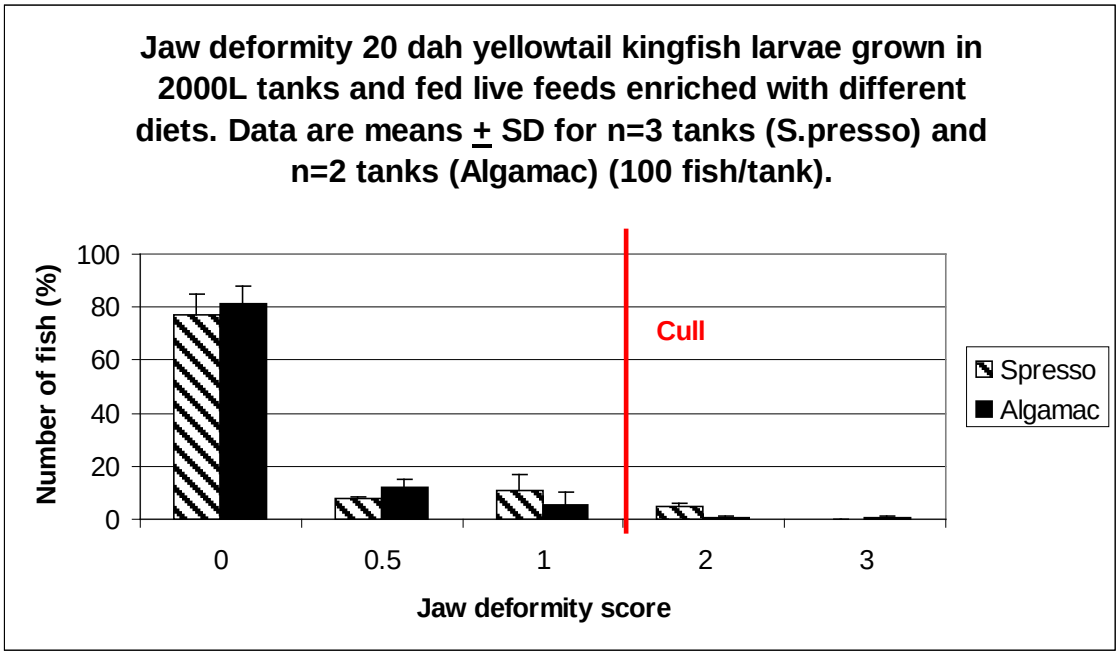

Figure 4. Average jaw deformity score of Yellowtail Kingfish (20 days after hatch, dah) fed rotifers enriched with different commercial products.

Experiment 3: Effect of light intensity

The SBI rates were not significantly different (*P* = 0.089) between low and high light intensities at 5 dph. However there was a significant difference in survival between light intensities (*P* = 0.008, Table 1). Larvae reared under high light intensity had higher survival (11.0 ± 2.3%) than those reared under the low light intensity (4.9 ± 3.6%). Similarly, at 18 dph length of larvae grown at high light intensity was greater (*P* = 0.001) than larvae grown at low light intensity (Table 1). There was no effect of light intensity on the level of larval jaw deformity which remained low, with only 2% of the population having excessive jaw deformity and necessitating culling for ongrowing (Fig. 5).

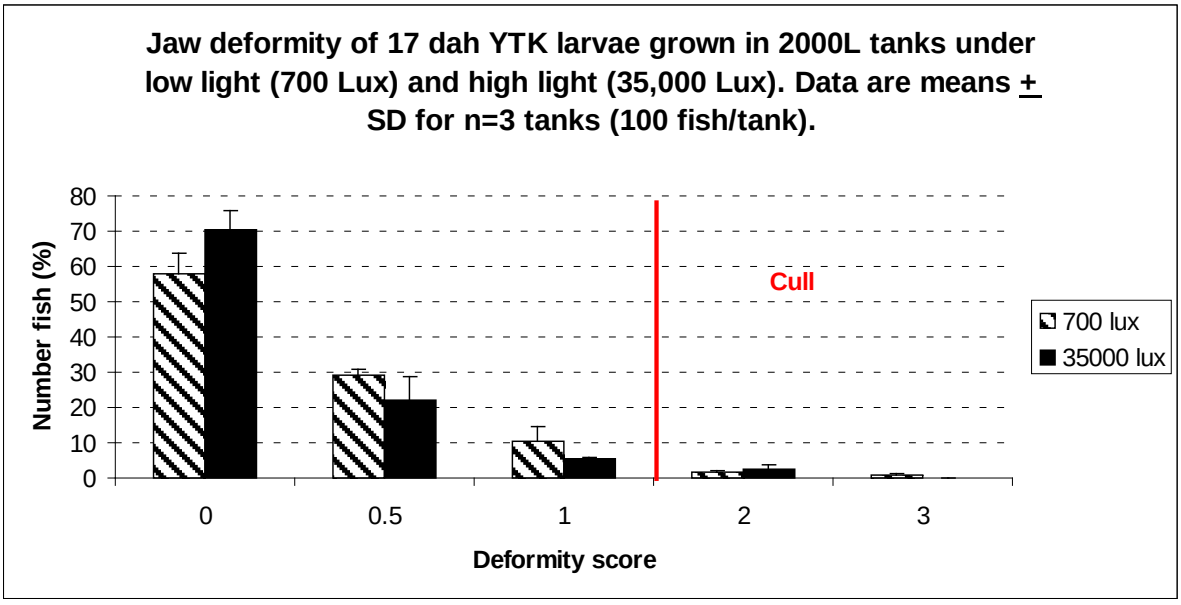

Figure 5. Average jaw deformity score of Yellowtail Kingfish (17 days after hatch, dah) reared under low and high light intensity.

Experiment 4: Effect of oxygen supersaturation

Oxygen supersaturation during early YTK larvae rearing did not significantly improve larval survival, SBI or growth to 12 dph (Table 1). Survival of larvae maintained in supersaturated dissolved oxygen (DO) conditions (25 ± 8%) was not significantly different to those larvae in ambient DO conditions (19 ± 6%) (*P* = 0.338). Swimbladder inflation rates were high in both treatments with inflation reaching 92 ± 3% in larvae reared under supersaturated DO levels compared with 85 ± 13% in larvae reared in ambient DO (*P* = 0.538). By 10 dph, there was no significant difference in swimbladder volume between DO levels (*P* = 0.108) and there was a positive correlation between swimbladder volume and standard length in both treatments (Fig. 6). There was no significant difference in growth between treatments (*P* = 0.323) with larvae reaching 6.6 ± 0.5 mm SL by 12 dph. There was no effect of DO concentration on deformity level with approximately 70% of larvae for both treatments having no jaw deformity. The number of larvae with excessive jaw deformity was <2% of the population for both treatments (Fig. 7).

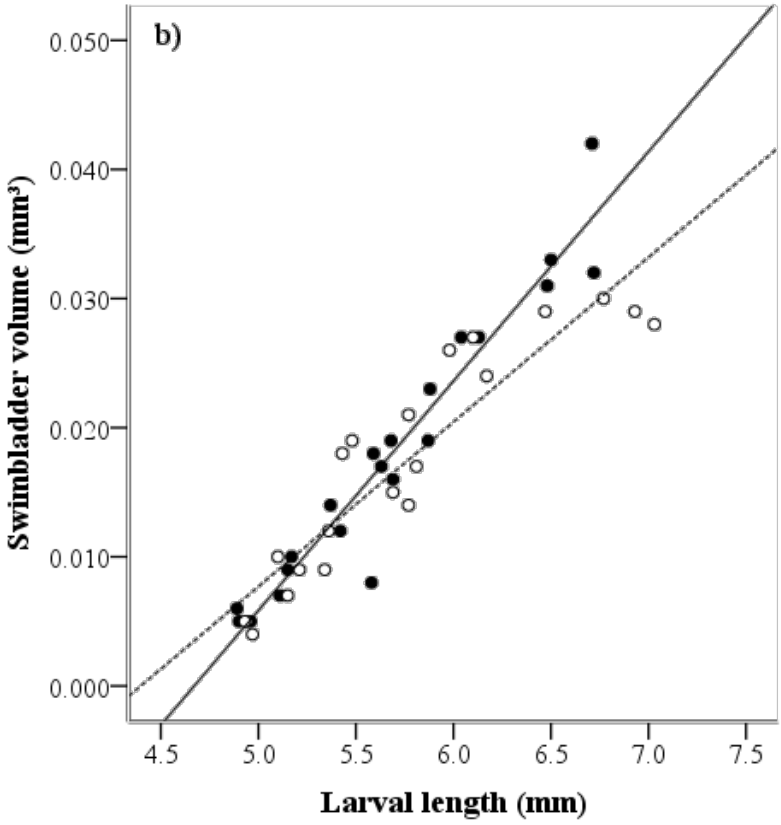

Figure 6. Correlation between swimbladder volume and standard length of *Seriola lalandi* larvae reared at ambient (—●—, R^2 = 0.858) and supersaturated oxygen (···○···, R^2 = 0.931) conditions.

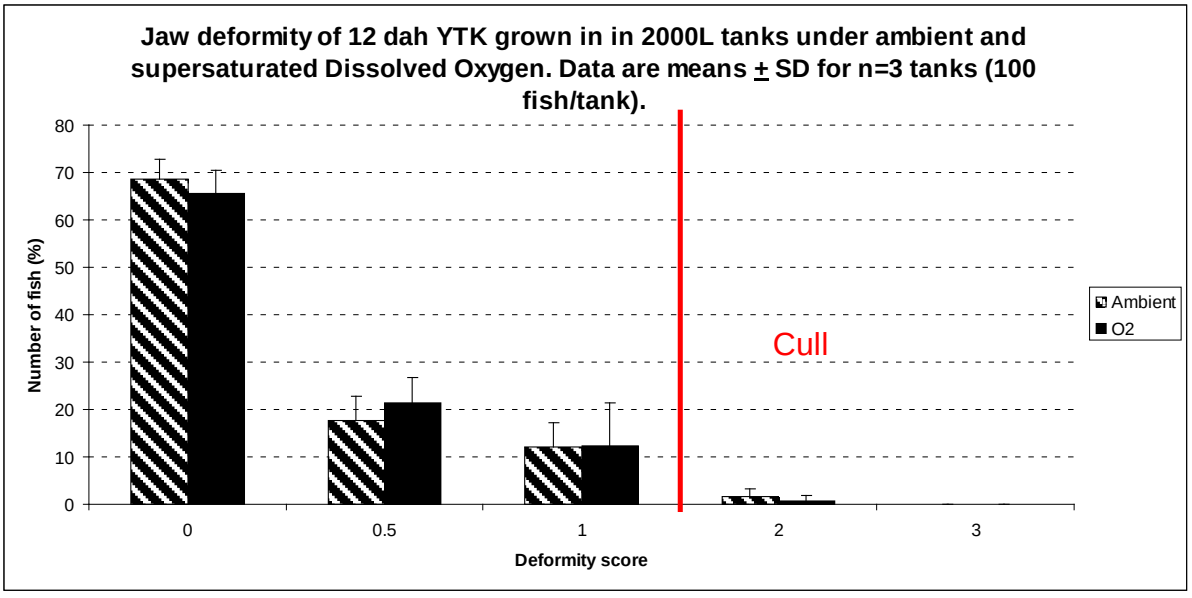

Figure 7. Average jaw deformity score of Yellowtail Kingfish (12 days after hatch, dah) reared with ambient or supersaturated dissolved oxygen.

Discussion

This study demonstrated that optimal conditions for swimbladder inflation, growth, survival and quality of YTK larvae include a rearing temperature of 24.5 °C, feeding of rotifers and *Artemia* enriched with S.presso, and provision of an artificial light source at high illumination intensity (32,000 lux). Supersaturation of dissolved oxygen did not improve performance of YTK larvae.

Swimbladder inflation occurs in a predetermined period when the pneumatic duct connects the swimbladder to the stomach (Woolley & Qin, 2010). Yellowtail kingfish are typical transient physostomes, whereby larvae gulp and swallow air bubbles at the water surface. The air bubbles enter the gut and then move into the pneumatic duct and finally into the primordial swimbladder to initiate inflation. Initial SBI occurs within a discrete window of time before the pneumatic duct degenerates. The window of SBI varies between fish species from as short as 1 day post hatch (dph) in angelfish (Zilberg, Ofir, Rabinski & Diamant, 2004) to 11 dph in Australian bass (Battaglene & Talbot, 1990) and striped trumpeter (Trotter, Pankhurst & Hart, 2001). In YTK the window of SBI is relatively short, ranging from 3 to 5 dph (Woolley *et al.*, 2012). Therefore, identification of optimal conditions for SBI during the early developmental stage was essential to achieve high inflation rates. Initial inflation in transient physostomes is severely impacted if access by larvae to the water surface is restricted by failure to remove the surface oil produced by enriched live feeds (Chapman, Hubert & Jackson, 1988). In this study, swimbladder inflation rates in experiments were generally above 80% (except Experiment 2 which compared the effects of Algamac and S.presso on YTK performance– approximately 50%) across experimental manipulations demonstrating that the rearing conditions in the larval tanks were conducive for swimbladder inflation. The facts that there was no treatment difference in Experiment 2 and that S.presso was used with high success in subsequent experiments suggests some other factors including newly-hatched larval quality and maternal broodstock nutrition may have influenced SBI.

Temperature affects virtually all aspects of reproduction in fish (van Der Kraak and Pankhurst 1996) and can influence many other aspects of early larval development, including size at hatching, efficiency of yolk utilization, growth, feeding rate, time to metamorphosis, behaviour and swimming speed, digestion and gut evacuation rates, and metabolic demand (Blaxter 1988; Rombough 1996). Our results agree with previous research on temperature tolerance of YTK in the embryonic stages. Moran et al. (2007) reported that 24 °C was suitable for YTK egg incubation and first feeding larvae. The temperature range used in this study did not affect the rate of SBI or the correlation with the increase of swimbladder volume with larval growth. Inflation began on 2 dph in larvae at 24.5 °C but by the end of the inflation window (i.e., 5 dph) inflation rates had reached 100% in both 21.5 and 24.5 °C treatments. A possible explanation is that the alteration of rearing temperature to an optimal range increases the metabolic rate of larvae leading to size variation and larger, fitter larvae may easily reach and penetrate the water surface to gulp air for initial inflation (Marty et al., 1995). Hirata *et al*. (2009) reported no effect of temperature on SBI rates when amberjack, *Seriola dumerili* (Risso) larvae were reared at temperatures from 22 to 28 °C. Sanabria *et al*. (2009) found that optimal swimbladder inflation of freshwater

angelfish *Pterophyllum scalare* (Gunther) occurred when larvae were reared at their optimal temperature range for survival. Likewise, Trotter, Pankhurst, Morehead & Battaglene (2003b) reported overlapping temperature optima for initial SBI and survival in striped trumpeter, where SBI and survival were highest at 16 and 17 °C. Although only two temperatures were tested in our study, the high rates of SBI and survival suggest that a temperature range of 21.5-24.5 °C is within the optimal range for YTK larval culture.

In our study, YTK larvae cultured at 24.5^{o}C reached metamorphosis (~10mm SL) at approximately 20 dph; 7 days earlier than those larvae cultured at 21.0^{o}C. Similar positive relationships between increasing temperature and growth rate of marine fish larvae within the tolerated temperature range over an extended period of time have been demonstrated for gilthead seabream, *Sparus aurata* (Person le-Ruyet and Verillaud 1980; Tandler et al., 2989), greenback flounder, *Rhombosolea tapirina* (Hart et al., 1996), and Australian snapper, *Pagrus auratus* (Fielder et al., 2005). The increased growth rate of YTK larvae at higher water temperatures has important implications for the cost of production of juvenile YTK. Increased growth rate of larvae means that fish can be transferred earlier from the hatchery to the nursery and the larval rearing tanks then used for another hatchery cycle. Similar results for Australian snapper showed that improved growth of larvae due to identification of optimal rearing protocols meant that 11 hatchery cycles could achieved each year compared with 7 cycles on an original hatchery protocol (Fielder et al.,2008). In addition, hatchery operating and labour costs associated with *Artemia* production will be reduced because bigger, faster-growing larvae can be weaned earlier from live feeds onto a pellet diet (Candreva et al., 1996).

One of the most widely investigated parameters for improving larval rearing protocols is light intensity. Optimal light intensity can improve growth rate by improving the ability of larvae to detect prey (Boeuf and Le Bail 1999). The receptivity of larva to light is species specific and can change with ontogeny. Many larvae require a minimum light intensity to properly see and catch prey, while excessive light intensities can cause stress and mortality of larvae. Optimal light intensity for larval culture are often similar to that experienced in the natural environment. Summer flounder, *Paralichthys dentatus* performed best at low light intensities which were consistent to those found in the offshore spawning grounds at depths up to 200m (Watanabe and Freely 2003). In contrast, growth of black sea bass *Centropristis striata* increased as light intensity was increased, consistent with the light environment experienced in shallow near-shore areas where larvae recruit (Copeland & Watanabe 2006).

Yellowtail kingfish larvae are visual feeders (Carton, 2005) and low light intensity may restrict the larvae's ability to see and capture prey as most larvae require a minimum level of light intensity threshold for normal development (Boeuf & Le Bail, 1999). Although feed intake was not measured, the significantly higher growth in larvae reared under high light intensity implies feeding capacity was improved at the higher light level. Similarly, the high light intensity significantly improved larval survival rates compared to those larvae reared under low light intensity. Stuart and Drawbridge (2011) reported similar effects of light intensity on *S. lalandi*.

Swimbladder inflation, fish growth and survival significantly improved when larvae were reared at 14 850 lux compared with 360 and 1675 lux. Swimbladder inflation rates were not significantly different between the two light intensities used in this study. However, the larvae reared under the higher light intensity reached inflation rates near 100%. The SBI rates were higher in our study than those at all the light intensities reported by Stuart and Drawbridge, suggesting that YTK at the typical neustonic larval stage (Trotter *et al.*, 2003a) require high light intensities (~32,000 lux) for normal growth and development.

Oxygen supplementation, achieved by efficient injection of pure oxygen into the water to increase the level of dissolved oxygen is a widely used technology in hatchery tanks (Edsall & Smith, 1990). Limiting dissolved oxygen can significantly inhibit first-feeding and interfere with metabolic processes when larvae rely on cutaneous respiration (Wexler *et al.*, 2011). Although there was a trend for higher SBI rates within the supersaturated treatment, there was no significant difference in inflation rates for larvae held in either near-saturated or supersaturated dissolved oxygen conditions. Similarly, there was no improvement in growth or survival of YTK larvae held in supersaturated dissolved oxygen demonstrating that near-saturated oxygen conditions provide the necessary oxygen demands for metabolism and development of early-stage YTK larvae. Post initial inflation, physoclistous larvae develop a counter current capillary system, i.e. the rete mirabile, which alters the gas volume within the swimbladder (Jobling, 1998). Secretion of gases from the capillaries into the swimbladder occurs by a reduction in blood oxygen (O_2) carrying capacity with an increase in blood pH. The gas gland cells in the swimbladder release acidic metabolites and drive the O_2 from the haemoglobin cells through the swimbladder vessels at the rete mirabile (Pelster, 1997). The swimbladder volume increased in proportion with larval growth in both treatments suggesting the blood O_2 levels were sufficient in larvae reared at near- and supersaturated levels. Similarly, Hattori, Sawada, Kurata, Yamamoto, Kato & Kumai (2004) found there was no significant difference in SBI rates in red sea bream, *Pagrus major* (Temminck et Schlegel) when larvae were reared in dissolved oxygen between 90 to 119% saturation. Summerfelt (1991) found SBI in walleye *Stizostedion vitreum* (Mitchill) was lower in larvae reared in oxygen supersaturated water compared with those reared in unsaturated water, but that the difference was not significant. This implies larvae gulping air at the water surface is still the only mechanism to initially inflate of the swimbladder. However, in commercial hatchery environments where high larval stocking densities are routinely used, supersaturated dissolved oxygen levels may improve fish swimming and feeding activities (Clark & Seymour, 2006).

Algamac 3050 and S.presso are commonly used as enrichment diets for rotifers and *Artemia* in marine fish hatcheries (Demir and Dikem 2011). Both products are rich in lipids, and other nutrients such as protein, vitamins and minerals and are designed to be easily applied to enrichment tanks and their ingestion by live feeds alters (increases) the lipid content and composition (especially long chain Polyunsaturated fatty acids including docosahexanaeoic acid (DHA) and eicosapentaenoic acid (EPA) and to some extent the protein content of the live feed (Battaglene et al., 2006). In our study, survival was significantly greater in the S.presso-enriched treatment. In contrast growth of YTK larvae was greatest when fed Algamac-enriched live feeds

although differences in growth did not occur until the *Artemia* feeding phase which commenced at 12 dph. A recent study by Demir and Diken (2011) showed that despite Algamac and S.presso having different proximate analyses, rotifers enriched after recommended enrichment protocols had the same and increased levels of crude fat and protein compared with non-enriched rotifers. High DHA:EPA ratios have been associated with increased survival of several marine fish larvae including striped trumpeter (Battaglene et al., 2006). Algamac 3050 has a DHA:EPA of approximately 15:1, whereas S.presso has a DHA:EPA of about 7. The fact that survival of YTK larvae was increased when fed S.presso-enriched live feeds suggests that a DHA:EPA of 7 is adequate for YTK larvae and that other nutritional factors such as minerals, vitamins or amino acid profiles were better in S.presso-enriched live feeds than Algamac-enriched live feeds. The increased growth of YTK larvae when fed Algamac-enriched *Artemia* may reflect a difference between rotifers and *Artemia* and the way in which the enrichment diets are assimilated, especially the protein fraction of the diets. Further research is required to understand the effects of feeding enriched *Artemia* on growth of YTK larvae.

Jaw malformation in the 12-27 dph YTK larvae was similar to that described by Cobcroft et al. (2003). In normal fish, the mouth was closed and the maxilla extended posterior-ventrally from the rostral cartilage parallel to the palatine. The maxilla was twisted laterally so that the temporal edge was toward the middle of the mouth and the nasal edge was at the external edge of the upper jaw. In deformed fish there was no lateral twisting of the maxilla; dorsal processes of the maxilla and premaxilla were in different positions to the normal fish; jaws were in the normal closed position but the hyoid arch was below the bottom jaw.

Various environmental factors including incubation temperature, salinity, light intensity and nutritional factors including fatty acid deficiencies have been implicated in the occurrence of larval fish jaw deformities of species other than YTK (Kanazawa et al., 1981; Gapasin et al., 1998). A high DHA:EPA ratio in live feeds has been suggested as being essential to reduce jaw deformity in striped trumpeter larvae. In our study, although larval jaw malformation was not excluded in all experiments, in general 60-80% of the YTK larvae had normal jaw formation and the majority of larvae had only minor jaw deformities. Indeed in each experiment there was no treatment effect on the occurrence of larvae with excessively deformed jaws and in three experiments the percentage of fish which were commercially nonviable and were culled from the population was <5%. These results suggest that neither live feed nutritional quality, nor temperature and light intensity were deficient or suboptimal, respectively and that factors other than those tested in our experiments may be influencing the occurrence of larval jaw deformity. We only observed deformity of advanced YTK larvae, however jaw deformities in larvae as young as 4 dph have been observed previously suggesting that endogenous factors including yolk reserves, broodstock nutrition and genetics can play roles in influencing development of jaw deformity. In addition, infection by bacteria of larval oral membranes has been attributed to jaw deformity in Atlantic halibut (*Hippoglossus hippoglossus*) (Morrison & MacDonald 1995). The influence of bacteria on development of jaw deformity and the subsequent management of bacterial flora in YTK larval rearing tanks therefore remains to be investigated.

In conclusion, this series of experiments identified the optimal water temperature, live feed enrichment protocols, light intensity and dissolved oxygen parameters for promotion of optimal SBI rates, growth, survival and quality of YTK larvae. The results of this study contribute to the understanding of the roles of abiotic and biotic factors in the larviculture of *Seriola* species. Given the demonstrated importance of light intensity for SBI and fish survival, and the fact that quality of light may differ between natural ocean and artificial tank environments, further studies should focus on understanding the effects of light periodicity (photoperiod) and light source, including wavelength and spectrum, on performance of YTK larvae.

Acknowledgements

We are very grateful for the assistance from the following people who have helped with this project:
Geoff Allan (Industry and Investment NSW)
Luke Cheviot (technical support, PSFI)
Luke Vandenberg (technical support, PSFI)
Lachlan Bassett (technical assistance, PSFI)
Ben Morton (technical assistance, PSFI)
Justin Kelly (technical assistance, PSFI)
Morton Deichman (industry support, Clean Seas Tuna)
Alex Czypionka (packaging of eggs, Clean Seas Tuna)
Adam Miller (packaging of eggs, Clean Sea Tuna)
Jennifer Cobcroft (technical exchange, UTAS)
Helena Heasman (Industry and Investment NSW)

6 Effect of prey density and live feed enrichment on YTK – Flinders

Section written by Zhenhua Ma and Jian Qin (Flinders University)

These experiments were directly funded by this project and conducted at SARDI, West Beach. Full reporting of this work is in Zhenhua's PhD thesis (Flinders University) and in the Final Report for SfCRC project 2009/700.

Experiment 1: Testing food consumption of Yellowtail Kingfish larvae at different prey densities.

Hypothesis:

- Fish growth and survival rates depend on the prey density.
- Food selectivity during co-feeding period (rotifers and *Artemia*) is prey density dependent.
- Fish food consumption is prey density dependent.

Methods:

This experiment was divided into two stages. Stage one covered rotifer feeding + mixed feeding (i.e., rotifers co-fed with *Artemia* nauplii) and stage two included *Artemia* nauplii only. To reduce possible variation in food consumption, after stage one, all the fish larvae used in this stage were discarded and the experimental tanks were restocked with fish larvae of the same cohort from 2000 L rearing tanks. In this case, the difference of previous rotifer densities in stage one would not affect the trial in stage two.

In stage one, four rotifers densities were used as the experimental factor, including 1, 10, 20 and 40 rotifers/ml. The *Artemia* density in stage one during the co-feeding period was constant 0.5 *Artemia*/ml. In stage two, the feeding density of *Artemia* nauplii started at 0.8 *Artemia*/ml on 15 DPH, and then four incremental intervals were used at 50%, 70%, 90% and 110%. The amount of *Artemia* nauplii fed to each tank was readjusted according to the daily fish survival rates. For each treatment, there were four replicates and a total of 16 tanks (172L) were used in each test stage. The duration of stage one was 14 days, and at stage two, the duration was 8 days. Fish growth, survival rates, food selectivity (during co-feeding period), gut content and gut evacuation time were used to calculate food consumption of YTK larvae in different prey densities.

Results:

Results of fish survival and swimbladder inflation: In stage I (ie, rotifers + rotifers co-feeding with *Artemia*), at 8 DPH, the mean survival rate was >45% and swimbladder inflation was about 20%. The low swimbladder inflation rate was possibly due to the

small size (172L) of the culture tank. In stage II (*Artemia* only stage), fish were transferred from the 2000 L tanks into 172L tanks at 15 DPH). At 8 DPH, the mean survival rate was >60%, and the swimbladder inflation rate was 99%.

See the project final report for additional information (SfCRC 2009/700).

Experiment 2: Effect of live food enrichment on YTK growth and survival

Summary:

There have been high fish mortalities in CST YTK hatcheries, potentially due to bacterial proliferation in rotifer culture. The high fish mortality was believed to be related to the type of enrichment formula for live food in larval fish rearing. The comparison of six commonly used live food enrichment products will help the industry choose the most suitable product for live food enrichment.

The aim of this study was to evaluate the effects of commercial products used for the enrichment of live food on feeding incidence rates, growth, survival and lipid composition of yellowtail kingfish larvae. This experiment involved five treatments with five replicates each. These treatments were: (1) live microalgae (1.6 million cells as *Nannochloropsis* sp./ *Tetraselmis* = 2:1), (2) half dose S.presso, (3) full dose S.presso, (4) Algamac 3050, and (5) Nutrakol. Rotifers fed on S.parkle were enriched with above enrichment formulas for 12 hours before being transferred into rearing tanks. The rotifers were introduced into the tank on 2 DPH with a density of 15 rotifers/ml. On 8 DPH, swimbladder inflation was 60%. On 12 DPH, fish survival was 40% in tanks enriched with live microalgae, 33% with half dose S.presso, 31% with full dose S.presso, 10% with Algamac and 20% with Nutrakol.

See the project final report for additional information (SfCRC 2009/700).

7 Benefits and Adoption

The direct beneficiary of this research is Clean Seas Tuna Ltd (CST), in relation to the improvement of hatchery production efficiency for Yellowtail Kingfish, with complementary benefits to the CST Southern Bluefin Tuna research program. Other beneficiaries of the research include other companies engaged in or looking to establish Yellowtail Kingfish culture in Australia. This currently includes The Marine Fishfarmers Association (MFA) of Western Australia collaborating with ACAAR in a new SfCRC project 2011/754.

Significant findings to date and their implications

Section 3 – Yellowtail Kingfish commercial trials
The CST Standard Operating Procedures for Yellowtail Kingfish (YTK) hatchery processes were reviewed and updated during the YTK season in 2010, prior to the SBT season. CST had a successful YTK production season in 2010, although survival between runs was variable (6 to 11%), and all fingerlings adhered to the quality control standards. The low swimbladder inflation rates seen in 2009 were rectified and many protocols were tightened, updated and improved. Jaw malformation remained a significant issue in commercial production, with an average of 33% of juveniles affected. Three feeding strategies were compared at a proof-of-concept scale, as well as artificial versus natural lighting. Meal feeding resulted in lower survival and growth than the standard practise of feeding multiple times during the day to a target density. There was an indication that jaw malformation was lower in YTK grown under artificial light compared with sunlight, and temperature control was more consistent in inside tanks, although growth and survival were similar.

Section 4 – Effect of rotifer enrichment on YTK larvae
Trials conducted by SARDI in the CST R&D system to the end of this project achieved good survival but not adequate levels of swimbladder inflation (>70%) limiting the duration that trials were maintained (i.e. until 9 dph). Regardless, results of a substantial enrichment trial showed that the best performing commercial product was S.presso (INVE Aquaculture) followed by S.pirit (INVE Aquaculture) and Ori-Green (Skretting), then N-Rich Ultra PL (Reed Mariculture). This suggests that the products currently used by CST may be the best available for use during the early stage of YTK larval rearing. Consideration should be given to repeating this trial when operational improvements have been made to the R&D system to achieve high levels of swimbladder inflation, particularly with the aim to assess jaw deformities.

Section 5 – Effect of light intensity on YTK larvae
Survival of YTK larvae to 9 DPH was lower in the 1,000 lux treatment (12%) compared with 7,000 or 12,000 lux (46 and 52%, respectively). Likewise, swimbladder inflation was lower at 1,000 lux (23%) compared with 7,000 or 12,000 lux (73 and 60%, respectively). Light intensity did not affect growth to 9 DPH. Jaw malformation was higher at 12,000 lux (27%) than at 1,000 lux (12%), with an intermediate level at 7,000 lux. An illumination level of 7,000 lux is recommended for

YTK larval rearing to improve survival and swimbladder inflation and avoid the potential negative impact of higher light intensity on jaw deformity. Light intensity is a significant parameter for CST for consideration during future planning of indoor commercial production and R&D systems for use with YTK.

Section 6 – Effects of live feed enrichment, light and oxygen on YTK larvae
Four experiments were done to determine the effects of temperature (low, 21.5 °C and high, 24.5 °C), live feed enrichments (S.presso and Algamac), light intensity (low, 700 lux and high, 32,000 lux), and dissolved oxygen concentration (ambient and supersaturated). Fish growth was improved at the higher temperature and at the higher light intensity. The swimbladder inflation rate was 100% in larvae in both temperature treatments. Survival was significantly influenced by light intensity, with the highest survival (11.0 ± 2.3%) at the high intensity (32,000 lux). However, dissolved oxygen at supersaturated levels did not improve either swimbladder inflation or fish growth and survival compared with oxygen at ambient levels. Larval survival was significantly improved when fed rotifers and *Artemia* enriched with S.presso compared with Algamac. There were no effects of any treatment on the level of larval jaw deformity. Approximately 60-80% of larvae in each experiment had normal jaws while the remainder had low level jaw malformations. The percentage of larvae with excessively deformed jaws and thus requiring culling from the population was <5% in 3 of the 4 experiments. An optimal YTK larval rearing regime was recommended to include temperature of 24.5^{o}C, rotifer enrichment with INVE S.presso, light intensity of 32,000 lux and saturated dissolved oxygen.

Section 7 – Effect of prey density and live feed enrichment on YTK
Prey consumption of YTK larvae was assessed in a co-feeding phase (I), with rotifers at four different densities with *Artemia* at a constant density, and in an *Artemia*-only phase (II). In phase I, the mean survival rate was >45% and swimbladder inflation was about 20% at 8 DPH. The low swimbladder inflation rate was possibly due to the small size (172 L) of the culture tanks. In phase II, swimbladder inflation was 99% in 2000 L tanks prior to transfer to the experiment system. Full results are provided in the project final report SfCRC 2009/700.

YTK Run in SBT Hatchery (chapter in companion SBT report 2010/750)
The CST R&D team conducted a 'Proof-of-Concept' production-scale run of Yellowtail Kingfish (YTK) larvae in the SBT Hatchery before the start of the SBT spawning season in early 2011. The run tested three different tank environments, and found no effect of green or white based tanks, nor one or two metal halide lights on YTK performance. Swimbladder inflation of 60% was low, survival of 17% was high, jaw malformation of 29% and growth rates were similar in comparison to other commercial runs. Weaning was achieved earlier than in other commercial runs and the trial provided important baseline information for the product, equipment and personnel requirements of a YTK run in the SBT Hatchery. Improvements would need to be made to culture methods to increase swimbladder inflation and reduce jaw deformities in the SBT Hatchery system. The trial established that there were no fundamental issues or shortcomings with the new SBT larval rearing recirculation system that would cause larval mortality in the upcoming SBT season.

Analysis of data collected by SARDI during the YTK production run in the SBT hatchery indicates the following opportunities for improvements:

- Reduced larval stocking density (40 larvae L^{-1}) provided with the same daily rotifer ration supplied to tanks at the standard stocking density (80 – 100 larvae L^{-1}) produced higher growth and survival.
- Floating of larvae as early as 27 dph (0.2 g) is possible to remove fish with non-inflated swimbladders to save costs of maintaining poor larvae until 40-50 dph.
- Higher survival of YTK larvae at 23 dph (43% greater) was observed in green bottom tanks compared to white bottom tanks.

Appendix 3 – ACAAR Review of SOPs
ACAAR reviewed the existing SOPs in use in CST YTK hatcheries. Several recommendations were made to improve SOP content and improve training and adherence to the techniques. They also supported Proof-of-Concept trials in Arno Bay Run 1, with the outcomes of the meal feeding and light regime indicating that the current CST practise of density feeding provided for better growth and survival in the nursery, while artificial lights and indoor tanks have promise to stabilise temperature, and potentially increase survival and reduce jaw deformity.

It should be recognised that there were differences between the results testing the same factors (e.g. temperature and light intensity) in different systems, or at different scales, or to different age or size end points. These have been considered by the Larval Rearing Implementation Group and follow-on research recommended as necessary in the new project 2011/740.

Progress in extension of the results to CST

The project successfully investigated several factors considered key to optimising survival, swimbladder inflation and jaw malformation in YTK. All research results were communicated in a timely manner to CST and project partners. There are many examples of extension and adoption of project activity by CST, including:

- YTK performance with various factors with application to CST as outlined above
- Test run of YTK in SBT Hatchery
    - Demonstrated that the mechanics and biology of the system work.
    - Provided confidence that high survival in YTK (~20%) can be achieved as an average output with consistent performance between tanks.
    - Demonstrated potential higher growth rate of YTK, potential to reduce the duration of the larval cycle, and potential for larger fish produced to be easier to wean.
    - Indicator that larval density may have an effect on survival – requires testing of 40 vs 90 larvae/L underway in new project 2011/740.
    - Issues with higher deformity rate that other runs in 2011, and lower swimbladder inflation rate (further trials in new project 2011/740 to investigate).
    - Data available to calculate live feeds usage per fish and $/fish in different systems.

- ACAAR review of SOPs (Gavin Partridge and Andrew Tindale)
    - SOP review by ACAAR was a catalyst for CST staff to revise and add new SOPs (see Section 3, Table 3)
    - Demonstrated that artificial light can be used successfully for YTK production AB Run 1. Repeated by YTK Production in AB Run 2.
- Implementation of rigorous surface skimming during the swimbladder inflation period resulted in high swimbladder inflation rates in 2010 in the YTK Hatchery (both in inside and outside tanks), which eliminated the requirement to 'float' juveniles in the nursery in high salinity water to separate and cull fish without inflated swimbladders. (Note. Swimbladder inflation rate was low in the SBT Hatchery.)
- Contribution of the project funded CST staff, consultants and partners in the monitoring of commercial production runs, and production of YTK fingerlings transferred to sea in the 2010/2011 season.
- The test run of YTK in the SBT Hatchery by CST R&D staff allowed for the timely set-up of the production systems in preparation for the SBT season and demonstrated that the mechanics and biology of the system work. The test run also demonstrated that the SBT Hatchery could be used to produce commercial quantities of YTK and that there were several issues that need to be resolved to improve production efficiency (ie. swimbladder inflation and jaw deformity). Some operating methods were adopted by CST Production, including the whiteboard display of daily task checklists for larval rearing, which improved organisation and efficiency. The SBT Hatchery was used for commercial production of YTK in the following 2011 season.
- Quality staff were recruited to CST through the project (new to CST – Marcell Boaventura, Nicolas Mace, Antonio Lillo, Atefeh Ghaltaii, David Poppi), and three have remained for > 18 months, and additional highly qualified staff have been recruited in the new project (2011/740). CST has retained some project staff on the commercial production payroll.
- Having researchers and consultants on-site was beneficial to Production as well as R&D (incl. Jenny Cobcroft, Wayne Hutchinson, Bennan Chen, Andrew Tindale, Gavin Partridge, Melanie Evans, Lindsey Woolley, Erin Bubner). Some of their specific activities were assistance with proof-of-concept trials (YTK and SBT Hatcheries), jaw malformation assessment, surface skimming and data collection (monitoring swimbladder inflation, larval measures).
- The demonstration of the usefulness of proof-of-concept trials has led to adoption of this approach by CST. In 2012, proof-of-concept experiments are underway at Port Augusta and integral to commercial production.
- This 1-yr project (2010/753) and the companion SBT project (2010/750) achieved the building of an R&D team and system in Arno Bay that could deliver on future research in both YTK and SBT.
- In the companion SBT project, SARDI researchers completed trials demonstrating that EBMix® (INVE) is a tool that can be used to manage bacteria, which significantly reduced bacterial load in enriched, rinsed rotifers in preparation to feed to larvae in rotifers, with likely benefit to YTK tank hygiene and bacterial flora. The product is not available commercially, but is recommended for use when possible.

- Joint technical meetings were held for CST Production and R&D staff to allow cross-fertilisation of ideas

8 Further Development

The proposed overall project plan for the current project was achieved in full, although the YTK performance indicators were not reached for survival and jaw deformity within this 12-month project.

At the end of the 2010/11 season the remaining hatchery issues identified for ongoing research were:

- Ongoing high prevalence of jaw malformation
- Low swimbladder inflation of YTK in the SBT Hatchery tanks

A new project was funded in 2011, to continue research in SBT larval rearing, with a small component of YTK research (2011/740). The new project development process was started by Dr Cobcroft, with direction from RMAG, and taken to the approved Concept Proposal stage. Dr Chen then coordinated the preparation of the full application for the successfully funded new project (2011/740). During the development of the new project, several items were considered by CST and RMAG, including:

- Continued support of project partner participation (PSFI), and R&D based in Arno Bay (CST and SARDI). The number of experiments funded at PSFI was reduced to reflect the lower annual budget.
- Maintain the R&D team at CST, with the PI/R&D Manager also based at Arno Bay. Dr Chen relocated to Arno Bay, which was an important step to improve project management.
- A stronger link between YTK proof-of-concept and R&D is encouraged, but requires pro-active management to deliver, because staff are very busy during the YTK season, and there must be time allocated to collaborative discussion and activity.

The follow-up project application was submitted to SfCRC in July 2011 and was approved and contracts were signed in March-April 2012 (Seafood CRC Project 2011/740). This new project will extend CST research with SBT larval rearing for the following three SBT spawning seasons, with complimentary activity in YTK, until the conclusion of the SfCRC in 2014.

An associated project has been funded by SfCRC to support Yellowtail Kingfish industry development in Western Australia (2011/754). It is intended that researchers from ACAAR will communicate with the 2011/740 project team to ensure hatchery experimentation is complementary and builds on work from this project.

9 Planned Outcomes

Public Benefit Outcomes

The planned public benefit outcome is in support of Yellowtail Kingfish industry expansion in Australia, which is currently based in South Australia and Western Australia. This would facilitate regional employment, new business opportunity, and access of Australian and international consumers to YTK.

Proposed Outcomes (long-term)

Reduced variability and improved efficiency in the hatchery production of Yellowtail Kingfish to support increasing aquaculture production of this species. The current largest producer in Australia, Clean Seas Tuna Ltd, anticipates production increasing to a sustainable 5,000 T by 2020. In turn, this contributes to the achievement of the national target of finfish aquaculture of 100,000 T by 2015 (Hone, 2008).

Private Benefit Outcomes

The short-term outcomes are intended to benefit Clean Seas Tuna Ltd through improved hatchery production efficiency of Yellowtail Kingfish.

Proposed Outcomes (short-term)

Improved yield of YTK juveniles and lower production costs by refined culture conditions to achieve reliably higher survival (>15% from yolksac larvae to 5g fingerlings within 2 years, >25% within 4 years), reduced malformations (<10% commercial malformations up to 5g within 2 years, <5% within 4 years) and increased swimbladder inflation (>97% in 5g fingerlings within 1 year).

The progress of the project toward the proposed outcome is indicated in Table 1. Progress has been made in some areas, most notably the improvement of surface cleaning to achieve swimbladder inflation in commercial systems and increased and stabilized survival in the SBT Hatchery system. This project provides optimal levels of some factors (temperature, rotifer enrichment, light intensity) to maximize yield, although there are apparent difference between the success of these in different locations. Additional research is required to identify factors to improve survival, swimbladder inflation and most critically reduce jaw deformity.

Table 1. Outputs of research toward the short-term outcome to improve the yield and reduce the hatchery production costs of YTK production to the 5g fingerling stage.

KPI	Result	Experiment/Trial
Survival >15% from yolksac larvae to 5g fingerlings within 2 years, >25% within 4 years	Not achieved. 6% to 11% survival achieved across all runs in the larvae phase, with 3% to 17% the range for individual tanks (stocking yolksac larvae to transfer to the nursery ~20-25 DPH). Survival in the nursery to sea (5g) 80 to 90%.	CST Production 2010
	Partially achieved. No effect of enrichment. 38% survival to 9 DPH with S.presso Affected by light intensity. 52% survival to 9 DPH with 12,000 lux	SARDI 2010/11 in CST R&D system
	Partially achieved. No effect of temperature. 6% at 21.5°C and 8% at 24.5°C. Affected by enrichment. 3% with Algamac and 12% with S.presso. Affected by light intensity. 5% at 700 lux and 11% at 32,000 lux. No effect of dissolved oxygen saturation. 19% at ambient and 25% at 150% saturation.	PSFI
	Partially achieved. ~45% at 8 DPH in 172 L tanks ~60% at 8 DPH in 2,000L tanks Affected by enrichment. 40% live microalgae and 10% Algamac at 12 DPH.	Flinders at SARDI
	Partially achieved. 17% to 22 to 25 DPH.	CST R&D in SBT Hatchery
Reduced jaw malformations <10% commercial malformations up to 5g within 2 years, <5% within 4 years	Not achieved. Average 8 to 55% at end of larval tank phase. Potential indication of artificial inside vs sunlight outside effect. 35% in Arno Bay and 31% in Port Augusta to 5g.	CST Production 2010
	Partially achieved. No effect of enrichment. 8% with S.presso and 15% with S.pirit at 9 DPH (note early assessment age). Affected by light. 13% at 1,000 lux and 27% at 12,000 lux at 9 DPH (note early assessment age).	SARDI 2010/11 in CST R&D system
	Partially achieved *. No effect of temperature. 14% at 21.5°C and 7% at 24.5°C (11 mm).	PSFI

	No effect of enrichment. 3% with Algamac and 4% with S.presso. No effect of light intensity. 2% at 700 lux and 2% at 32,000 lux. No effect of dissolved oxygen saturation. 2% at ambient and 1% at 150% saturation. * Note – relatively low jaw deformity although the factors driving this were not determined and are the subject of research in the new project 2011/740.	
	Not determined in these experiments	Flinders at SARDI
	Not achieved. 29% to 22 to 25 DPH.	CST R&D in SBT Hatchery
Swimbladder inflation >97% in 5g fingerlings within 1 year	Achieved. 3 runs all >97% at 5g 1 run, 3% culled to reach >97%.	CST Production 2010
	Partially achieved. Affected by enrichment. 69% and 65% with S.pirit and S.presso Affected by light intensity. 73% and 60% with 7,000 and 12,000 lux	SARDI 2010/11 in CST R&D system
	Partially achieved. No effect of temperature. 100% at 21.5°C and 24.5°C (6 DPH). No effect of enrichment. 53% with Algamac and 47% with S.presso. No effect of light intensity. 93% at 700 lux and 67% at 32,000 lux. No effect of dissolved oxygen saturation. 90% at ambient and 80% at 150% saturation.	PSFI
	Partially achieved. 20% and 60% at 8 DPH in 172 L tanks. 99% at 8 DPH in 2,000L tanks. SBI related research in L. Woolley thesis and final report 2009/733.	Flinders at SARDI
	Not achieved. 53 to 66% at 8 DPH. 95% had been achieved in one tank in a previous trial – improved management of 'SBT hatchery' tanks required.	CST R&D in SBT Hatchery

The progress towards the objectives and KPIs for the project was as follows:

Objective 1. To identify key factors which can be manipulated in the hatchery to increase Yellowtail Kingfish survival and swimbladder inflation rate (>97% in 5g fingerlings in 2010), and reduce malformations

Objective 2. To identify key factors which can be manipulated to increase production of high quality rotifers

Objective 3. To rapidly apply research findings to production scale systems for Yellowtail Kingfish at Clean Seas Tuna

1 KPI. Identification of key factors which can be manipulated to increase YTK survival (>15% from yolksac larvae to 5g fingerlings within 2 years) and swimbladder inflation rate (>97% in 5g fingerlings in 2010), and reduce malformations (<10% commercial malformations up to 5g within 2 years). At least seven (7) experiments and three (3) production-scale trials.

Achieved in full. Eight replicated experiments and four proof-of-concept production scale trials were completed in this project. Experiments confirmed that S.presso was an appropriate enrichment for YTK, that 24.5°C is an appropriate rearing temperature, and that artificial lighting may be used to culture YTK. Additional research is required to reduce jaw deformity, and the low prevalence at PSFI indicated this is possible. Two workshops were held to prioritise R&D for this (2010/753) and the next project (2011/740). Potential key factors were identified at the beginning of the project with a comprehensive list of prioritised research areas.

2 KPI. Rapid application of research findings to production scale systems for YTK at Arno Bay (at least three proof-of-concept trials in 2010-2011).

Achieved in full. Findings of trials in live feeding strategy, and artificial lights indoor compared with ambient sunlight outdoors, and lighting systems and tank colour in the SBT Hatchery were all immediately applied by CST.

3 Refined Standard Operating Procedures for live feeds and YTK fingerling production.

Achieved in full. Done through the review by ACAAR (Appendix 3) and SOPs revised by CST (Chapter 2, Table 3). Increased swimbladder inflation in commercial runs was testament to the adoption of improved SOPs.

4 Increased skill level of staff (recruitment and training) and R&D infrastructure capacity at CST (medium-scale system constructed).

Achieved in full. The physical R&D infrastructure was delivered and this is a useful long term resource to CST as it gives the capacity for replicated trials. Good quality staff were recruited, all with a minimum tertiary degree qualification.

5 Revised management system for communication and commercial adoption of R&D results.

Met in part. The system was an improvement but the level of engagement with CST remained below what is required for effective integration of R&D into the commercial hatchery environment. This was significantly better in relation to YTK than SBT research and communication, although 'commercial' pressure on R&D in Arno Bay hampers progress.

- There were 9 meetings of the YTK Larval Research Implementation Group during the project, with two of those meetings held face-to-face in Arno Bay to facilitate communication between hatchery staff and researchers.
- Communication – A monthly report on CST Fingerling R&D activity was provided to the CST Executive, RMAG, the Larval Research Implementation Group and the SfCRC Program Manager (Table 2).
- Communication – Contribution of R&D to CST technical meetings.
- Communication – Irregular meetings with Hatchery General Manager.
- Structured professional training course. A Communications workshop for CST hatchery staff was held on 13/01/2011, presented by Aubrey Warren, Pacific Training & Development, at Arno Bay. 15 CST staff attended.

6 Strong on-going links developed between the research partners in the project, contributing to the outcome and strategies of the CRC's finfish theme business plan and leading to further joint research proposals in the area of larval rearing and marine fish juvenile production.

Achieved in full. Noting that the new project (2011/740) contracted the scale of the collaboration, recruiting a key researcher into CST, and reduced the number of collaborations and the locations for the research to CST and PSFI. This approach was dictated by a reduced on-going budget and the necessity to focus on SBT. A complementary project was developed for YTK industry development in WA (SfCRC 2011/754), which will build linkages between work at CST and PSFI to the hatchery research occurring in ACAAR and it is intended the YTK LRIG will be expanded to include WA researchers.

Extension outputs achieved

1) Brief reports monthly to CST, SfCRC and YTK Larval Research Implementation Group, and Seafood CRC milestone reports

Monthly reports were delivered to the CST Managing Director and General Manager Hatcheries, to RMAG, and to the Seafood CRC Program Manager (Table 2). The reports were also provided to all project researchers from partner organisations and to the CST Hatchery Managers. Copies of the reports can be obtained through and with the approval of CST.

Table 2. Monthly reports from the CST hatchery R&D program to CST, SfCRC and research partners.

Report filename	**Date provided**
CST Fingerling R&D Report_11Oct2010.docx	12/10/10
CST Fingerling R&D Report_9Nov2010.docx	9/11/10
CST Fingerling R&D Report_6Dec2010.docx	17/12/10
CST Fingerling RD Report_14Feb2011.docx	14/02/11
CST Fingerling RD Report_7Mar2011.docx	7/03/11
CST Fingerling RD Report_11Apr2011.docx	12/04/11
CST Fingerling RD Report_6May2011.docx	6/05/11
CST Fingerling RD Report_13June2011.docx	13/06/11
CST Fingerling RD Report_11 July2011.docx	10/07/11
CST Fingerling RD Report_15 Aug2011.docx	15/08/11
CST Fingerling RD Report_12 Sept2011_JennyBennan_jc.docx	12/09/11 (sent by Bennan)

2) Research seminars to industry and research groups, oral and poster presentations (e.g. at CRC conferences, national workshops and international conferences)

Cobcroft, J.M. 2011. 'Marine finfish larval rearing – challenges and solutions.' Presentation to staff and students of Kinki University, Shirahama Station, Japan. 18 June 2011. (Oral presentation only)

Cobcroft, JM, Chen, B, Deichmann, M, Fielder, S, Hutchinson, W, Knuckey, R, Qin, J, Schipp, G, Thomson, M. 2011. 'Progress in understanding larval culture requirements of Southern Bluefin Tuna and Yellowtail Kingfish.' Australian Seafood CRC Science Day, Adelaide, 11th July 2011. (Abstract and oral presentation)

Hilder, PI, Cobcroft, JM, and Battaglene, SC. 2011. 'The first-feeding response of southern bluefin tuna and yellowtail kingfish larvae exposed to different prey density, prey size and larval density.' 3rd annual Australian National Network in Marine Science (ANNiMS) Conference, Perth, 29th November to 1 December 2011. (Abstract and poster presentation)

Hilder, PI, Cobcroft, JM, and Battaglene, SC. 2012. 'The effect of prey density, prey size and larval density on the first-feeding response of Southern Bluefin Tuna and Yellowtail Kingfish larvae.' Australasian Aquaculture Conference 2012. Melbourne 1st to 4th May 2012. (Abstract and oral presentation)

Woolley,L., Partridge, G., Qin, J. 2012. 'Mortality reduction in Yellowtail Kingfish (*Seriola lalandi*) larval rearing by optimising *Artemia* feeding regimes.' Australasian Aquaculture Conference 2012. Melbourne 1st to 4th May 2012. (Abstract and oral presentation) (work based on project 2009/733, with collaboration and some travel support from this project)

3) Articles for industry newsletters and magazines

Cochrane, P. 2011. 'From little things big things grow'. Research to Reality, University of Tasmania. Edition 8, June 2011. p.2. (Article highlighting J. Cobcroft (UTas) leadership of the SBT and YTK propagation research through Seafood CRC.)

CST made stock exchange announcements with information related to the YTK R&D program. All releases available from http://www.cleanseas.com.au/main/investor-information.html

4) Contribution to updating of current YTK hatchery protocols (note not a hatchery manual from this project)

Project researchers and members of the CST R&D team participated in meetings with CST Production to discuss YTK Hatchery protocols. Key meetings were the YTK Larval Research Implementation Group meetings, especially those held face-to-face in Arno Bay on 30 Nov – 1 Dec 2010 and 5 – 6 July 2011. On both occasions, all standard practice parameters were discussed and baseline values agreed, and factors for experimentation were prioritised.

In addition, the Standard Operating Procedures used by CST were revised which included live feeds protocols (Chapter 2, Table 3).

5) Manuscripts prepared for submission to peer-reviewed international journals

Woolley L.D., Partridge G.J. & Qin J.G. (2012) Mortality reduction in yellowtail kingfish (*Seriola lalandi*) larval rearing by optimising *Artemia* feeding regimes. *Aquaculture* 344-349, 161-167. (work based on project 2009/733, with collaboration and some travel support from this project)

Woolley L.D. & Qin J.G. (2010) Swimbladder inflation and its implication to the culture of marine finfish larvae. *Reviews in Aquaculture* **2**, 181-190. (work based on project 2009/733)

Two manuscripts prepared by PhD candidate Polly Hilder for submission following final editing and approval by CST and SfCRC.

Hilder, P.I., Cobcroft, J.M., Battaglene, S.C. The early-feeding response of Southern Bluefin Tuna *Thunnus maccoyii* and Yellowtail Kingfish *Seriola lalandi* larvae to prey density, prey size and larval density. For submission to 'Aquaculture'.

Hilder, P.I., Cobcroft, J.M., Battaglene, S.C. The effect of light intensity, turbidity, turbulence and tank colour on the early-feeding response of Southern Bluefin Tuna *Thunnus maccoyii* and Yellowtail Kingfish *Seriola lalandi* larvae. For submission to 'Aquaculture'.

Linkages with CRC Milestone Outcomes

This research project has contributed to successfully addressing selected bottlenecks in YTK Hatchery production, to support an increase in the production and profitability of the Australian YTK aquaculture industry. Other researchable constraints were identified, and some larval culture factors were eliminated to allow future research to focus on continued improvement of hatchery procedures.

10 Conclusion

There was progress toward all performance indicators (see section 9 Planned Outcomes). A summary of the results of the project under the objectives is provided below.

Overview of achievement within each objective

Objective 1. To identify key factors which can be manipulated in the hatchery to increase Yellowtail Kingfish survival and swimbladder inflation rate (>97% in 5g fingerlings in 2010), and reduce malformations

Through the eight experiments and four proof-of-concept trials conducted in this project, factors were identified that affected the performance of Yellowtail Kingfish. Larval survival was affected by light intensity and enrichment, with 32,000 lux and S.presso providing for the highest levels at PSFI to 18 to 20 DPH. In Arno Bay, 12,000 lux provided for the highest survival to 9 DPH in the R&D system and the run in the SBT Hatchery had the highest commercial-scale survival (17%) compared to other runs in 2010. Proof-of-concept trials in Arno Bay revealed meal feeding resulted in lower survival than the standard practise of feeding multiple times during the day to a target density. Swimbladder inflation of >97% in 5g fingerlings was achieved in three of four CST commercial runs in 2010, achieved through improved surface skimming of larval tanks. Swimbladder inflation remained problematic in the SBT Hatchery and in small tank experiment systems. There was no effect of temperature, enrichment, light intensity or dissolved oxygen saturation on YTK swimbladder inflation at PSFI. However light intensities of 7,000 lux and 12,000 lux and enrichment with S.presso and S.pirit resulted in higher swimbladder inflation in YTK in the R&D system in Arno Bay. Jaw malformations remained problematic in all commercial runs at 29 to 35%. There was an effect of light intensity in the R&D system in Arno Bay, with higher jaw deformity prevalence at higher light intensities, and there was a similar indication in commercial-scale trials. There was no effect of temperature, enrichment or light intensity on the prevalence of jaw deformity in YTK ≥ 10 mm, nor dissolved oxygen saturation in YTK at 6.6 mm at PSFI. However, the overall levels of jaw malformation were low compared to other facilities (2 to 14% at the size equivalent to the end of the larval tank phase, which was 3 to 18 times lower than the CST commercial runs), and other factors varying between PSFI and CST will be compared in the next project (2011/740).

Objective 2. To identify key factors which can be manipulated to increase production of high quality rotifers

Intensive culture methods with alternative commercial products (OriCulture, Skretting and Nanno 3600, Reed Mariculture) were identified as having potential to improve hygiene in culture and have been adopted in batch cultures by CST. A new product, EBMix, INVE, was demonstrated to reduce the load of culturable bacteria in rotifers ready to feed to larval fish tanks. This product is not available commercially, but is recommended for use when it is released to the Australian market. Details of this research are presented in the companion project final report (SfCRC 2010/750). Revised SOPs are in use for improved commercial rotifer production at CST.

Objective 3. To rapidly apply research findings to production scale systems for Yellowtail Kingfish at Clean Seas Tuna

Four proof-of-concept commercial scale trials were implemented in the 2010 YTK season, with designs based on research from previous projects (2007/718 and 2009/749) and through collaboration with researchers from ACAAR. Results were applied in the immediate production season (density feeding strategy, artificial lights for some tanks, utilisation of the SBT Hatchery for YTK production). This has been extended in the new project 2011/740, with commercial scale trials to test and adopt best rearing methods now integral to hatchery operations.

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12 APPENDIX 1: Intellectual Property

PROJECT INTELLECTUAL PROPERTY

Project Name	Improving hatchery production of Yellowtail Kingfish larvae and juveniles		
CRC Project Number	2010/753	**Principal Investigator**	Jenny Cobcroft (UTAS)

Short title of Item of intellectual property
(<10 words; separate sheet for each discrete item of IP)

Knowledge of rearing conditions which enhance swimbladder inflation and survival, and influence jaw deformity in YTK larvae

Description of the Project IP:-

- *Specific and unambiguous, but not disclose full enabling detail of Confidential Information;*
- *If subject to Restricted Access, this should define the nature and scope of the IP but not disclose the substance*

Increased understanding of some environmental, nutritional and microbial management factors important in larval rearing of Yellowtail Kingfish that enhance swimbladder inflation and survival, and influence the prevalence of jaw deformity, in medium-scale research tank systems and large scale production tanks.

Where and by whom is full enabling detail of the Project IP recorded?
(location, identification of documents, person in charge)

Cleanseas Tuna Ltd, Arno Bay, and electronic files in password protected systems. Contact Dr Bennan Chen (CST R&D Hatchery Manager).

Who developed the Project IP?
(Inventors, other project team members who are involved, any other contributions?)

CST Hatchery staff (Production and R&D) and collaborating researchers from partner organisations (UTas–IMAS, SARDI, NSW Fisheries, Flinders University) in project 2010/753 and consulting researchers from ACAAR.

Method of IP protection used so far
(any doubts about security of confidential information must be disclosed here)

Files held in locked facilities, electronic data in password protected servers, presentation and discussion of information in confidence in project and CST meetings.

Proposed method of IP protection
(E.g. patents, confidentiality, registered design, etc)
Confidentiality

Agreements, licences, assignments or disclosures planned or carried out in relation to this IP *(Reference relevant documents)*

Agreement between CST and Seafood CRC re IP and commercialisation.

DATA REGISTER

Project Name	Improving hatchery production of Yellowtail Kingfish larvae and juveniles		
CRC Project Number	2010/753	**Principal Investigator**	Jenny Cobcroft (UTAS)

The purpose of the Data Register is to identify Processed Data, i.e. assessed as reliable, statistically analysed, and processed into useable form. It is not for tracking all raw data.

Location of data	**Topic(s)**	**Author/custodian**	**Access to data**
Larval rearing data sheets. YTK Production	Effect of light type (artificial and natural) and feeding strategy on YTK larval performance.	Morten Deichmann	Confidential
Larval rearing data sheets. YTK Production	Effect of light intensity on YTK larval performance and trial of SBT Hatchery with YTK.	Bennan Chen (formerly Jenny Cobcroft)	Confidential
Rotifer Production Datasheets	Effect of culture method and diet on rotifer production.	Bennan Chen (formerly Jenny Cobcroft)	Confidential
R&D System Manual	R&D System design & operation manuals	Bennan Chen (formerly Jenny Cobcroft)	Confidential
Electronic files – in password protected	SBT Research planning and interim reports, meeting notes, data summaries and analyses – including SBT growth & performance	Bennan Chen (formerly Jenny Cobcroft)	Confidential

Intellectual Property issues arising:

Protection: All data is maintained commercial in confidence. With permission of CST and SfCRC it will be distributed as necessary to progress larval rearing research with current and future project partners.
Background IP: The study used some background IP from project partners as indicated in the original IP Register for 2010/753.
IP exploitation after project: As agreed in the Industry Partnership Agreement (1 July 2010 to 30 June 2014), between CST, FRDC, PIRSA and Seafood CRC, “All parties to this agreement agree that all Yellowtail Kingfish research developed from programs under this IPA will be made public through the normal process of external publication, other than for specific information that CST elects to maintain confidential by implementing a Commercialisation Agreement as provided for in the SCRC Participants Agreement.”

13 APPENDIX 2: Staff

Organisation	Position	Funding
IMAS, University of Tasmania		
Dr Jennifer Cobcroft	Principal Investigator & CST Fingerling R&D Manager (15 Sept 2010 – 14 Sept 2011 & 20 days in 2012 for Final Report completion)	Project funded 25%
Assoc Prof Stephen Battaglene	Researcher	Project funded 1%
Ms Melanie Evans	Technical Officer	Project funded 15%
CST RMAG		
Dr Craig Foster	RMAG Chair	In-kind 2%
CST		
Mr Morten Diechmann	General Manager Hatcheries	In-kind 2%
Mr Nicolas Mace	Assistant Fingerling R&D Manager (Start – 6 Dec 2010 End – 16 June 2011)	Project funded 25%
Mr Alex Czypionka	R&D Hatchery Technician & Hatchery Health (Start – 9 August 2010 End – 13 April 2011)	Project funded 25%
Mr David Poppi	Live Feeds Technician (Start – 2 Sept 2010 End – 9 Nov 2011)	Project funded 25%
Ms Atefeh Ghaltii	Live Feeds Technician (Start – 22 Dec 2010 End – 28 Sept 2011)	Project funded 25%
Mr Thomas Moyle	Live Feeds Technician (Start – 1 Aug 2011 End – 9 Nov 2011)	Project funded 25%
Ms Nahid Shokri Bousjein	Live Feeds Technician (Start – 29 Aug 2011 End – 13 Oct 2011)	Project funded 25%

Mr Marcell Boaventura	SBT Hatchery Manager (Start – 5 May 2011 End – 28 Sept 2011)	Project funded 25%
Mr Antonio Lillo	SBT Hatchery Technician (Start – 23 Oct 2010 End – 28 Sept 2011)	Project funded 25%
Mr Adam Miller	Broodstock Manager (Start – 15 Sept 2010 End – 28 Sept 2011)	Project funded 25%
Mr Jamie Crawford	YTK Hatchery Manager – Arno Bay	In-kind 2%
Mr Travis Dymmott	YTK Hatchery Manager – Port Augusta	In-kind 2%
PSFI		
Dr Stewart Fielder	Research Scientist	Project funded 12% In kind 10%
Mr Luke Cheviot	Fisheries Technician	In kind 12%
Mr Luke Vandenberg	Fisheries Technician	In kind 12%
Mr Lachlan Bassett	Fisheries Technician	In kind 12%
Mr Ben Morten	Technician	Project funded 25%
Mr Brendan Findlay	Technician	Project funded 25%
SARDI		
Mr Wayne Hutchinson	Senior Research Scientist	Project funded 12%
Dr Bennan Chen	Post-doctoral Scientist – Larval Rearing	Project funded 6%
Flinders University		
Dr Bennan Chen	Post-doctoral Scientist – Larval Rearing	In-kind Flinders 4%
Assoc Prof Jian Qin	PhD & Postdoc supervisor, LRIG Member	In-kind 3%

14 APPENDIX 3: Review of SOPs by ACAAR

Report appended as a PDF document.

Partridge, G., Tindale, A., 2010. A Review of Clean Seas Tuna's Yellowtail Kingfish Hatchery Procedures and Protocols. Australian Centre for Applied Aquaculture Research, Challenger Institute of Technology, Fremantle, Western Australia. 16pp. (Confidential document)