Evaluation of Cobia and Giant Groper production and health in multiple growout systems, as an alternative species to farm in WSSV affected areas of South East Queensland

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Abbreviations

SGR	Specific growth rate
SFR	Specific feed rate
eFCR	economic feed conversion ratio
bFCR	biologic feed conversion ratio
GHH	glycogenic hepatopathy and hepatomegaly
RPA	Rocky Point Aquaculture
BSL	Biosecurity Sciences Laboratory
TCO	The Company One
FFVS	Future Fisheries Veterinary Services
RAS	Recirculating aquaculture system
ORP	Oxidative reduction potential
DO	Dissolved oxygen
CV	Co-efficient of variance

Executive Summary

What the report is about

The report details the production performance and health performance of Cobia (*Rachycentron canadum*) and giant grouper (*Epinephelus lanceolatus*) reared in tanks, ponds and cages within a farm in the previously white spot syndrome virus affected region of the Logan River, Queensland.

Background

Cobia (*Rachycentron canadum*) and giant grouper (*Epinephelus lanceolatus*) are two highly valued aquaculture species. These two species were explored as potential candidates for aquaculture in the previously white spot syndrome virus affected regions in the Logan River, Queensland. The production and health performance of giant grouper and cobia in the climatic conditions of the Logan region had yet to be explored. Detailed performance data in various types of grow-out infrastructure had yet to be quantified.

Aims/objectives

1. Generate data on the production and health performance of Giant Grouper and Cobia in recirculating aquaculture system (RAS), cages in a saline lake and in outdoor pond growout systems.
2. Field test production outcomes from an experimental recombinant autogenous vaccine for prevention of nodavirus in the giant grouper.
3. Assess market potential for various formats of cobia and grouper

Methodology

Twelve farm visits were performed between December 2018 to Jan 2020 to investigate the health and production performance of giant grouper and cobia. Data was entered into Microsoft Excel spreadsheet.

Previous attempts at farming giant groupers in open pond systems in Australia have been impacted by high mortalities associated with nodavirus infections. A recombinant nodavirus vaccine was developed by Professor Andy Barnes at University of Queensland (FRDC Project 2018-101 Project 2018-098 - Vaccination for emergency and long-term control of nodavirus in Australian marine aquaculture) and produced with a commercial boutique vaccine manufacturer under an APVMA research permit. Production data of vaccinated and unvaccinated giant groupers were recorded to explore outcomes of vaccination.

At the conclusion of each trial run, the fish were sold into different markets to assess the potential of various formats of cobia and giant grouper.

Results/key findings

Findings of this report include:

- Vaccinated and unvaccinated giant groupers performed (FCR, SGR, SFR) similarly
- The experimental recombinant nodavirus vaccine appears to be efficacious in reducing mortalities and clinical signs in face of an outbreak.
- Giant groupers can be grown to market size in RAS tanks, cages in lakes, and ponds.
- Juvenile cobia can be ongrown in RAS tanks and grown out to market size in cages within a brackish water lake.
- Year round market placement and demand for the two species were identified.
- Cobia and giant grouper production could be achieved in the previously affected white spot syndrome virus regions in South East Queensland.

Implications for relevant stakeholders

A handbook outlining various disease/conditions/symptoms has been compiled from this project to assist producers in identifying and managing fish health and production problems that may be faced in the culture of giant grouper and cobia in land-based systems. This report and production data within will assist investors and stakeholders in appraising the opportunities for further development of cultured giant grouper and cobia. The findings will also assist in identifying potential future areas of research and development.

Recommendations

Additional research and development would be beneficial to improve production performance of stock through improving growth rate and reducing the food conversion rate. Potential future research projects include:

- Investigation into the cause and prevention of glycogenic hepatopathy and hepatomegaly in giant groupers.
- Investigation into causative factors for cobia slow growth syndrome and approaches to improve performance.
- Dietary optimisation of cobia and giant grouper amino acid, lipid, energy requirements.
- Strategies to reduce condition loss of cobia through winter.
- Exploring non-nutritional factors influencing the growth in giant groupers in ponds including pond design and feeding strategies.

Keywords

Health, production, cobia, *Rachycentron canadum*, giant grouper, *Epinephelus lanceolatus*, white spot syndrome virus, nodavirus, Logan River, Queensland

1 Introduction

The detection of white spot disease (WSD) caused by white spot syndrome virus in the Logan River in 2016 led to a disease eradication program which had profound impacts on the production of tiger prawns (*Penaeus monodon*) in the area. A WSD control zone within Morton Bay including the prawn farms along the Logan River is maintained and the disease continues to be a source of major concern for the Australian prawn farming industry. Diversification of species under culture is one strategy for risk reduction for farms in the disease zone.

Cobia (*Rachycentron canadum*) and Queensland giant grouper (*Epinephelus lanceolatus*) are two highly valued species that are emerging aquaculture species. Cobia are a pelagic fish that can be found in waters of the tropical Atlantic and Indo-Pacific with their endemic wild population range contiguous with South East Queensland. A sporadic wild harvest industry exists. Farmed production of cobia began in Taiwan in the 1990s and has since expanded to other countries including USA, Panama, China and Vietnam. Cobia are being farmed in ponds and sea cages successfully overseas. The production of cobia in Australia was explored in the previous FRDC funded project (2014-242) which found cobia to be a feasible species for production in ponds. FRDC Project 2014-242 highlighted the need on-farm veterinary disease investigation and health training to facilitate health management operations on farm to control production impacts from disease events.

The Queensland Giant Grouper are endemic in the Indo-Pacific region, including South East Queensland and can populate within estuaries and offshore reef. This species of grouper is a protected species in Queensland, New South Wales, Western Australia, and Northern Territory (any grouper greater than 120cm). Previous attempts to farm giant groupers in Australia have been adversely impacted by outbreaks of nodavirus associated with high mortalities in affected populations. An experimental nodavirus vaccine is being developed for the control of nodavirus in giant groupers. This project sought to evaluate how this vaccine would impact on the health and growth of giant groupers.

As an aquaculture species, giant grouper are known for their high value and rapid growth rates. Reliable seed stock production of giant groupers was initially developed in Taiwan. In Australia, The Company One has been successful in producing quality, reliable fingerlings for the giant grouper industry in Australia and overseas in Hong Kong, China and Taiwan. The fish are easy to handle and tolerate high stocking densities and perform across a wide range of salinities which are favourable characteristics for development as an aquaculture species in Australia.

Production of both giant grouper and cobia in Australia have previously not been performed in the Logan River region. As such, it is important to understand the production performance and health concerns these new aquaculture species may face. The domestic market for these species has yet to be fully explored. This report describes factors that may influence the success of the two species in the Logan River region and generates data to support the assessment of the suitability of other sites.

2 Objectives

1. Generate data on the production and health performance of giant grouper and cobia in RAS, cages in a saline lake and in outdoor pond growout systems.
2. Field test production outcomes from an experimental recombinant autogenous vaccine for prevention of nodavirus in the giant grouper.
3. Assess market potential for various formats of cobia and grouper

3 Method

3.1 Experimental Animal

Cobia larvae were sourced from Bribie Island Research Centre (BIRC). Giant grouper fingerlings were sourced from The Company One (TCO). Additional details of the stocking are detailed in Table 1 and Appendix 1.

Table 1. Source, stocking numbers, stocking date, and batch numbers of giant groupers and cobia stocked during the Nov 2018 to Nov 2019.

Species	System type	Number of separate systems	Source	Hatchery run number	Number of fish stocked	Stocking date into system
Grouper	Pond	2	TCO	1, 2	10,315	Nov – Dec 18
Grouper	Cage	4	TCO	1, 2	8,855	Nov – Dec 18
Grouper	Tank	5	TCO	3	9,617	Mar 19
Grouper	Tank	4	TCO	4	19,189	Jun 19
Grouper	Pond	2	TCO to RPA tank batch 4 to RPA pond	4	10,000	Sep 19
Grouper	Pond	1	TCO	5	19,200	Oct 19
Grouper	Cage	1	TCO to RPA tank batch 4 to RPA cage	4	5,256	Sep 19
Grouper	Cage	1	TCO	5	1,314	Oct 19
Cobia	Cage	3	BIRC 2018 run 1 through RPA tanks 2018 (prior to start of project) transfer to RPA cages 2018	1	4,535	Oct 18
Cobia	Tank	5	BIRC 2019 run 1	2	6,550	May 19
Cobia	Cage	4	BIRC 2019 run 1 through RPA 2019 run 1 tanks	2	6,059	Oct - Nov 19

3.2 General animal husbandry

3.2.1 System

Giant groupers and cobia were held in three different production systems (cage, pond, tank). Due to the lack of availability of cobia fingerlings, no cobia were stocked into ponds. Additional details of the stocking are detailed in Table 1.

3.2.1.1 Cage

Floating cage systems were placed into enclosed saline lakes. Water within the lakes is a combination of groundwater and marine water from the intake channel of the farm. No new water has been introduced onto the growout farm since the lake system was closed off. Additional aeration, paddlewheels and current movers are placed adjacent to the cages to promote water movement and water oxygen saturation. Cage dimensions are 9 x 9 x 2m (162m^3). Water exchange rates in ponds was approximately 10% water volume each day.

3.2.1.2 Pond

The pond systems dimensions are approximately 3000m^2 x 1.5m (4,500m^3). The pond systems intakes water from the lake system and discharges into the lake system. Paddlewheels are placed in the ponds.

3.2.1.3 Tank

Cobia and giant groupers were housed in repurposed prawn larval rearing parabolic tanks (20m^3 volume). These tanks are located in a separate location (hatchery) from the growout farm. The water temperature in the hatchery ranged between 22.1 - 31.0^{o}C depending on ambient water temperature and capacity of heating systems. Two separate hatchery systems with the same design were used in the trials. Water flow into and within the hatchery are as follows:

Incoming Water Treatment.

1. Incoming water is pumped up on the top of the tide into 3 storage ponds (about 900T) which are used alternately.
2. Chlorine is added at a rate of 30ppm (30mg L^{-1}) and the pond is left for a minimum of 48 hours prior to use.
3. Water is pumped from the storage ponds through 2 Waterco sand filters.
4. Water is pumped through 10 µm Seafar cartridge filters followed by 1 µm bag filters and 1 µm cartridge filters.
5. Water is then pumped into 3 x 34,000L header tanks and heated or cooled via electric heat pumps before being used in the various areas of the hatchery.
6. The filtration system, tanks and lines are chlorinated at 200ppm (200 mg L^{-1}) once per week.

Recirculating systems for fish rearing

1. Water gravity flows the tank outlets to a 48 µm drum screen filter and then into a sump.
2. The water is then pumped into a protein skimmer injected with ozone.
3. Water gravity flows into an aerated moving bed biofilter.
4. Water is pumped to return to the tanks via UV filtration and low head oxygen concentrators.
5. Water turnover rate in each System is 100% volume turnover per hour.
6. Water was buffered with daily soda ash addition to increase pH.
7. 10% of the system's water is exchanged daily and replaced.

3.2.2 Photoperiod

The photoperiod for each system was that of the normal day length. No artificial lighting was given to the systems.

3.2.3 Stocking density

Stocking density was calculated by dividing the biomass of the system by the water volume. The biomass was calculated from the monthly average weights. Initial fish densities are detailed in Table 2, Table 4, Table 5.

3.2.4 Feed usage

Giant grouper and cobia were fed commercial extruded pellets up to twice a day to satiation. Feed volume (kg) consumed, feed size, feed type were recorded daily in a Microsoft Excel spreadsheet. Feed was stored in either the growout farm shed or hatchery shed until required.

3.2.5 Mortalities

The number of mortalities removed were recorded in a Microsoft Excel spreadsheet daily. The survival of each fish population within each system was calculated at the end of the production cycle for that cohort. Mortalities were removed from tanks daily. In cages, floating mortalities were removed daily. Cage bottoms were swept 1-3 times/week to remove any mortalities. The cages were also dived at least once every two weeks to remove any mortalities at the bottom of the cage. In ponds, floating mortalities or mortalities near the edge of the ponds were removed daily.

3.2.6 Water quality

All water quality data were collected up to twice daily by RPA staff and recorded in a Microsoft Excel spreadsheet. For each system, temperature, dissolved oxygen, pH, salinity, nitrate, nitrite, ammonia data were recorded. Additionally, oxidative reduction potential (ORP) was recorded for all indoor RAS systems and secchi was recorded for outdoor systems (cages and ponds) daily. The typical water quality ranges of the different systems are detailed in Table 2. These ranges should not be necessarily considered to be optimal, rather they serve to inform the reader about what production performance was achieved under these water quality conditions.

Table 2. Typical water quality range parameters at the farm. Ponds production occurred between September - April

Water quality	*Cobia*	*Giant grouper*
DO	*Lake 3.5 - 5.3* *Tank 4.9 - 6.1*	*Pond 5.6 - 8.4* *Lake 3.5 - 5.3* *Tank 4.7 - 6.1*
Temperature	*Lake 15.9 - 31.2* *Tank 22.1 - 31.0*	*Pond 19.4 - 33.0* *Lake 15.9 - 31.2* *Tank 22.1 - 31.0*
pH	*Lake 7.1 - 7.7* *Tank 6.1 - 7.4*	*Pond 7.5 - 8.2* *Lake 7.1 - 7.7* *Tank 6.0 - 7.7*
Salinity	*Lake 25.4 - 30.4* *Tank 31.9 - 40.7*	*Pond 22.9 - 33.4* *Lake 25.4 - 30.4* *Tank 31.5 - 40.4*

3.3 Monitor the health status of giant grouper and cobia under commercial culture conditions using different production systems

The trial sought to document and investigate diseases and production syndromes of giant grouper and cobia through structured monthly health and production observations at a SE Queensland farm over a one year period in parabolic tanks, cages in saline lakes and ponds.

Skin mucus and gill biopsy samples were collected from approximately 6 fish from each cage/tank/pond each visit and examined microscopically for evidence of disease. Each fish was anaesthetised with AQUI-S which was titrated to effect prior to examination. An approximate rate of

0.01mL AQUI-S L^{-1} water (5.4mg isoeugenol L^{-1} water) was required to anaesthetise both giant groupers and cobia. Sick or poor performing fish were targeted for sampling for laboratory diagnostics (histology, virology, microbiology) provided by BSL. Findings were reported in monthly health reports which are appended to this final report.

Additionally, data collected from monthly reports were compiled to develop a health management handbook for cobia and giant groupers in different growout systems. The frequency of detection and potential control, monitoring, biosecurity, treatment strategies for the conditions are outlined in the health management manual.

3.4 Performance Monitoring

The trial sought to compare the production performance of giant grouper and cobia in three different aquaculture systems: ponds, cages in a saline lake, and indoor tanks on a heated RAS. The SGR, SFR, FCR, survival (performance indicators) of each system was recorded and compared. The final harvest weight and feed consumption data was used to calculate each performance indicator where available. Where the final harvest weight and feed consumption is not available, the performance indicators were calculated off the most recently available average weight obtained through monthly weight check monitoring.

Weight checks were performed monthly on each system. The average weight and length of the fish was collected monthly from a sample size of approximately 20-60 fish. The co-efficient of variance (CV) of length, weight and condition index was calculated and a distribution curve was generated. The condition index of the fish was calculated from length and weight of the fish sampled. Prior to weighing, fish were sedated with AQUI-S to an approximate rate of 0.005mL AQUI-S L^{-1} water (2.7mg isoeugenol L^{-1} water). Once sedated, individual weight and tail lengths were collected. Results were transferred to Microsoft Excel and an average weight, length, and condition factor was calculated. Monthly FCR, SFR, SGR were calculated with the average weight obtained during the month. Results and trends were documented in each monthly health report. See Appendix 1 for additional production performance details.

3.4.1 Evaluate production performance and fish health of giant grouper within indoor tank RAS, free-ranging outdoor pond, cage systems in a saline lake:

Ponds, cages in a saline lake, and indoor tanks on a heated RAS were stocked during the trial. Timing of the stockings was constrained by timing of fingerling availability from the hatchery. All giant grouper were sourced as fingerlings from TCO. All cages, ponds and tanks were fed commercially available extruded pellets to apparent satiation by hand by farm staff up to twice a day (0600 – 0800 and 1500-1700). None of the giant grouper systems were graded once stocked. Each cage and pond were farmed until they were fully harvested (or transferred to winter growout cages). Below market size fish (<600g) in cages and ponds transferred to winter growout cages over winter and were considered as harvested fish. These fish were weighed prior to transfer. In tanks, giant groupers were kept in heated RAS systems over winter.

The final production performance of giant groupers in various systems was document and the production performances of each system was calculated and compared to one another.

The production performances of the giant groupers were recorded at each weight check. Additionally, due to the weight size differences at stocking between cohorts of giant groupers in different systems, comparisons between systems were made from weight check data of fish of an average weight of approx. 130-150g to approx. 500g. Data from fish which were not above an average weight of 500g at the conclusion of the trials or which were stocked at an average weight above approx. 150g were excluded from the analysis.

2019

A total of 10,315 fish were stocked into two ponds in the 2019 season. Stocking of ponds occurred during Spring and Summer as water temperatures were suitable for growth of giant groupers. 5,600 unvaccinated giant groupers of 155g average weight were stocked into Pond 6 on 3/11/2018 (batch 1). 4,715 vaccinated giant groupers of 157g average weight were stocked into Pond 9 on 19/12/2018 (batch 2).

Stocking of cages occurred during Spring to Summer as water temperatures were suitable for growth of groupers. A total of 8,855 fish were stocked into four cages in the 2019 season. 303 unvaccinated giant groupers of 155g average weight were stocked into Cage 1 on 3/11/2018 (batch 1). A total of 8,552 vaccinated giant groupers were stocked into Cages 7, 8, 9 at 139g, 130g, 248g respectively on 8/12/2018 (batch 2), 8/12/2018 (batch 2), 4/12/2018 (batch 1) respectively.

Two separate batches of giant groupers were stocked into parabolic tanks. Stocking of tanks occurred during Autumn to Winter as water temperatures in the lake and ponds decreased. Stocking occurred on 14/3/2019 (batch 3), and 18/6/2019 (batch 4) and 22/6/2019 (batch 4).

In batch 3, a total of 9,617 fish were stocked in the 2019 season. Vaccinated and unvaccinated fish were mixed amongst the tanks in this cohort. 743 giant groupers of 48g were stocked into Tank 3 on 14/3/2019. A total of 8,874 giant groupers were stocked into Tanks 4, 5, 6, 7 with 2,206, 2,202, 2,234, 2,200 fish respectively at 41g on 14/3/2019.

In batch 4, a total of 19,189 fish were stocked in the 2019 season. 3,845 unvaccinated giant groupers of 30.5g were stocked into Tank 12 on 16/6/2019. Vaccinated giant groupers were stocked into Tanks 13, 14, 15 with 5255, 4703, 5386 fish respectively on 22/6/2019 at 32g.

2020

In the 2020 season, batch 4 fish were transferred to growout farm. Fish were stocked into Cages 6 and 7, and Pond 12. Cage 6 was stocked with 5,256 vaccinated giant groupers at 93g on 17/9/2019. Pond 12 was stocked with 10,000 vaccinated giant groupers at 93g on 17/9/2019. Upon stocking, all fish in Cage 7 escaped through a hole in the netting. Cage 7 was stocked with additional fish from TCO with fish from batch 5. 1,314 unvaccinated giant groupers were stocked into Cage 7 at 109g on 15/10/2019. Pond 6 was stocked with 10,499 vaccinated giant groupers at 79g (batch 5). Pond 9 was stocked with 8,701 unvaccinated giant groupers at 90g (batch 5).

3.4.1.1 Evaluate performance of giant grouper that have been vaccinated using an experimental autogenous recombinant nodavirus vaccine.

Additional to the assessment of the performance of giant groupers in different systems, the effects of the experimental nodavirus recombinant vaccine on the production performance of giant groupers were evaluated concurrently in batches 1-5. The final production performance of giant groupers in various systems with various vaccination status was document and compared to one another to assess the effects of vaccination on production of giant groupers. This data was collected in conjunction with “Project 2018-098 - *Vaccination for emergency and long-term control of nodavirus in Australian marine aquaculture*” which will report separately with more detail on the outcomes of vaccination.

The vaccination status of giant grouper was divided into three separate groups. These are vaccinated, unvaccinated and mixed. All vaccinated giant groupers were vaccinated with an experimental nodavirus recombinant vaccine at TCO as fingerlings of approximately 20 - 50g. Unvaccinated giant groupers did not receive any vaccine or placebo. Mixed vaccinated cohort consisted of vaccinated and unvaccinated giant groupers. The mixed vaccination status cohort arose through an error in transport packing and were excluded from the subsequent performance comparison.

3.4.2 Evaluate production performance and fish health of cobia within indoor RAS, open outdoor cage systems in a saline lake:

Cages in a saline lake, and indoor tanks on a heated RAS were stocked during the trial. Insufficient fingerling supply precluded the stocking of cobia into ponds, so data is unavailable for that species within that growout system. All fish were sourced from Bribie Island Research Centre as larvae and grown to approx. 200-300g in size in parabolic tanks over winter prior to being transferred to outdoor growout cages in saline lakes. Each cage was farmed until they were fully harvested. Below market size fish (<4kg) were transferred to winter growout cages over winter and were considered as harvested fish for the purpose of the trial. Cages were stocked during Spring to Summer as water temperatures were suitable for cobia growth. Tanks were stocked during Autumn and Winter as water temperatures decreased.

The final production performance of cobia in various systems was document. Due to the size class difference of the systems and the lack of stock availability from BIRC, comparisons between different production performance of the systems could not be made. However, the production performance trends, and production strategies are detailed.

A total of 3 cages and 5 tanks were stocked in the 2019 season and 4 cages were stocked in the 2020 season. In 2019, Cages 4, 5, 6 were stocked with 1266, 1636, 1633 cobia fingerlings respectively of 177g, 312g, 305g respectively on 10/10/2018. Two separate stockings of cobia occurred in the hatchery tanks these are on 14/5/2019 and 16/7/2019. Both stockings were from fish from the same hatchery cohort. The 16/7/2019 stocking occurred as there was an excess of trial fish available from BIRC. During the 14/5/2019 stocking, 3 tanks were stocked. These were Tanks 8, 9 ,10 which were stocked with 1,500, 1,400, 1200 cobia larvae respectively of 2.2g, 1.2g, 1.2g size respectively. During the latter stocking, an additional tank (Tank 11) and additional fish were added into Tank 10. An additional 300 fish of 42g size were added to Tank 10. Tank 11 was stocked with 1350 cobia fingerlings of 42g. On 26/9/2019, 200 fish from each cobia tank (800 fish total) was transferred to a separate tank (Tank 12) to reduce stocking density in the tanks. These fish were estimated to be 189.8g at the time of transfer.

3.4.3 Calculations

SGR = ln([Final average weight (g)] / [Initial average weight (g)]) / [number of days in production]*100
SFR = ([Cumulative sum of daily feed volume (kg)] / [number of days in production]) / (([Initial biomass (kg) + [Harvested Biomass (kg)])/2))
eFCR = [Cumulative sum of daily feed volume (kg)] / ([Harvested Biomass (kg)] – [Initial biomass (kg)])
bFCR = [Cumulative sum of daily feed volume (kg)] / ([Harvested Biomass (kg)] – [Initial biomass (kg)] + [Cumulative Mortality biomass (kg)])
Stocking density = [Biomass (kg)] / [water volume (m^3)]
Survival = 100% - ([Cumulative sum of daily mortality count] / [initial stocking number] *100%)
Condition index = ([Body weight (g)] / [length (cm)^3]) * 100

3.5 Marketability and seasonal supply of cobia and giant grouper

The marketability of cobia and giant grouper and the ability of indoor and outdoor aquaculture systems to be stocked and managed to achieve supply to markets year-round were assessed by farm staff.

The acceptance of cobia and giant grouper supplied to markets were monitored by farm staff to estimate the potential market for these species. Different sized fish and different forms of processed products were trialled for this purpose.

Results achieved from indoor and outdoor production trials were used to assess the development of production systems to allow year-round production of market size fish.

4 Results and Discussion

The final production data for giant groupers and cobia is detailed in Appendix 1. Analysis of production performances and fish health are detailed in Appendix 2-8 and in the handbook. A summary of the health reports are detailed in Appendix 9.

The on-farm data suggests that cobia and giant groupers are viable species for farming in the South East Queensland region. No pond data for free-ranging cobia are available as a lack of fingerling supply prevented completing this aspect of the study.

4.1 Significant diseases of concern

The list of disease, syndromes noted in the surveillance program are detailed below in Table 3. Additionally, a brief summary of the conditions and disease seen are described below.

Table 3. List of disease/pathogens/syndromes observed in the project period at Rocky Point Aquaculture.

Disease/pathogen/syndrome	Seen in giant grouper during the project	Seen in cobia during the project	System type
Viral nervous necrosis disease	Yes	No	Cages
Trichodinosis	Yes	Yes	Cages, Ponds
Brooklynellosis	No	Yes	Tanks
Vorticella	Yes	Yes	Tanks
Leech	Yes	No	Cages, Ponds
Gill fluke	Yes	No	Cages
Gas bubble disease	No	Yes	Cages
Gill necrosis (gill foul)	No	Yes	Cages, Tanks
Exophthalmos (pop eye)	No	Yes	Tanks
Cloudy eyes (cataract)	No	Yes	Cages
Melanisation of viscera in vaccinated giant groupers	Yes	No	Cages, Ponds, Tanks
Epizoic diatom	No	Yes	Cages
Deformity	Yes	Yes	Cages, Ponds, Tanks
Glycogenic hepatopathy and hepatomegaly in giant groupers	Yes	No	Cages, Ponds, Tanks
Cobia slow growth syndrome	No	Yes	Cages
Granulomatous syndrome in cobia	No	Yes	Cages
Gastric bloat	No	Yes	Cages

4.1.1 Glycogenic hepatopathy and hepatomegaly

Hepatomegaly (enlarged liver) was found in 100% of farmed giant groupers necropsies (Figure 1). Figure 3 illustrates the normal size of a grouper liver for comparison, in a wild fish on a natural diet. Histology of the aquaculture reared giant grouper livers of these fish show enlargement of liver cells with severe accumulation of glycogen (glycogenic hepatopathy) and some lipid, with no infectious causes evident across multiple laboratory submissions (Figure 2).

The liver is responsible for metabolism of proteins, carbohydrates and lipids. It makes of blood proteins, forms and secretes bile to facilitate the digestion of lipids in the diet. The liver also plays a primary role in the elimination of some toxicants through cellular processing and excretion via faeces.

Glycogenic hepatopathy and hepatomegaly (GHH) may contribute to increased morbidity and reduced growth performance through impairment of some or all of the above listed metabolic roles of the liver. The cause of this condition is yet to be elucidated and warrants additional targeted research.

Potential causes include: fish meal substitution with plant proteins (Ye et al., 2019), inclusion of excessive carbohydrates in the diet (Castillo et al., 2018; Gao et al., 2019; Luo et al., 2016; Wang et al., 2017), glycaemia (Castillo et al., 2018), dietary starch type (Ismail et al., 2018), substitution of fish meal with animal based proteins (Ye et al., 2019), diet entrained toxins (e.g. pesticide residues like glyphosate and AMPA) (Mesnage et al., 2019) anti-nutritional compounds (Roehm et al., 1970).

Figure 1. Hepatomegaly in a juvenile farmed giant grouper

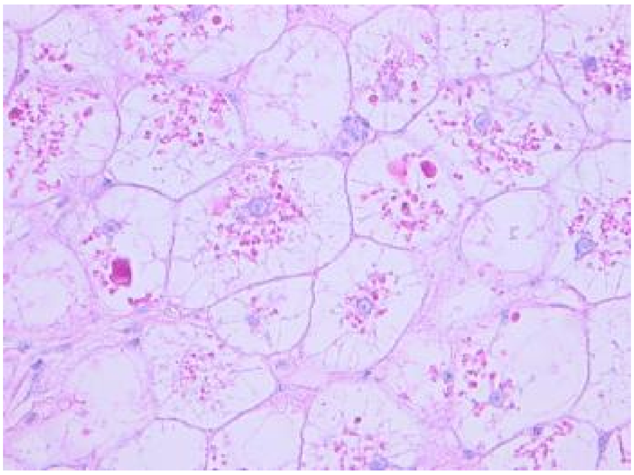


Figure 2. Histological section of the liver stained with PAS stain (x400 magnification) – multiple red (PAS positive), intracytoplasmic glycogen granules (Source: BSL – Dr. Ian Anderson)

Figure 3. The liver size of wild grouper.

4.1.2 Nodavirus

Nodavirus is a significant disease that can cause significant stock loss in farmed giant grouper. Nodavirus is responsible for the clinical disease viral nervous necrosis disease (VNN). The disease has been reported in more than 50 species of fish including groupers and cobia. This virus is a β-nodavirus that is non-enveloped, spherical, 25nm diameter sized with two segments of single-stranded RNA.

Eradication of nodavirus within a farm is challenging as wild fish populations can act as asymptomatic reservoirs for the disease and it is difficult to sanitise the high volumes of water needed for growout cost-effectively. Nodavirus is stable outside the host and can survive for extended durations which can be influenced by environmental temperatures. At 25 °C, nodavirus persisted in cell cultures for at least 4 weeks and potentially up to 2 months (not detected after 3 months). At temperatures below 15 °C, nodavirus persisted for more than 1 year (Frerichs et al., 2000).

Nodavirus is usually transmitted horizontally (from one fish to another fish) in water, and occasionally vertically (from broodstock to fry). Once infected with nodavirus, the virus infects its target organs (mainly brain, spinal cord, retina) and replicates resulting in extensive vacuolations. Intracytoplasmic inclusion bodies may be observed. Fish affected with VNN show signs of blindness, lethargy, erratic behaviour/movement, corneal opacity, hyper-buoyancy, skin colour changes and mortalities. Stock loss upwards of 100% can be seen in populations affected by VNN and was observed at the study farm in previous attempts at giant grouper culture.

An experimental recombinant nodavirus vaccine was developed by University of Queensland. This vaccine appears to be efficacious in reducing the clinical symptoms and mortalities in face of a nodavirus outbreak. Survival of approximately 80% was observed in the lake caged vaccinated populations during the 2019 clinical outbreaks of the disease (Figure 4). Previous outbreaks in unvaccinated stock had led to much lower survival around 29% (however, in systems where stock were too small for harvest, survival was 0%). However, it must be noted that survival was calculated based on the number of mortalities removed from the systems and do not take into account clinically affected fish that were emergency harvested and not tallied as a mortality. It was noted by the farm that 100% of the unvaccinated populations were affected (i.e. showing clinical signs) by nodavirus in the previous season prior to the commencement of this project. Very high mortalities have also been anecdotally reported from nodavirus outbreaks in other giant grouper farming trials in ponds in north Queensland in previous years.

These outbreaks correlated with prolonged exposure to low dissolved oxygen and subsequently with an acute stressor from an equipment cage side fire which was extinguished by extinguisher. The unvaccinated and mixed fish were held in different locations, due to insufficient cage infrastructure within the one area. These stock did not appear to suffer from a clinical disease outbreak, in contrast to the site where vaccinated stock were held.

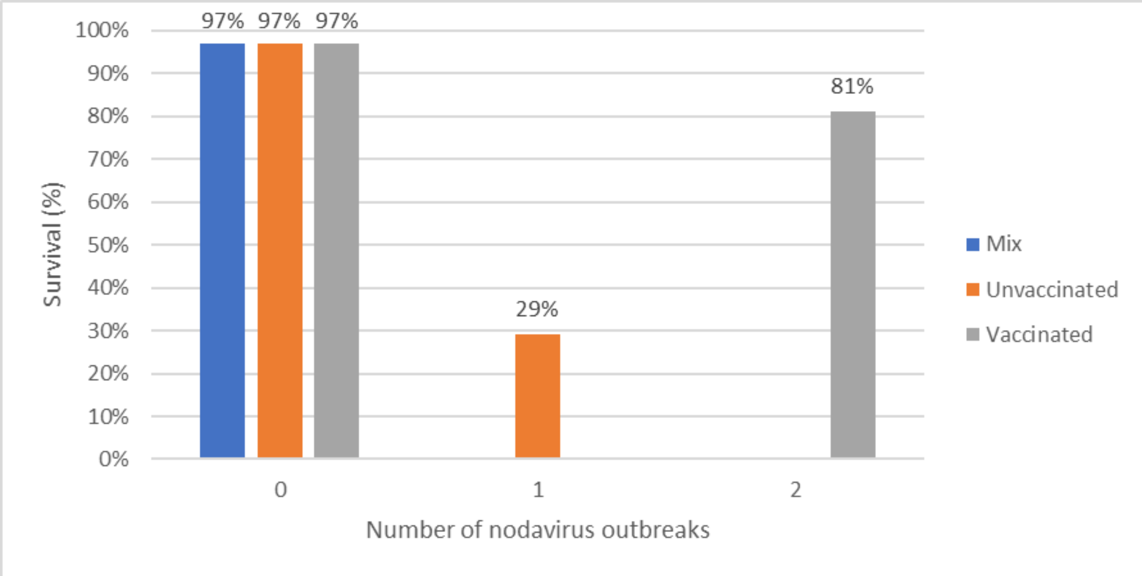


*Figure 4. Survival of vaccinate and unvaccinated giant groupers. *Note: harvested fish that were clinically affected were considered as fish that survived until harvest.*

4.1.3 Cobia slow growth syndrome

Cobia affected with slow growth syndrome grow significantly slower than their counterparts and are usually shorter, lighter and are in poorer body condition score compared to the normal sized fish in the population. These fish are of economic significance as they take significantly more time (months) to reach market size. Approximately 30% of the lower grade fish were affected by this condition from the first run of cobia in 2018. The 2019 cohort were not able to be classified due to stage of their production at conclusion of project.

This condition was more prevalent in one cage in the 2018-2019 season. The affected cage consisted of lower grade fish from the hatchery. Pathology of affected fish did not reveal any obvious consistent tissue aberrations which could explain the reduced growth or indicate that the condition was of an infectious origin. At necropsy a small proportion (approximately 8%) of affected fish had signs of gastric dilation with enlarged flaccid stomachs with loss of rugal folds.

Exploration of factors such as tank rearing conditions, genetics, feeding methods and grading should be considered.

4.2 System performance

4.2.1 Growth of giant groupers in different systems

The growth of giant groupers to market size (>600g) was achieved in ponds, cages and tanks. However, the production of giant groupers showed wider variance between ponds, than was evident between cages or tanks. The cause of variance is unclear as the poorer performing pond was from the same hatchery cohort as the vaccinated caged giant groupers which grew faster.

The SFR (specific feed rate) and SGR (specific growth rate) was lowest in the ponds (Table 4, Figure 5, Figure 6). Ponds also had the highest bFCR (biological feed conversion ratio, Figure 8). In the cages, the eFCR (economical feed conversion ratio) (Figure 7) and SFR was the highest (Figure 5). The bFCR in cages was lower than that of ponds (Figure 7). The best (lowest) FCR were seen in the tank populations which also had the highest SGR, which largely relates to the smaller sized fish in the tanks.

Comparisons are made between systems for the period of culture where fish of common sizes were present as there was no comparison population of the same weight range fish in the different production systems. Table 5 shows the start and finish weight of stock in various system types where similar size classes of fish are present in each system. The SFR, SGR, FCR, survival of giant groupers were recalculated in Table 4 (see Table 5 for overall production performances).

From the recalculated production performances of similar sized fish, the best FCR (lowest) was seen in the 2020 cage population, however it is important to note that significant infrastructure improvements had been made in 2020. The best FCR in 2019 was seen in the tank populations. The tank populations also required more days in production than the other two systems to produce 500g fish. SGR and SFR of the tanks were the lowest of the three system types. The best (highest) SGR was seen in the cage populations. The cage populations had higher SGR and SFR than the pond and tank populations. The FCR of cages was higher than that of ponds. The standard deviation (SD) of the tanks was higher than the pond and cage populations as the fish were stocked at smaller sizes (approx. 40g) and were never graded since the initial stocking into other systems. This differs from the cage and pond populations as they were graded at hatchery until they were approx. 130-150g when they were transported to the receiving farm. The cage population has the lowest survival of the three systems as they were the only system that experienced outbreaks of nodavirus during the trials.

Low stocking densities have been associated with poorer growth rates and FCR in other *Epinephelus* groupers (Agus et al., 2014; Shao et al., 2019). One trial cage population which had low stocking density did have higher SFR and FCR compared to other cage populations, however it had similar SGR. This cage was stocked with larger fish which may explain some of the variation. The stocking density in ponds was much lower than tanks and cages, and experienced lower SGR. Within each production system there was no obvious effect of stocking density on growth suggesting maximal densities had not been exceeded within tanks, ponds or cages (see Cage 1 in Appendix 2).

The coefficient of variance (CV) for weight, length and condition factor was highest in the tank populations (Figure 9, Figure 10, Figure 11). The tank populations were stocked at a smaller size and were not graded at any time during the trial which has likely resulted in the elevated CV in the tank populations. Giant grouper are yet to have substantial domestication breeding programs established, which may help reduce size variation with cohorts. More frequent feeding and grading may assist in reducing the variation in size in the tank cohorts, however trials to test this are yet to be conducted.

Table 4. Average growth performance of giant grouper in different systems

Giant grouper	**Cage**	**Pond**	**Tank**
Number	4	2	9
Average Start weight (g)	169.00 ± 46.46	156.00 ± 1.00	37.61 ± 5.76
Average End weight (g)	996.76 ± 215.21	849.49 ± 250.51	378.19 ± 252.69
Average farming period (days)	175.25 ± 25.87	210.00 ± 21.00	171.33 ± 73.74
SFR	1.50 ± 0.51	0.79 ± 0.11	0.93 ± 0.40
SGR	1.03 ± 0.13	0.78 ± 0.07	1.18 ± 0.09
eFCR	1.73	1.59	1.19
bFCR	1.53	1.59	1.18
Survival (%)	84.9	99.95	96.4
Max Stocking density (kg m^{-3})	9.26 ± 4.21	0.76 ± 0.14	37.59 ± 20.97
Average fish density (fish m^{-3})	12.99 ± 6.57	1.14 ± 0.52	158.74 ± 78.28

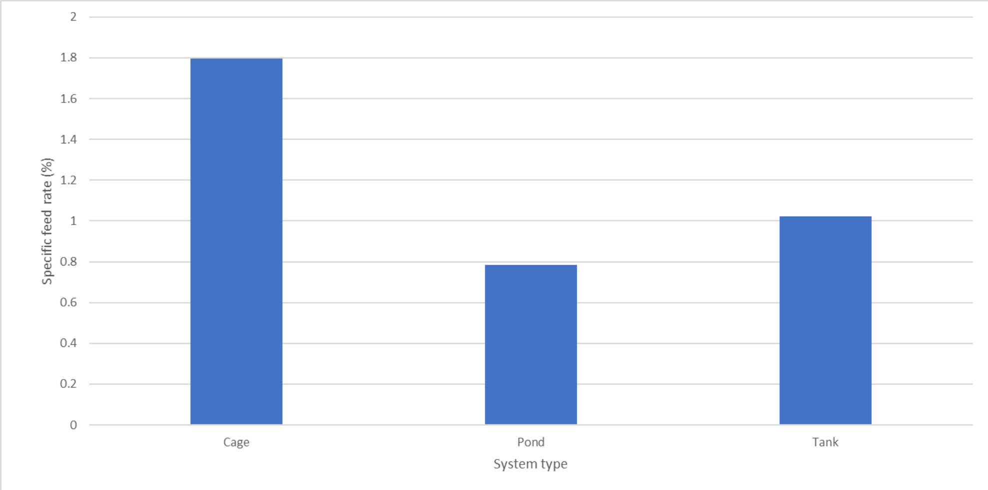


Figure 5. Specific feed rate of giant groupers cultured in different production systems

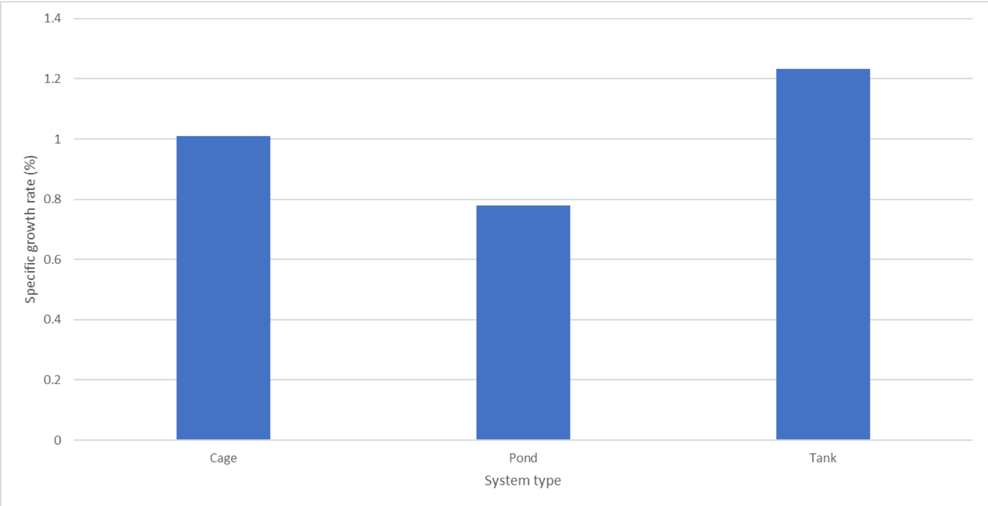


Figure 6. Specific growth rate of giant groupers cultured in different production systems

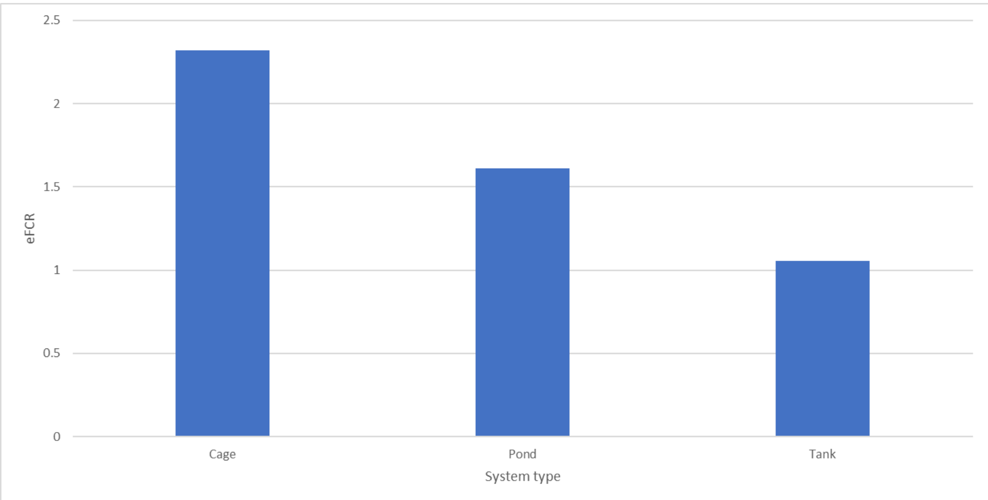


Figure 7. eFCR of giant groupers cultured in different production systems

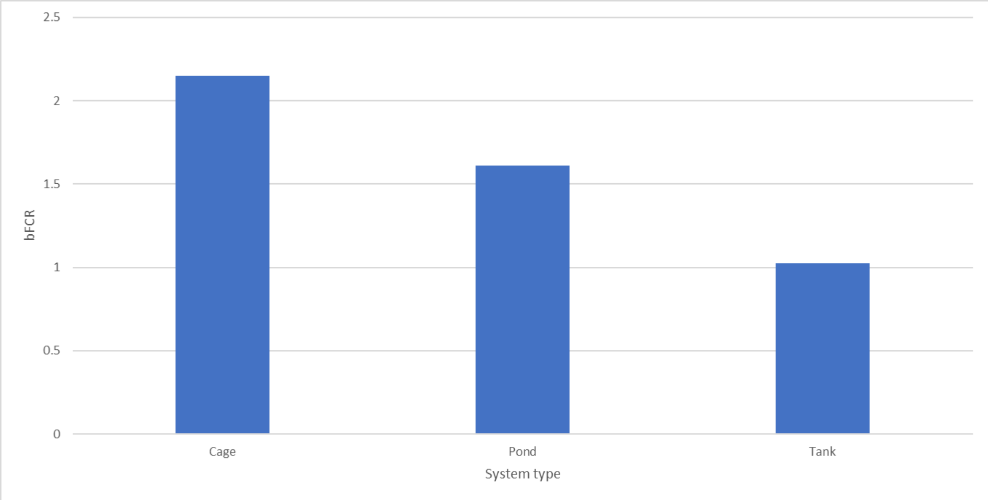


Figure 8. bFCR of giant groupers cultured in different production systems

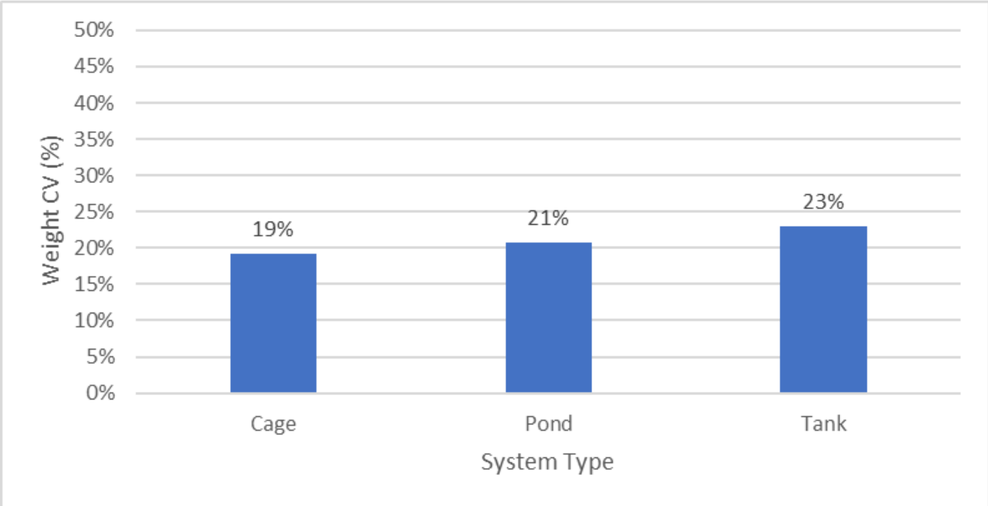


Figure 9. Co-efficient of variance for weight (g) of giant groupers cultured in different production systems

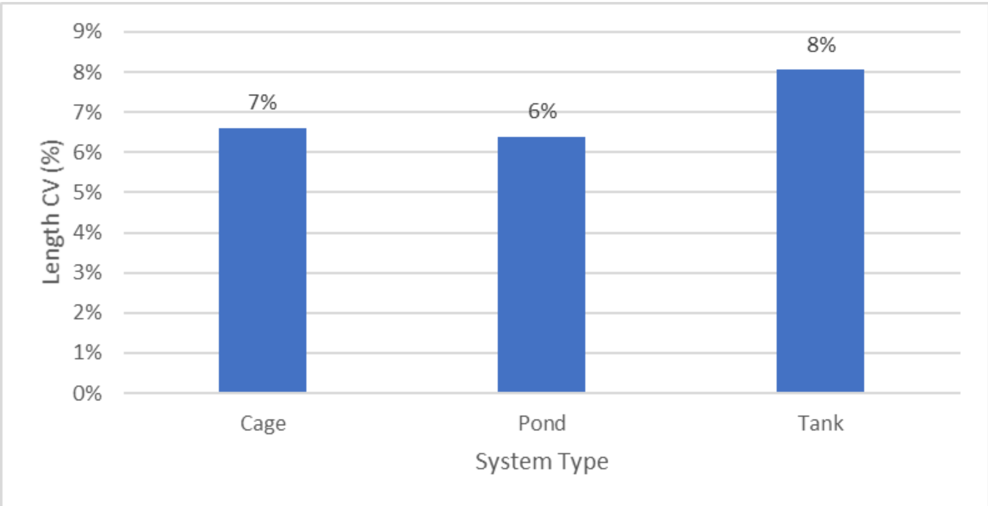


Figure 10. Co-efficient of variance for length of giant groupers cultured in different production systems

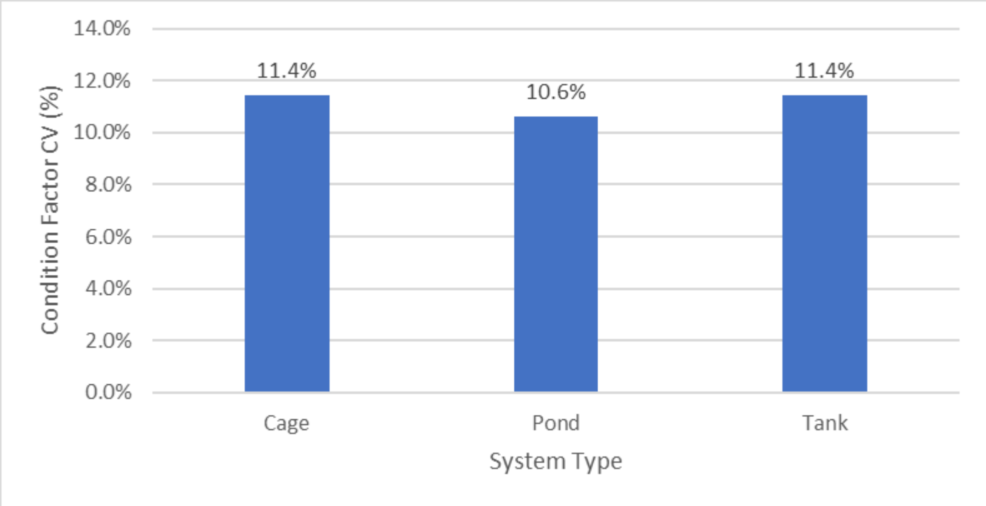


Figure 11. Co-efficient of variance of condition factor of giant groupers cultured in different production systems

Table 5. Growth performance of approx. 130-150g fish grown to approx. 500g in different systems

	Pond 2019	Tank 2019	Cage 2019	Cage 2020
Numbers	2	5	2	1
Starting weight (g)	156	128	135	137
End weight (g)	514	533	515	533
Starting numbers	10315	7265	5614	5256
End weight SD (g)	96	149	80	138
Production time (days)	86	154	82	120
FCR	1.35	1.17	1.41	0.91
SGR	1.39	0.93	1.63	1.13
SFR	1.68	0.93	1.86	0.90
Survival (%)	100.00%	99.99%	88.28%	99.92%

4.2.2 Growth performance of vaccinated and unvaccinated giant groupers

The growth performance of the vaccinated and unvaccinated groupers was similar to one another (Table 6). The SFR of giant groupers in cages, ponds and tanks was higher in the unvaccinated cohort than the vaccinated cohorts (Figure 12). This difference in feed rate did not always correlate to a better (higher) SGR (Figure 13). The FCR was highest (i.e. least efficient food conversion) in the unvaccinated cage cohort (largest sized fish stocked at a low density) (Figure 14, Figure 15). The lowest FCR was noted in the tank cohorts which performed similarly to one another (Figure 7.14, Figure 15). In Atlantic Salmon IP vaccines have been associated with reduced growth performance (Midtlyng et al., 1998). One difference in the growth performance of the unvaccinated compared to the vaccinated giant groupers was with the co-efficient of variance (CV). The CV of the length, weight, and condition factor of vaccinated giant groupers was on average higher than the CV of unvaccinated giant groupers (Figure 16, Figure 17, Figure 18). It remains plausible that the vaccine may have some production impact on a small proportion of animals in the cohort, which needs to be weighed against the benefit of disease protection of the population.

*Table 6. Production performance of vaccinated and unvaccinated giant groupers in different production systems. *Unvacc: unvaccinated; Vacc: vaccinated; Mixed: mix of vaccinated and unvaccinated fish*

System type	**Cage**		**Pond**		**Tank**		
Vaccine status*	**Unvacc**	**Vacc**	**Unvacc**	**Vacc**	**Mixed**	**Unvacc**	**Vacc**
Number	1	3	1	1	5	1	3
Average Start weight (g)	155.00 ± 0.00	173.67 ± 52.83	155.00 ± 0.00	157.00 ± 0.00	42.40 ± 2.80	30.50 ± 0.00	32.00 ± 0.00
Average End weight (g)	1300.00 ± 0.00	895.68 ± 144.51	1100.00 ± 0.00	598.97 ± 0.00	603.07 ± 33.24	108.00 ± 0.00	93.47 ± 5.35
Average farming period (days)	217.00 ± 0.00	161.33 ± 10.84	231.00 ± 0.00	189.00 ± 0.00	237.20 ± 4.58	93.00 ± 0.00	87.67 ± 0.94
SFR	2.39 ± 0.00	1.20 ± 0.01	0.90 ± 0.00	0.67 ± 0.00	0.77 ± 0.06	1.19 ± 0.00	1.11 ± 0.02
SGR	0.98 ± 0.00	1.04 ± 0.15	0.85 ± 0.00	0.71 ± 0.00	1.12 ± 0.02	1.36 ± 0.00	1.22 ± 0.07
eFCR	2.98 ± 0.00	1.66 ± 0.27	1.56 ± 0.00	1.66 ± 0.00	1.12 ± 0.18	1.03 ± 0.00	1.02 ± 0.07
bFCR	2.85 ± 0.00	1.45 ± 0.16	1.56 ± 0.00	1.66 ± 0.00	1.12 ± 0.18	0.97 ± 0.00	0.98 ± 0.06
Survival (%)	96.0 ± 0.0	80.9 ± 6.9	99.9 ± 0.0	100.0 ± 0.0	97.5 ± 1.0	93.7 ± 0.0	96.4 ± 0.3
Max Stocking density (kg m^{-3})	1.98 ± 0.00	11.69 ± 0.17	0.89 ± 0.00	0.62 ± 0.00	49.86 ± 15.60	19.39 ± 0.00	23.21 ± 2.09
Average fish density (fish m^{-3})	1.75 ± 0.00	16.73 ± 1.23	1.24 ± 0.00	1.05 ± 0.00	93.95 ± 29.01	191.95 ± 0.00	255.67 ± 14.82

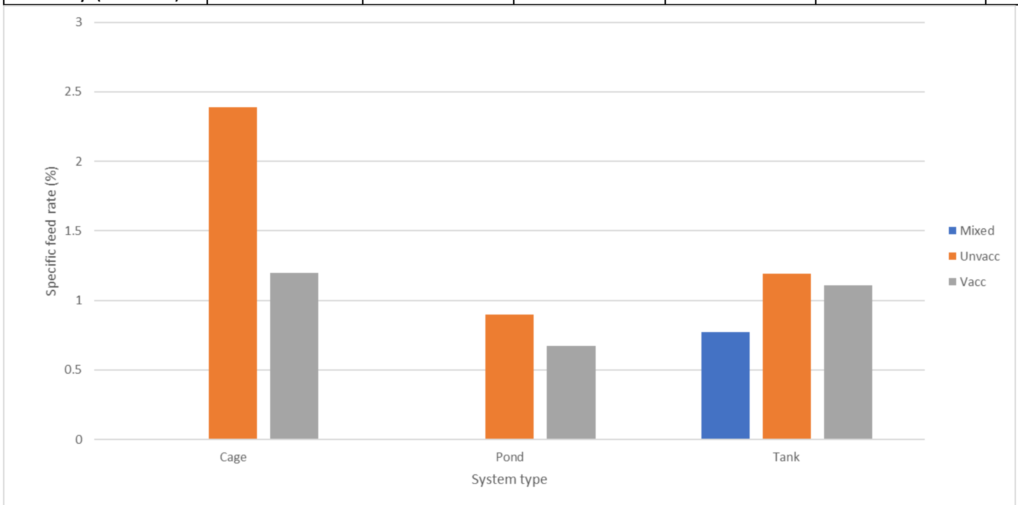


Figure 12. Specific growth rate of giant groupers with different vaccination status in different systems

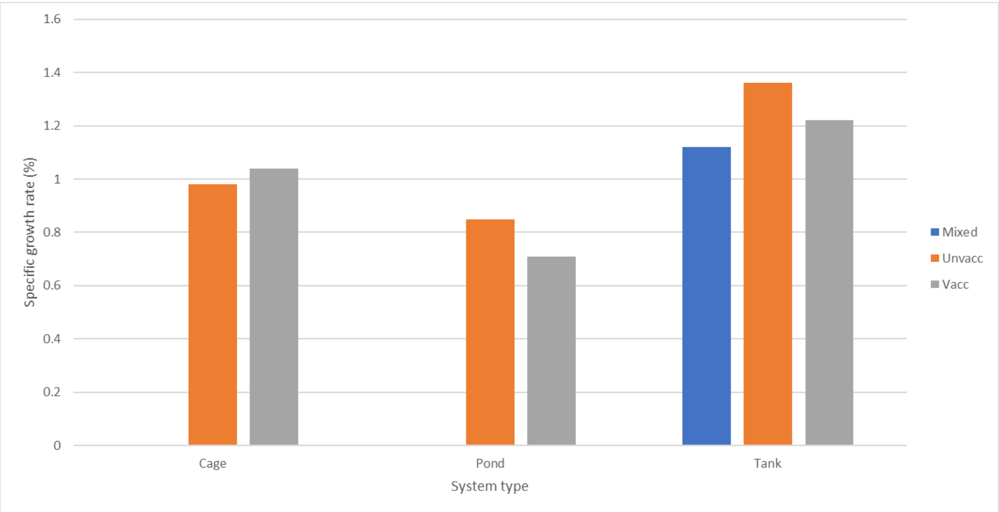


Figure 13. Specific growth rate of giant groupers with different vaccination status in different systems

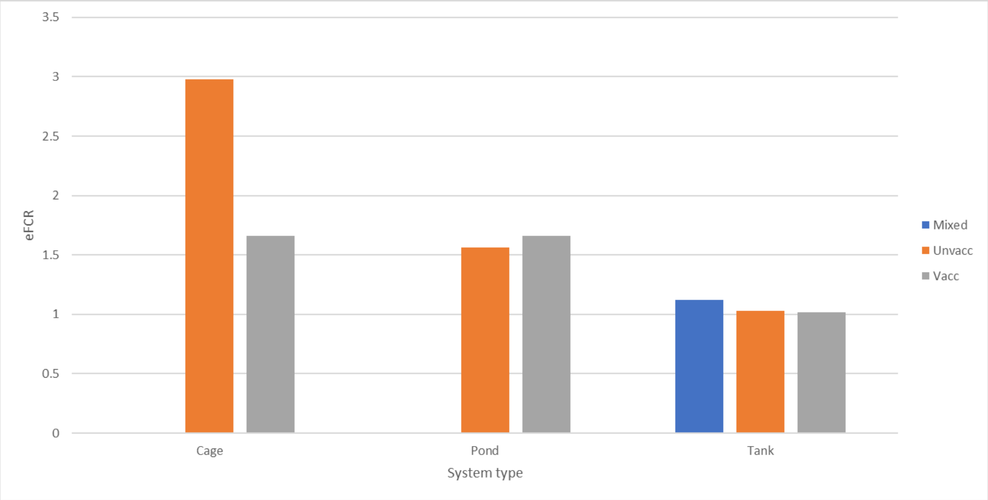


Figure 14. Economic feed conversion ratio of giant groupers with different vaccination status in different systems

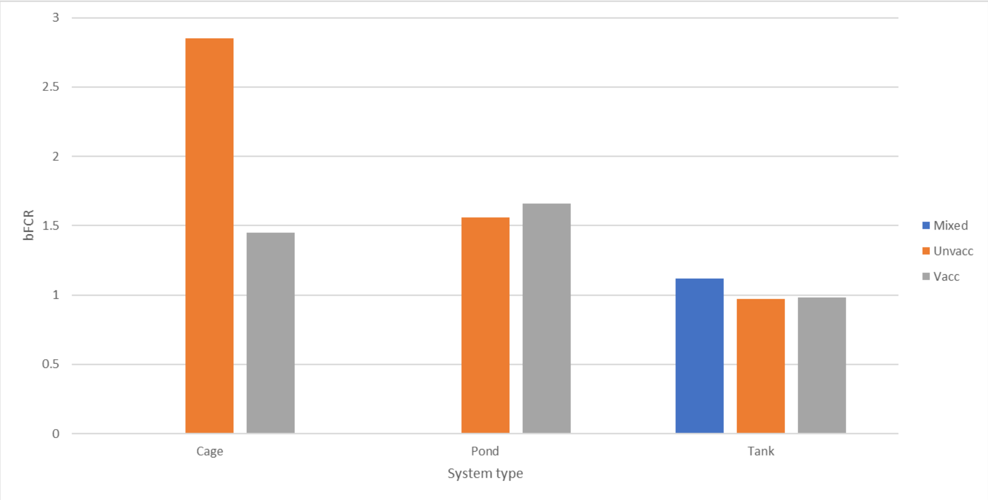


Figure 15. Biological feed conversion ratio of giant groupers with different vaccination status in different systems

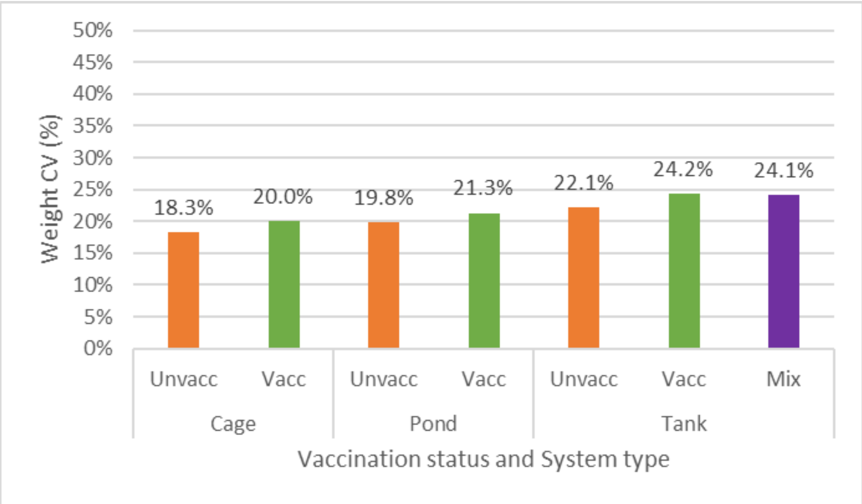


Figure 16. Co-efficient of variance between weight of vaccinated and unvaccinated giant groupers in different production systems.

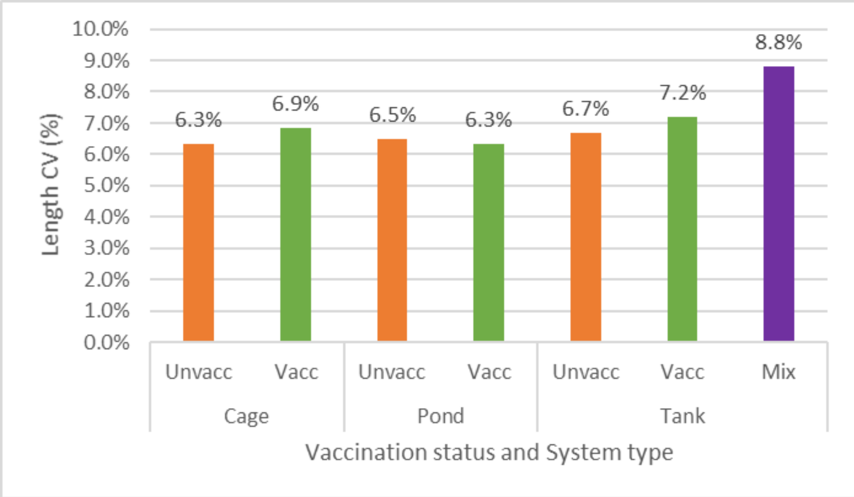


Figure 17. Co-efficient of variance between length of vaccinated (yes) and unvaccinated (no) giant groupers in different production systems.

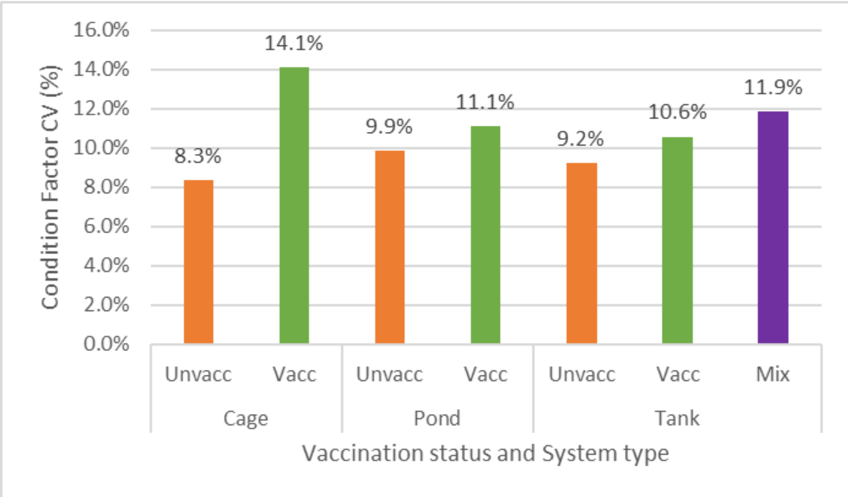


Figure 18. Co-efficient of variance between condition factor of vaccinated (yes) and unvaccinated (no) giant groupers in different production systems.

4.2.3 Growth of cobia in different culture systems

Cobia production within the project was limited by hatchery supply of fingerlings. There appeared to be an association between small grade fish and subsequent poorer performance in cages. When small grade fish were stocked into grow out cages from the hatchery, slow growth was observed. These smaller fish had higher SFR (~ 2.57 c.f. ~2.00 – 2.13) and higher FCR (~5.5 - 6 c.f. ~3 - 4) than larger grade fish at the end of the season. During the production season, the estimated SFR of the smaller cobia was lower than the larger grade cobia (Appendix 1). This effect was observed in cobia of approximately 300-500g in size onwards (approximately 30 days post stocking into cages). The condition was classified as cobia slow growth syndrome and is discussed elsewhere in this report (7.1.3 Cobia slow growth syndrome).

In the 20,000L parabolic tanks, cobia fingerlings of approximately 200g to 280g were produced in the hatchery. In the previous season, fish of approximately 300g were produced from the hatchery. The parabolic tanks were not assessed for production of fish all the way through to market size fish (>4kg) as they were utilised to provide an over-wintering strategy, when outdoor ponds and cages were too cool for cobia to grow. This allowed for cages to be stocked in spring. Generally, the SGR (Figure 20), FCR (Figure 21, Figure 22) of cobia in tanks were higher than that of cages (Table 7). This is likely due to the difference in size classes between the two groups. The SFR (Figure 19) was higher in cages than tanks which may be due to differences in water quality between the cage and tanks.

*Table 7. Average growth performance of cobia in different systems. *Ponds were unable to be stocked due to the lack of fingerlings.*

Cobia	Cage	Pond*	Tank
n value	n=3	n=0	n=5
Average Start weight (g)	264.67 ± 62.06	N/A	47.27 ± 72.95
Average End weight (g)	3680.73 ± 489.11	N/A	218.80 ± 31.34
Average farming period (days)	337.33 ± 15.97	N/A	112.40 ± 55.99
SFR	2.24 ± 0.25	N/A	2.13 ± 0.35
SGR	0.79 ± 0.05	N/A	2.48 ± 1.02
eFCR	4.09	N/A	2.20
bFCR	3.94	N/A	2.18
Survival (%)	97.0	N/A	98.4
Max Stocking density (kg m^{-3})	34.17 ± 5.82	N/A	13.86 ± 3.67
Average fish density (fish m^{-3})	9.33 ± 1.07	N/A	61.97 ± 12.08

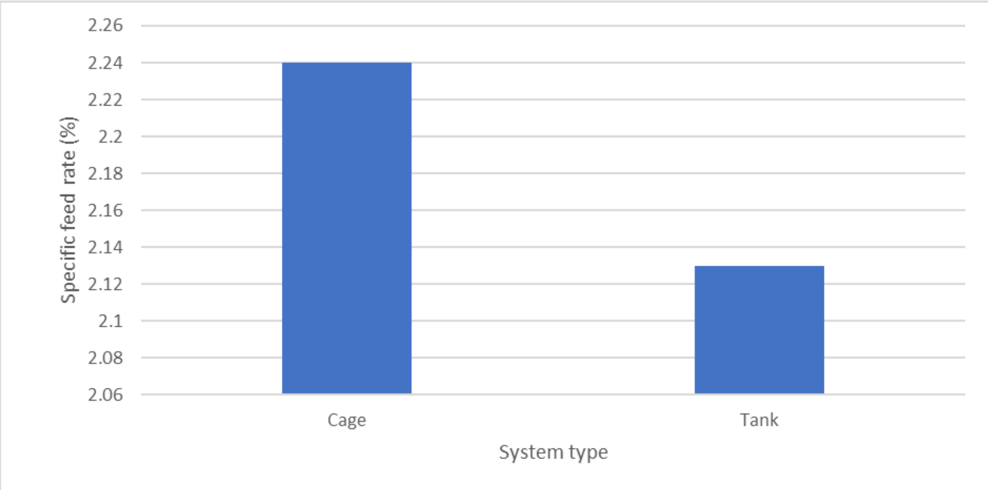


Figure 19. SFR of cobia cultured in different production systems

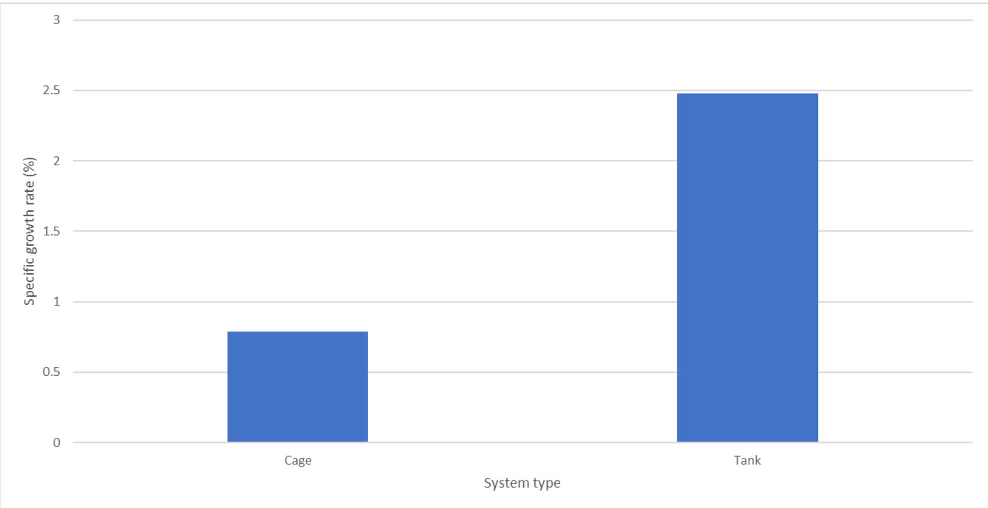


Figure 20. SGR of cobia cultured in different production systems

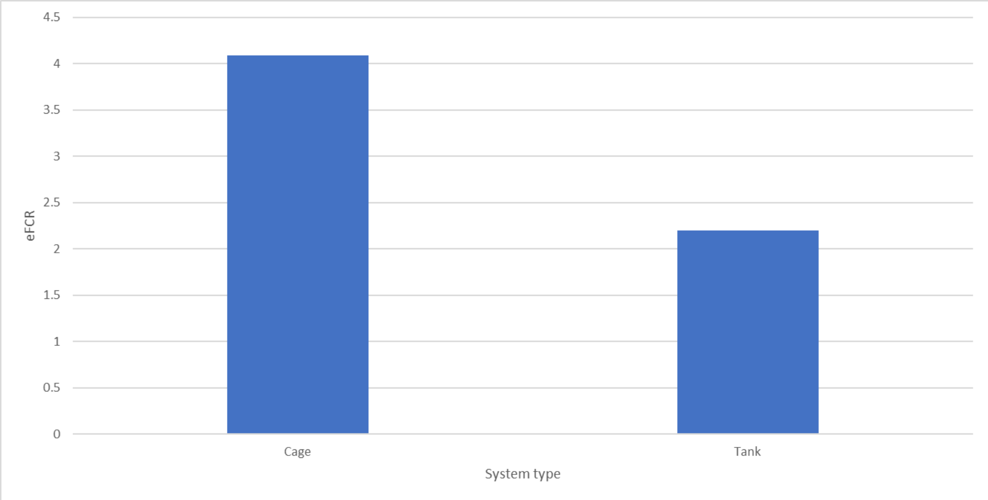


Figure 21. eFCR of cobia cultured in different production systems

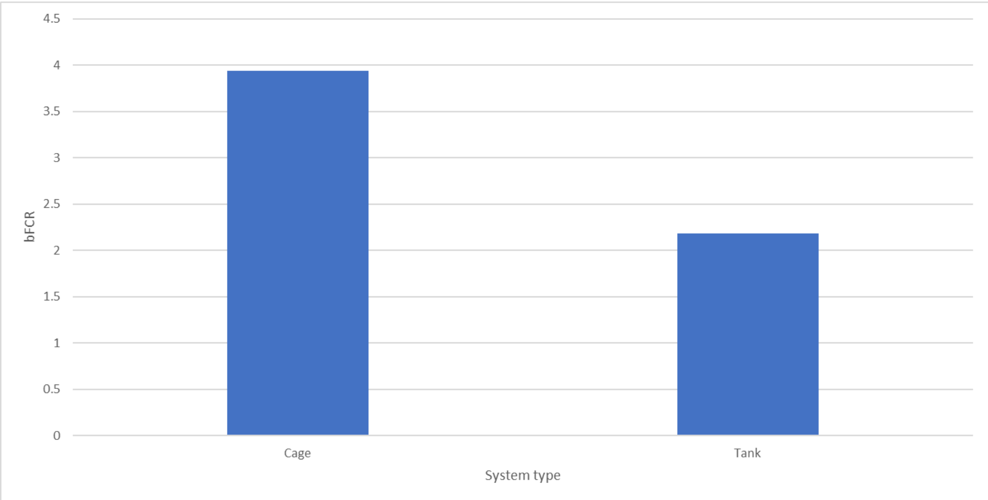


Figure 22. bFCR of cobia cultured in different production systems

4.3 Water quality

4.3.1 Thermal tolerance

Cobia

Over the one-year period, the growth in cobia appeared to decrease as water temperature fell below 20^{o}C. Growth and feed rate in cobia ceased or were significantly diminished when temperatures decreased below 18^{o}C. Over winter, the body condition of cobia decreased. Some increase in mortalities occurred when temperatures decreased below 18^{o}C. These findings are similar to findings reported in the literature for cobia. At temperatures below 16^{o}C, growth has been reported to be slow and mortalities may be expected (Liao et al., 2004). Cobia were observed to recover from injuries more slowly during winter and few fish that had been handled were noticed to develop skin lesions. Granulomas in the viscera were observed in slow growing cobia (cobia slow growth syndrome) and were not seen in the faster growing cobia which were harvested before the conclusion of the winter period. During winter some cobia were noticed to have patchy algal growth on the skin. These lesions did not appear to affect the health of the fish and no inflammation was seen over the affected areas. Histologically, these areas also did not appear to be affected. Skin scrapings taken over these regions did not reveal any obvious parasitic organisms that may be contributing to disease, and diatoms were seen over the region. These diatoms appear to be epizoic diatoms (pers com. Gustaaf Hallegraeff, 2019) and were self-limiting in the cobia. Epizoic diatoms have not been previously reported in cobia.

At warm water temperatures growth is reported to increase as temperature increase towards the optimal growth temperature (31°C to 35°C) and declined after the optimal growth temperature is exceeded (Sun et al., 2014). In the tank systems, water temperature can be regulated and maintained, as such the improving growth during winter periods offer a good opportunity to provide larger cobia fingerlings for stocking in spring. An approximately 200g weight cobia fingerling can be produced from a starting weight of 1g in approximately 150 days at 24°C. In ponds, an approximately 250 days (mid-September to late-May) production window of adequate growth could be achieved in ponds in the Logan region (above 18°C water temperature), however it may not be viable to farm cobia in ponds over the winter period in the Logan region as water temperatures in the ponds were approximately 2°C lower than the saline lake temperatures during winter. This difference in temperature may be beyond the minimum thermal tolerance of cobia (16°C) resulting in mortalities (Liao et al., 2004). In lake systems (cages), the water temperature was above 18°C for approximately 270 days (early-September to early-June).

Giant Grouper

Lower grade giant groupers (below market size fish from Pond 9) were cultured through winter. Fish from this group were slow to feed and did not grow well prior to the decrease in water temperature. These smaller fish were transferred to cages to be on grown during winter. Through the winter period, minimal growth was observed in this cage. Mortalities were low through winter (0.17%) for this cage. In *Epinepelus fuscoguttatus x E. lanceolatus*, survival at 9°C, 14°C, 19°C, 24°C, 29°C, 34°C were 0%, 0%, approx. 60%, approx. 85%, 100%, 100% respectively (Zhang et al., 2018). Though there may be minimal growth through winter, holding of fish over the winter period appears to be feasible for giant groupers in the Logan region in lake cages. No giant groupers were cultured in the pond systems during winter.

Giant grouper could also be produced in the hatchery from 40g fingerlings to market size in approximately 230-240 days at 24°C. Approximately 90 days is required for 30g giant groupers to reach 90g average size in the hatchery systems at 24°C.

4.3.2 Dissolved Oxygen (DO)

It appears that the feed rate of cobia can be influenced by dissolved oxygen. Prior to moving to the outdoor cages, the average DO in the tanks was 4.8 mg L^{-1}. This corresponded to a satiation feed rate of 2.0-2.5% biomass (at 27°C). When moved into the outdoor cages (average DO of 5.8 mg L^{-1}), the satiation feed rate of the increased (Figure 23). This finding suggests that additional growth performance benefits may be achieved by delivering additional oxygen to the tank systems to support greater metabolic function.

DO is an important factor to consider in giant groupers. In the 2019 season, prolonged low DO (below 75% saturation) for greater than two weeks was associated with a nodavirus outbreak in vaccinated giant groupers.

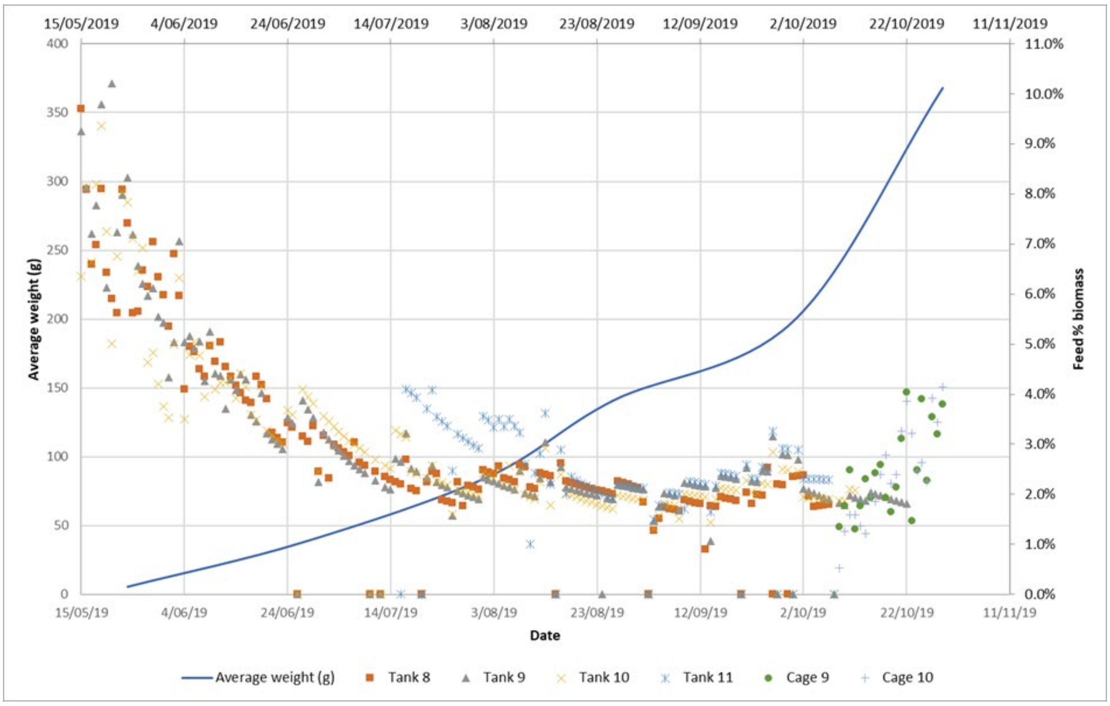


Figure 23. Feed rate of the same cobia cohort in hatchery tanks and in cage systems.

4.4 Disease/Syndromes

4.4.1 Trichodinosis

Trichodina parasites were seen on the skin and gills of giant grouper and cobia in outdoor systems. These parasites appear to vary in abundance from month to month. Water quality appeared to correlate with the numbers of Trichodina parasites seen on the giant grouper and cobia. Trichodina are able to proliferate more readily in waters with more organic matter and bacteria (Randy MacMillan, 1991). Potentially, overtime with increase organic inputs to the lake and pond systems, the water quality may favour proliferations of Trichodina.

4.4.2 Vorticella

Vorticella is another parasite seen in both cobia and giant groupers. Infections with these parasites were strictly limited to the hatchery tank systems. High burdens of *Vorticella* are generally associated with poor water quality (high organic loads). When infection burden of *Vorticella* is high, these protozoa can trigger bacterial infections by feeding on tissues of infected fish and compromise the skin of fish. This has been reported in association with mass mortalities in groupers in Indonesia (Palm et al., 2015; Salem et al., 2011). Chronic mortalities were observed in juvenile giant groupers with *Vorticella* protozoa. Treatment with 20mg L^{-1} formalin for 24 hours under the Australian Pesticides and Veterinary Medicines Authority (APVMA) Minor Use Permit, appeared to be effective in controlling the disease when detected in routine health surveillance.

4.4.3 Brooklynellosis

Brooklynellosis is caused by the pathogenic protozoa *Brooklynella hostilis. B. hostilis* infects and causes significant damage by feeding on the epithelial debris of the gill and skin of fish. These parasites are approximately 3-4 times larger than the red blood cells of cobia. Clinical signs often seen in infected fish include respiratory distress, increased mucous production, lethargy and sudden death. Histologically, the gills of infected fish may display signs of haemorrhage, cellular proliferation and desquamation (Lom et al., 1970). This disease is often seen in stressed fish (i.e recently handled/transported fish) in aquaculture. In Puerto Rico, *Brooklynella sp.* was detected in cobia shortly after transport which resulted in complete stock loss (Bunkley-williams et al., 2006). In the wild, this parasite can be seen in low numbers in asymptomatic fish, however, when stressed outbreaks of Brooklynellosis can occur (Roberts, 2002). *Brooklynella* have been observed in recently transported cobia from Bribie Island Research Centre. These fish were treated with 20 mg L^{-1} formalin for 24 hours under the Australian Pesticides and Veterinary Medicines Authority (APVMA) Minor Use Permit. Treatment appeared to be effective based on post-treatment skin mucous and gill biopsy examination.

4.4.4 Leeches

Leeches have yet to be detected in the cobia populations on the farm but were regularly detected on grouper populations cultured in ponds and occasionally in cages, but not in tanks

Zeylanicobdella arugamensis is one leech that has been documented to infect orange spotted groupers, hybrid groupers and cobia. This parasite can survive in a wide range of salinities ranging from 10-40ppt and temperature ranges between 25-35°C (Kua et al., 2010). However, salinities between 20-30ppt and temperatures between 25-27 °C provide more suitable environments for the parasite to grow and reproduce. *Z. arugamensis* leeches are self-fertile and lay multiple cacoons (housing a single egg) which are resistant to many chemicals, at the dose which is safe to use on fish (Murwantoko et al., 2018). Cacoons hatch into juvenile leeches 7-12 days after they are deposited, which then develop into adults 9-10 days after hatching (Kua et al., 2010). This life cycle pattern means that multiple treatments are required to control leeches. Based on life cycle treatment is recommended on Day 0, 7 and 14 (three treatments applied one week apart) to ensure no juveniles reach adulthood to produce further eggs.

Leeches feed on blood of their host fish. So heavy loads cause blood loss and drive the fish into an anaemic state, where their gills may begin to appear pale. They can also drive skin and mouth ulcers and open the fish up to secondary bacterial infections (Mahardika et al., 2018).

Leeches are also known to transmit blood parasites (trypanosomes). It is speculated that leeches have been responsible for the transmission of trypanosomosis in brown-marbled groupers (*Epinephelus fuscoguttatus*) in China and areolate groupers (*Epinephelus areolatus*) in Hong Kong (Su et al., 2014). Trypanosomes are very small flagellate parasites about 10-15 microns in size (roughly as long as a red blood cell is wide). Trypanosomes can be detected on gill preparations in the blood which drains from the gills. They are motile and can be seen moving swiftly through the blood. These parasites damage blood cells within the fish and can contribute to anaemia (pale gills). These blood parasites have not been detected in any health examinations or pathology submissions from the farm to date.

Cleaner shrimp have been found to be effective in reducing adult and juvenile stages of *Z. arugemensis* and their chemically resistant cocoon stage (Vaughan et al., 2018). Anecdotally, in Taiwan, *Penaeus monodon* (stocked at about 2g size) have been used to control leeches with great efficacy with small crops of *P. monodon* harvested at the end of the season (pers com Ralph Wang, 2019). It is expected that *P. monodon* are likely to be more efficacious at removing leeches than cleaner shrimps due to their increased size (pers com Vaughan, 2019). Potentially, the use of *P. monodon* may be an efficacious alternative to copper sulphate or formalin use to control leeches. Pond trials have been initiated at Rocky Point Aquaculture (10 m^{-2}), and early results suggest that *P. monodon* can suppress the abundance of infection from *Z. arugemensis* but appear insufficient to eradicate all leeches from pond cultured giant groupers.

4.4.5 Gill flukes

Dactylogyrid flukes (also known as gill flukes) are monogeans that infect the gills and skin of fish. To date, gill flukes have only been observed once in low numbers on one fish in the caged grouper populations. These flukes have yet to be seen in any of the caged cobia populations directly adjacent to the infected groupers. The fluke infestation appeared to be self-limiting in this instance and may be an aberrant infection from another fish species which is present in the lake ecosystem.

4.4.6 Gill necrosis

Gill necrosis was observed in the cage cobia population during winter in one darkened coloured fish (see Figure 24 and Appendix 9). Pathology did not suggest it was a case of primary bacterial gill disease. The cage history suggested some harvesting had been occurring which may have induced this lesion through trauma. The gills of fish are important structures responsible for maintaining oxygen levels in the body and excretion of metabolic waste. Irritation/damage to the gills may result in impairment of these functions. Gill necrosis may be caused by several factors such as trauma, bacterial disease, toxic algae exposure, jellyfish nematocyst exposure, toxin exposure, parasitism, fungal disease, viral disease, excessive total gas pressure. Factors that affect the fish's ability to produce and secrete mucus may also have an influence on the development of gill necrosis.

Figure 24. Severe bacterial gill necrosis of a cobia

4.4.7 Eye conditions

Various eye conditions have been observed during health surveillances. These include gas bubble disease (excessive total gas pressure), cataracts (cloudy lens within the eye) and exophthalmos (pop eye).

Gas bubble disease
Gas bubble disease was observed once in one fish in a cage cobia population. The lack of other affected fish within the cage cohort suggest that individual fish factors, rather than total gas pressure issues in the water body may be implicated.

Gas supersaturation can occur when the total pressure of the dissolved gases exceeds the ambient atmospheric pressure. When the dissolved gas is absorbed across the fish gill, the excess gas may equilibrate with the atmospheric pressure inside the fish and escape as gas bubbles in the gas phase into the blood stream leading to intravascular thrombi and trauma.

Generally, total gas pressures (TGP) above 110% saturation is considered very dangerous to fish (Noga, 2010). TPG levels of >105% can lead to chronic effects from total gas pressure rather than acute mortality. The origin of gas supersaturation varies from excessive algal production with dense blooms on still sunny days, to injection of air at too greater depth, leading to increased dissolution of nitrogen into the water. Gas supersaturation is also common where air is drawn into the suction side of a pressure pump. Oxygen can contribute to excessive gas pressure if supersaturated levels are maintained for extended periods.

In ponds with heavy loads of macrophytes, or intense microalgae blooms, gas bubble disease may develop in fish when the production of oxygen through photosynthesis drives supersaturation of the pond water and elevates the total gas pressure in the pond. At no stage in the experiment was there substantial macrophyte or dense microalgal blooms in the water bodies which contained the cages.

Exophthalmos
Exophthalmos is term given to the condition where the eyeball protrudes from the orbit (eye socket). This can be caused by a number of factors such as bacterial infections (*Streptococcus*, *Vibrio*, *Mycobacterium*, *Photobacterium*) (Luo et al., 2019; Nagai et al., 2008; Noga, 2010; Zamri-Saad et al., 2010), viral infection (*Lymphocystis*) (Dukes et al., 1975), excessive gas pressure (supersaturation, barotrauma) (Humborstad et al., 2017; Xue et al., 2019), trauma, neoplasia (Hargis, 1991), nutritional deficiency (Hargis, 1991). Exopthalmus was observed in the tank cobia population post transport and it is possibly attributed to supersaturation during transport although total dissolved gas was not able to be measured at this time.

Cataracts
Cataracts were observed in the cobia populations. Potential causes of cataracts include: nutritional deficiencies, bacterial disease, water quality and trauma (Bjerkås et al., 2004, 2003; Bunce et al., 1990; Houng-yung et al., 2014; Kantorow et al., 2004; Karvonen et al., 2004; Maage, 1990; Neves et al., 2015; Sambraus et al., 2017; Wegener et al., 2002). It is unknown what impact cataracts have on the cobia populations; however, it is expected that the condition may have some impact on growth due to impaired sight feeding ability. Currently, the presence and severity of the lesion did not appear to correlate to the body condition of the cobia.

4.4.8 Deformities

Deformities were seen in both giant groupers and cobia. Deformities may result in decreased performance and decreased marketability. Deformities can be caused by a number of factors including nutrition, genetics, handling, husbandry, developmental abnormalities, trauma, infections, inflammation and toxins. In one cobia cage stocked with lower grade fish, the prevalence of deformities was more prominent and deformed fish were also graded into this cage. This cage performed poorer than the other two cages (see Cage 4, 5, 6 in Appendix 1). Improved hatchery production is likely to facilitate more rigorous grading and culling of the bottom end of fish runs, which should minimise the commercial impact of deformities.

4.4.9 Melanisation of viscera in vaccinated giant groupers

Melanisation has been observed in the abdomen of vaccinated groupers. This has not been observed in the non-vaccinated populations. Melanin deposits can be caused by stimulation of the immune response of the fish to components in the vaccine, such as the LPS from the lysed bacterial cells and the adjuvant. The amount of immunological response can vary depending on the age/size at vaccination, adjuvant inclusion rate, volume and concentration of the lysed cells delivered. This condition is likely caused due to inflammatory reactions to the experimental recombinant nodavirus vaccine. Melanin deposits have also been observed in other fish species that have been vaccinated such as the Atlantic Salmon (Chalmers et al., 2020). No adverse market responses have been noted to date due to the presence of the melanin deposits.

4.4.10 Granulomatous syndrome in cobia

Granulomas are clusters of fish immune cells (macrophages). They typically form as a chronic response to bacterial infections, fungal infections, migrating parasites, and non-infectious causes such as nutrition and vaccination. In giant groupers, the formation of melanisation lesions in the abdomen has been associated with vaccination with a recombinant nodavirus vaccine. Granulomas with occasional rod shape bacteria within have been detected in the liver, spleen, kidney, pancreas and omentum of 81.8% of the slower growing population of cobia sampled post winter. Granulomas appear to be limited to cobia in the slower growing cohort in the cage system which have experienced one winter season. Histology and necropsy data from cases prior to winter did not detect the presence of granulomas. The pathogens/aetiology involved in the development of these lesions in cobia is not known, however it is speculated that these lesions may be induced in fish that have been previously handled during winter, and/or in slow growth syndrome cobias. Current understandings of the condition are insufficient to assess the impacts granulomas have on growth of cobia and its role in and association to cobia slow growth syndrome. Potentially, the occurrence of granulomas may be lower in faster growing fish.

The presence of large numbers of granulomas may have a negative impact on marketing of cobia. To date, the faster growing cobia and harvested fish from the slower growing population have not resulted in any negative market feedback regarding the granulomas.

4.4.11 Gastric bloat

Gastric bloat was observed in cage cobia populations. Aquaculture diets vary substantially from their wild counterparts. The physical properties of the feed can influence the ability for fish to process the nutrients offered to the fish. In the wild, fish digest their feed slowly by breaking down their consumed meal (small amounts of nutrients at a time) which is digested in dilute gastric secretions and absorbed in the intestines. In aquaculture, nutrient dense feeds are offered to the fish in the form of pellets. These pellets may have different breakdown times and differing rates of movement of soluble components. The speed of nutrient movement can also be impacted by the type of raw materials used in the formulation. When feed is rapidly broken down in the stomach, high concentrations of nutrients are contained within the stomach chyme (secretions). As there is a small amount of digestive secretions produced compared to the amount of nutrients available, the chyme would contain high concentrations of nutrients compared to the relatively nutrient-dilute digestive secretions in wild fish eating a non-aquaculture diet. When absorbed by the intestines, this highly nutrient dense chyme is more likely to trigger the fish’s intestines to activate its intestinal brake mechanism which temporarily shuts off further nutrients from passing through the stomach to the intestines(Forgan et al., 2007). In saline waters, fish constantly lose fluid through their gills which is replaced by constant drinking of water. In fish experiencing gastric dilation, there is delayed gastric movement due to the intestinal brake mechanism leading to feed retention in the stomach for extended periods and retention of the consumed water. Water is not absorbed from the stomach, only the intestine, so the fish’s thirst cannot be easily quenched when the exit to the stomach is restricted. The combination of the two factors can result in increased thirst due to reduced fluid replacement, which leads to increased drinking, and further distension of the stomach (Anderson, 2006).When this occurs over an extended period it can lead to bloating and stretching of the stomach musculature, leading to loss of muscle structures and functionality over time.

4.5 Marketability and seasonal supply of cobia and giant grouper

4.5.1 Marketability and supply of cobia

The market size for cobia was between 3kg to 6kg. These fish were sold into restaurants and supermarkets. In restaurants, the predominant use of the fish is for sashimi. These fish were sold as whole fish into the market after ike-jime and bleeding.

To achieve market size fish, broodstock spawning had to be induced between January and March. Fingerlings were stocked in parabolic tanks in a RAS system over winter to reach a size of 200g to 300g prior to stocking to outdoor cages in September.

Cobia which did not reach market size were kept through winter in cages to be on-grown into the following spring and summer for sale. This also ensured that there was continuous supply of cobia through the year to the market. During the winter periods, the condition of the fish decreased, and skin lesions were more prevalent due to repeated harvesting and handling of fish. This resulted in difficulties in marketing of the product. In warmer temperatures, this did not prove to be an issue as body condition improved and harvest trauma appeared to heal rapidly.

There are also additional market opportunities for smaller fish (1kg to 2kg) to the live market. This has yet to be commercially explored.

4.5.2 Marketability and supply of giant grouper

The market size for giant groupers was between 600g to 4kg. These fish were sold into restaurants and supermarkets. Most restaurants preferred plate sized fish (600g to 800g) which were humanely killed by the ike-jime technique. To ensure continuous supply to the market, two main stockings of giant groupers were required. The first stocking was of the cages and ponds which occurred during September. These fish were stocked at approximately 150g to 200g. This allowed for market size fish to be produced by late January and for continuous supply until late winter/ early spring. In the trial, an additional stocking during March with 40-50g fish in the RAS system allowed to produce market size fish by October. As such, to ensure continuous supply of giant groupers to the market, a stocking of giant groupers into the RAS system in January would assist in the supply during the late winter to early summer period.

Alternative markets for giant groupers also exist. Currently, some restaurants have shown interest in larger giant groupers. These fish vary between 2kg to 4kg. To produce giant groupers of this size, two years of production is required. This appears to be feasible in the cage systems. Some restaurants have also expressed interest in live fish of 600g to 800g. Some live fish sales were trialled during this period with success.

4.5.3 Acceptance of cobia in the market

The preferred market size for cobia was 4kg and above. In the restaurant market, this size fish allowed for optimal flesh recovery and for easier utilisation of other organs, carcass and skin for other culinary purposes. Fish under 4kg were less optimal for the restaurant market.

To the supermarket chain, 1kg to 2kg fish may potentially be able to be sold whole, however, this market has yet to be established. Generally, supermarkets prefer larger fish (above 4kg) which is then sold as value-added packaged fillets and/or cutlets. Cobia cutlets and fillet are valued in supermarkets due to their versatility and stability.

4.5.4 Acceptance of giant grouper in the market

There were two main markets for giant groupers – the supermarket and restaurant market. The restaurant market preferred 600g to 800g fish. These are sold both live and whole. Larger giant groupers were also sold into the restaurant market (2kg to 4kg fish). These fish were either portioned or used as a banquet whole fish. Giant groupers of 600g to 1.2kg were suitable for supermarkets. These fish were scaled, gutted and gilled prior to packaging in trays for sale.

Generally, giant groupers were widely accepted by the market due to their sweet white flesh. It is expected that this market would continue to improve with time and expanded product availability.

5 Conclusion

The findings of this project have shown that cobia and giant groupers can be successfully reared in the Logan region of southern Queensland. For giant groupers, these fish can be housed in tanks, ponds and cages to produce fish for the market all year round. For cobia, only tanks and cages were trialled. The current parabolic tank design in the RAS system are suitable for market size cobia production. Market sized cobia can also be produced in cages located within a saline lake and previous FRDC studies have documented that cobia can also be produced in ponds.

The production of cobia and giant groupers in the Logan region has also highlighted two health issues that may be of concern to the production of the two species. Glycogenic hepatopathy and hepatomegaly in giant groupers and cobia slow growth syndrome in cobia are significant disease/conditions which may impact on the expansion of the industry. These areas should be of focus for further research and development.

Nodavirus is a pathogen of significance to the giant grouper industry. Early production data suggest that the vaccine is able to achieve above 80% survival in face of an outbreak of VNN. In Atlantic salmon, vaccinated fish tend to perform poorer than unvaccinated fish. This did not appear to be the case in the giant groupers vaccinated with the recombinant nodavirus vaccine. The CV of the vaccinated fish did however appear to be higher than unvaccinated fish. Potentially, there may be some fish in the vaccinated populations that are more affected by the vaccine than others. This does not appear to be widespread in the populations. Cobia appear to be significantly more resistant to nodavirus infections than giant grouper.

The market has accepted both cobia and giant grouper produced to date. It is expected that this market would continue to develop with expansion of the industry.

6 Implications

This project has highlighted health and production factors that may be faced by producers of cobia and giant grouper in the Logan region. A handbook outlining various disease/conditions/symptoms has been compiled from this project to assist producers in identifying and managing problems that may be faced.

For investors and stakeholders, this document offers an insight into the risks that are present with farming giant grouper and cobia in the Logan region. It also highlights areas where additional research is likely to advance and improve the production of giant groupers and cobia. One such example is with glycogenic hepatopathy and hepatomegaly in giant groupers. This condition is 100% prevalent in the giant grouper populations. It is likely that additional growth performance could be achieved in giant groupers if the condition is managed.

7 Recommendations

This project has shown the potential for giant groupers and cobia to be an excellent candidate for farming in the Logan region. It has also highlighted the need for further research and development for these new farmed species in Australia.

8 Further development

Further potential research and development projects include:

- Investigation into the cause and prevention of glycogenic hepatopathy and hepatomegaly in giant groupers.
- Investigation into causative factors for cobia slow growth syndrome and approaches to improve performance.
- Dietary optimisation of cobia and giant grouper amino acid, lipid, energy requirements.
- Strategies to reduce condition loss of cobia through winter.
- Exploring non-nutritional factors influencing the growth in giant groupers in ponds including pond design and feeding strategies.

9 Extension and Adoption

Early production data from this project was presented at the 2019 FRDC Australasian Scientific Conference on Aquatic Animal Health and Biosecurity in Cairns, Queensland. Final findings of the project are to be presented at future conferences.

A regularly comprehendible handbook on the health and production of giant grouper and cobia was compiled.

10 Project materials developed

A handbook on diseases, diagnosis, treatment, prevention of cobia and giant groupers was developed from the materials gathered from this project.

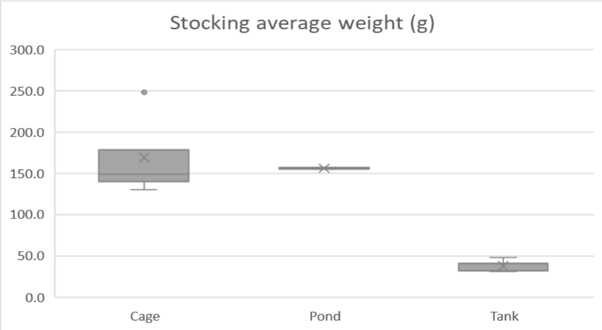
Monthly health reports were generated from the data and information gathered during the monthly visits to Rocky Point Aquaculture.

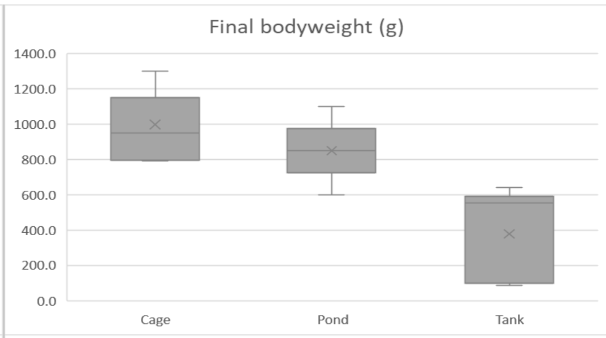
11 Appendices

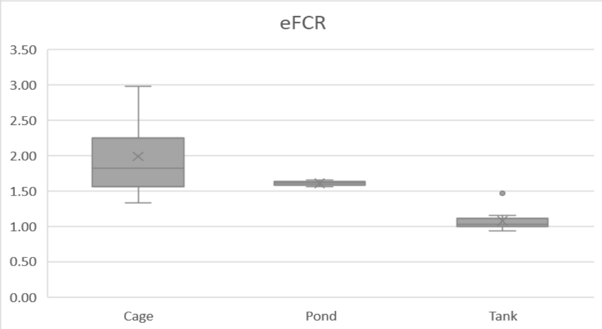
Appendix 1. Production performance of giant grouper and cobia

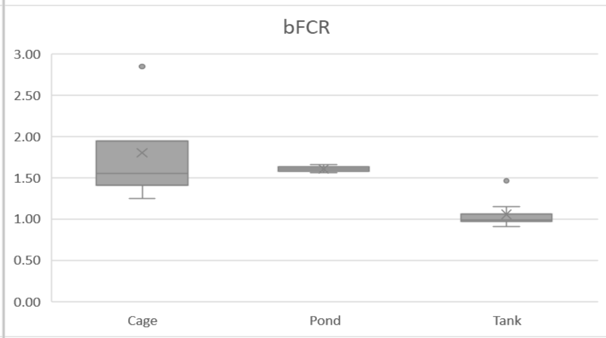
YC	Run	System	Fish	Vaccination status	Number of nodavirus outbreaks	Hatchery ID	Farm period	Growing days	Start weight	End weight	SGR	SFR	eFCR	bFCR	Survival	Max Stocking density
2018	1	Cage 1	Grouper	Unvaccinated	0	EL0418	Nov 2018 - Jun 2019	217	155.0	1300.0	0.98	2.39	2.98	2.85	96.0%	1.98
2018	1	Cage 7	Grouper	Vaccinated	2	EL0518	Dec 2018 - May 2019	169	143.0	789.7	1.01	1.18	1.64	1.46	74.2%	11.45
2018	2	Cage 8	Grouper	Vaccinated	2	EL0518	Dec 2018 - May 2019	146	130.0	797.4	1.24	1.21	1.33	1.25	90.4%	11.83
2018	1	Cage 9	Grouper	Vaccinated	2	EL0418	Dec 2018 - May 2019	169	248.0	1100.0	0.88	1.22	2.00	1.65	78.0%	11.79
2018	1	Pond 6	Grouper	Unvaccinated	0	EL0418	Nov 2018 - Jun 2019	231	155.0	1100.0	0.85	0.90	1.56	1.56	99.9%	0.89
2018	1	Pond 9	Grouper	Vaccinated	0	EL0518	Dec 2018 - Jun 2019	189	157.0	599.0	0.71	0.67	1.66	1.66	100.0%	0.62
2019	1	Tank 12	Grouper	Unvaccinated	0	EL02_19	Jun 2019 - Sep 2019	93	30.5	108.0	1.36	1.19	1.03	0.97	93.7%	19.39
2019	1	Tank 13	Grouper	Vaccinated	0	EL02_19	Jun 2019 - Sep 2019	87	32.0	86.9	1.15	1.13	1.11	1.06	96.2%	22.42
2019	1	Tank 14	Grouper	Vaccinated	0	EL02_19	Jun 2019 - Sep 2019	89	32.0	93.5	1.20	1.12	0.94	0.91	96.2%	21.14
2019	1	Tank 15	Grouper	Vaccinated	0	EL02_19	Jun 2019 - Sep 2019	87	32.0	100.0	1.31	1.08	1.01	0.97	96.9%	26.08
2019	1	Tank 3	Grouper	Mix	0	EL1018	Mar 2019 - Nov 2019	231	48.0	589.6	1.09	0.77	1.00	0.99	96.8%	18.79
2019	1	Tank 4	Grouper	Mix	0	EL1018	Mar 2019 - Nov 2019	235	41.0	553.7	1.11	0.88	1.47	1.46	98.7%	57.48
2019	1	Tank 5	Grouper	Mix	0	EL1018	Mar 2019 - Nov 2019	237	41.0	591.5	1.13	0.73	1.02	1.02	98.4%	57.20
2019	1	Tank 6	Grouper	Mix	0	EL1018	Mar 2019 - Nov 2019	245	41.0	638.0	1.12	0.79	1.15	1.15	97.6%	60.04
2019	1	Tank 7	Grouper	Mix	0	EL1018	Mar 2019 - Nov 2019	238	41.0	642.5	1.16	0.70	0.97	0.96	96.0%	55.82
2019	1	Tank 8	Cobia	Unvaccinated	0	N/A	May 2019 - Oct 2019	148	2.2	203.0	3.06	2.01	1.52	1.52	99.7%	15.55
2019	1	Tank 9	Cobia	Unvaccinated	0	N/A	May 2019 - Oct 2019	162	1.2	280.0	3.37	1.84	1.56	1.50	99.7%	19.54
2019	1	Tank 10	Cobia	Unvaccinated	0	N/A	May 2019 - Oct 2019	152	1.2	206.0	3.39	1.72	1.32	1.31	97.3%	13.70
2019	1	Tank 11	Cobia	Unvaccinated	0	N/A	Jul 2019 - Oct 2019	86	42.0	192.0	1.77	2.57	1.89	1.86	95.7%	11.97
2019	2	Tank 12	Cobia	Unvaccinated	0	N/A	Sep 2019 - Oct 2019	14	189.8	213.0	0.83	2.51	1.97	1.97	100.0%	8.52
2018	1	Cage 4	Cobia	Unvaccinated	0	N/A	Oct 2018 - Oct 2019	356	177.0	3120.2	0.81	2.57	5.99	5.46	95.3%	25.94
2018	1	Cage 5	Cobia	Unvaccinated	0	N/A	Oct 2018 - Sep 2019	339	312.0	3610.0	0.72	2.00	3.84	3.75	97.9%	38.42
2018	1	Cage 6	Cobia	Unvaccinated	0	N/A	Oct 2018 - Aug 2019	317	305.0	4312.0	0.84	2.13	3.20	3.16	97.6%	38.15

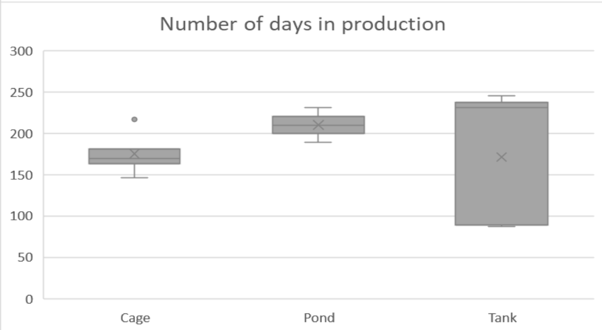
Appendix 2. Giant grouper performance comparison graphs

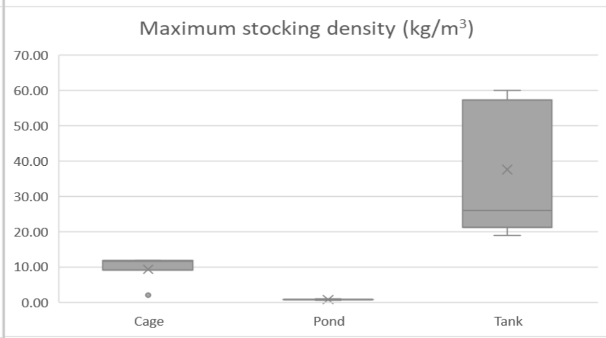


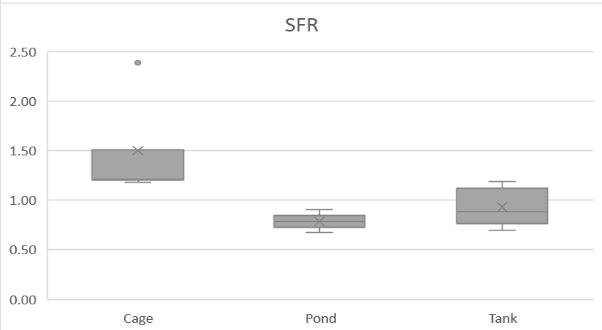


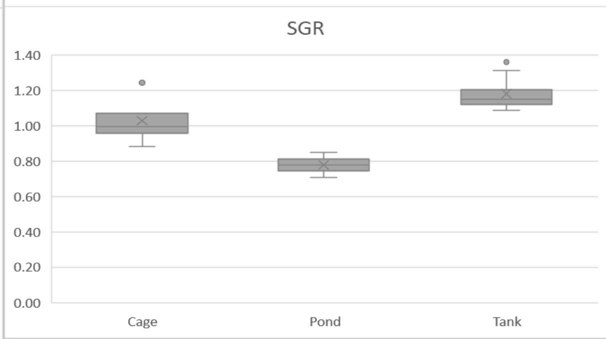


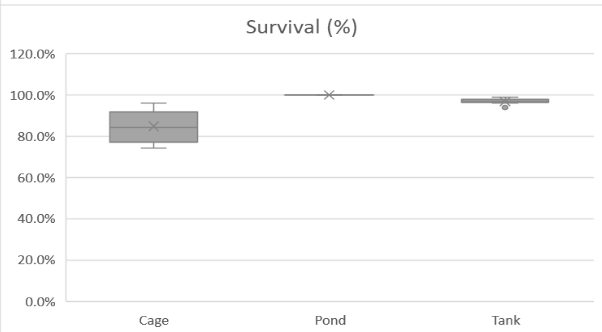


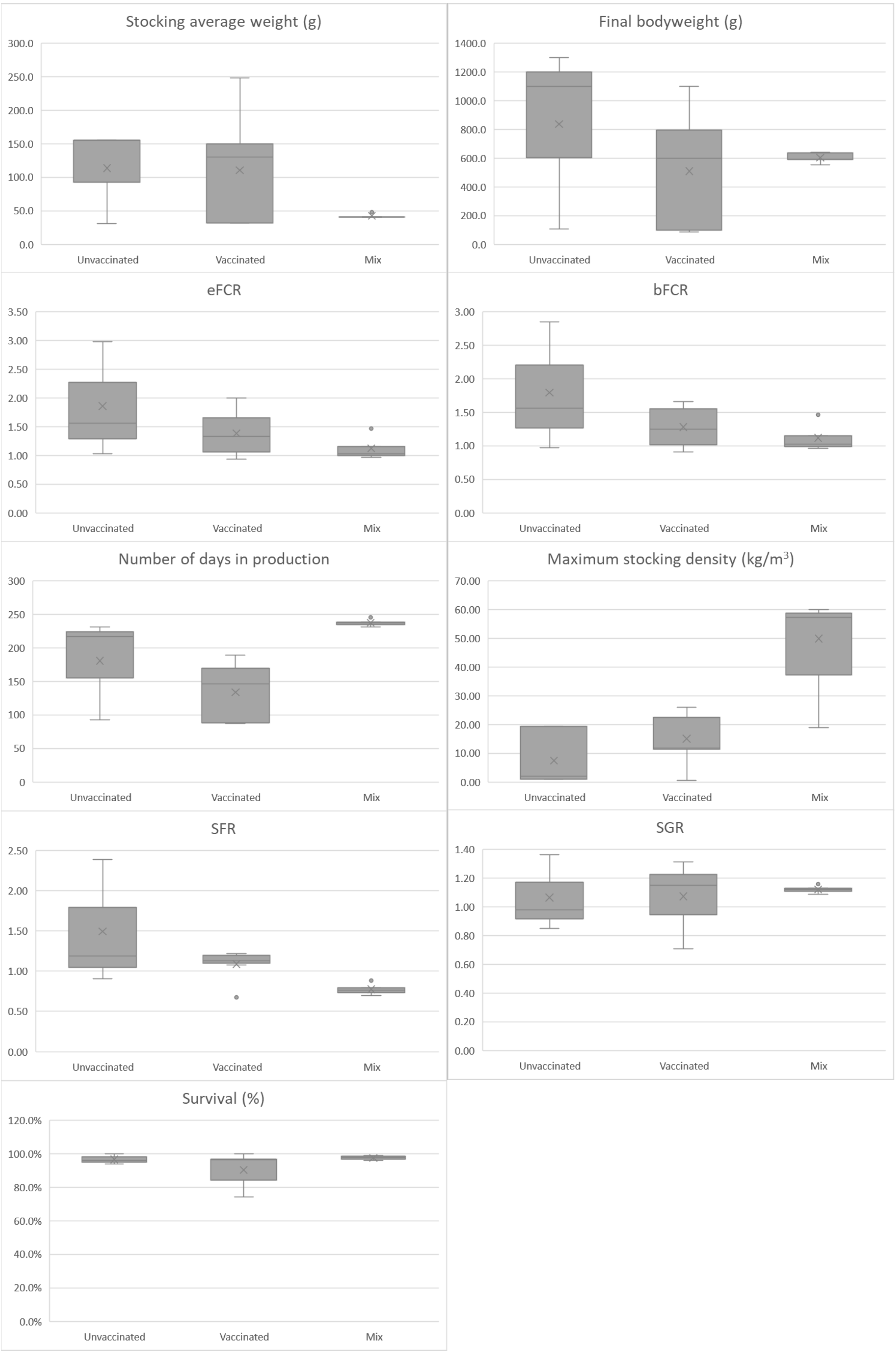


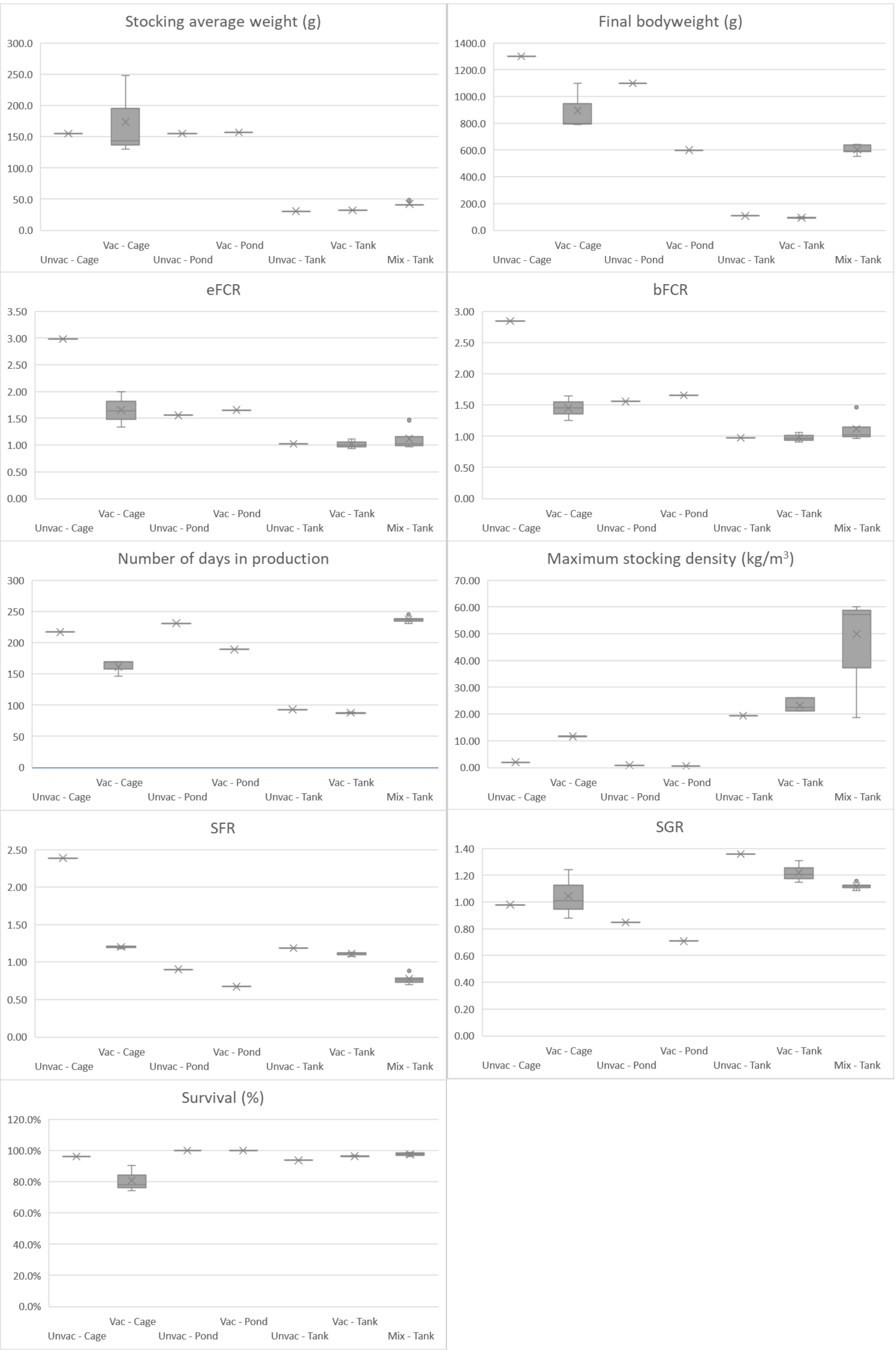




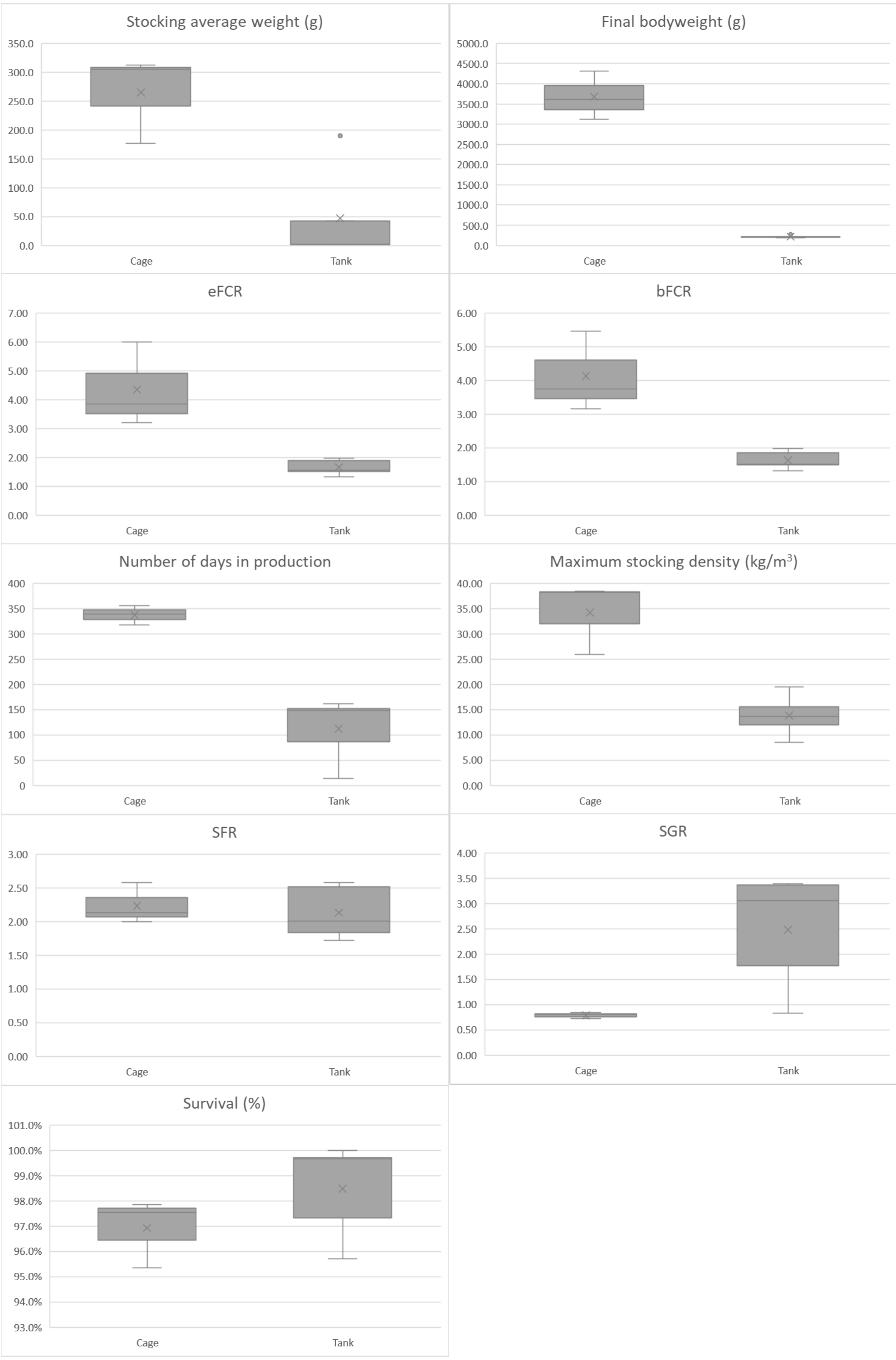




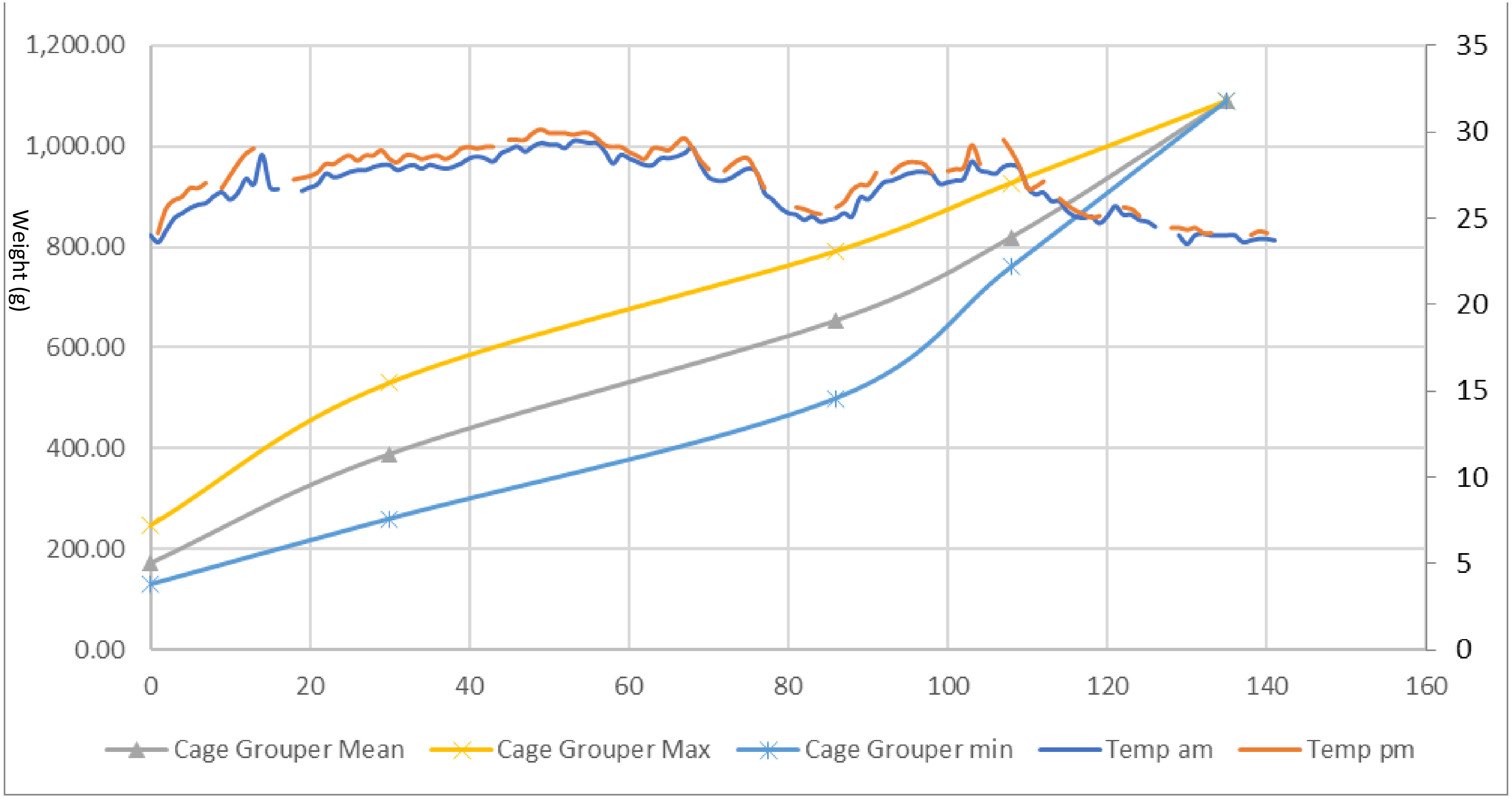

Stocking average weight (g)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Unvaccinated
Vaccinated
Mix
Final bodyweight (g)
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Unvaccinated
Vaccinated
Mix
eFCR
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Unvaccinated
Vaccinated
Mix
bFCR
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Unvaccinated
Vaccinated
Mix
Number of days in production
300
250
200
150
100
50
0
Unvaccinated
Vaccinated
Mix
Maximum stocking density (kg/m³)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Unvaccinated
Vaccinated
Mix
SFR
2.50
2.00
1.50
1.00
0.50
0.00
Unvaccinated
Vaccinated
Mix
SGR
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Unvaccinated
Vaccinated
Mix
Survival (%)
120.0%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
Unvaccinated
Vaccinated
Mix


Stocking average weight (g)
Final bodyweight (g)
eFCR
bFCR
Number of days in production
Maximum stocking density (kg/m³)
SFR
SGR
Survival (%)
Unvac - Cage
Vac - Cage
Unvac - Pond
Vac - Pond
Unvac - Tank
Vac - Tank
Mix - Tank

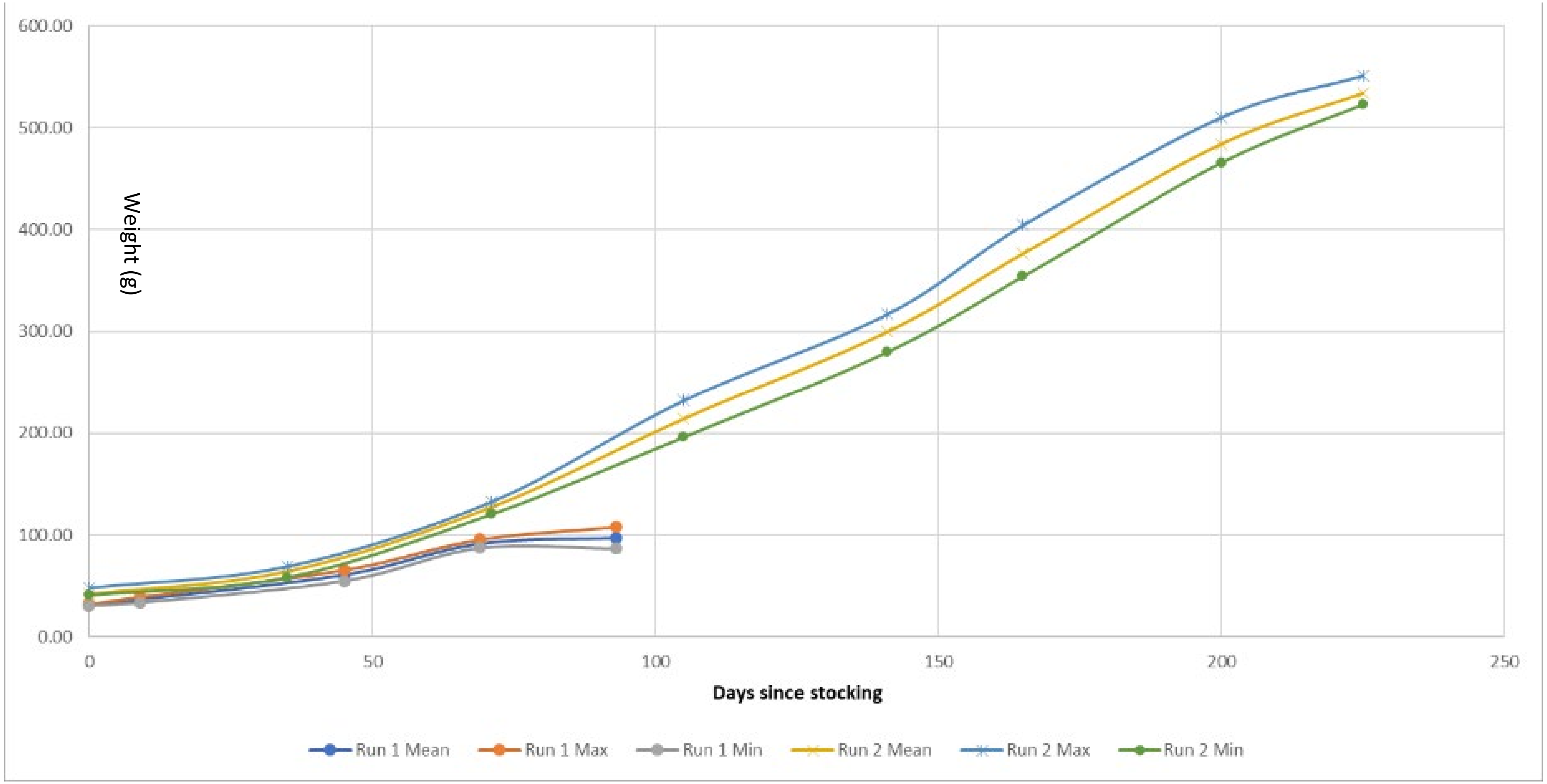
Appendix 3. Cobia performance comparison graphs



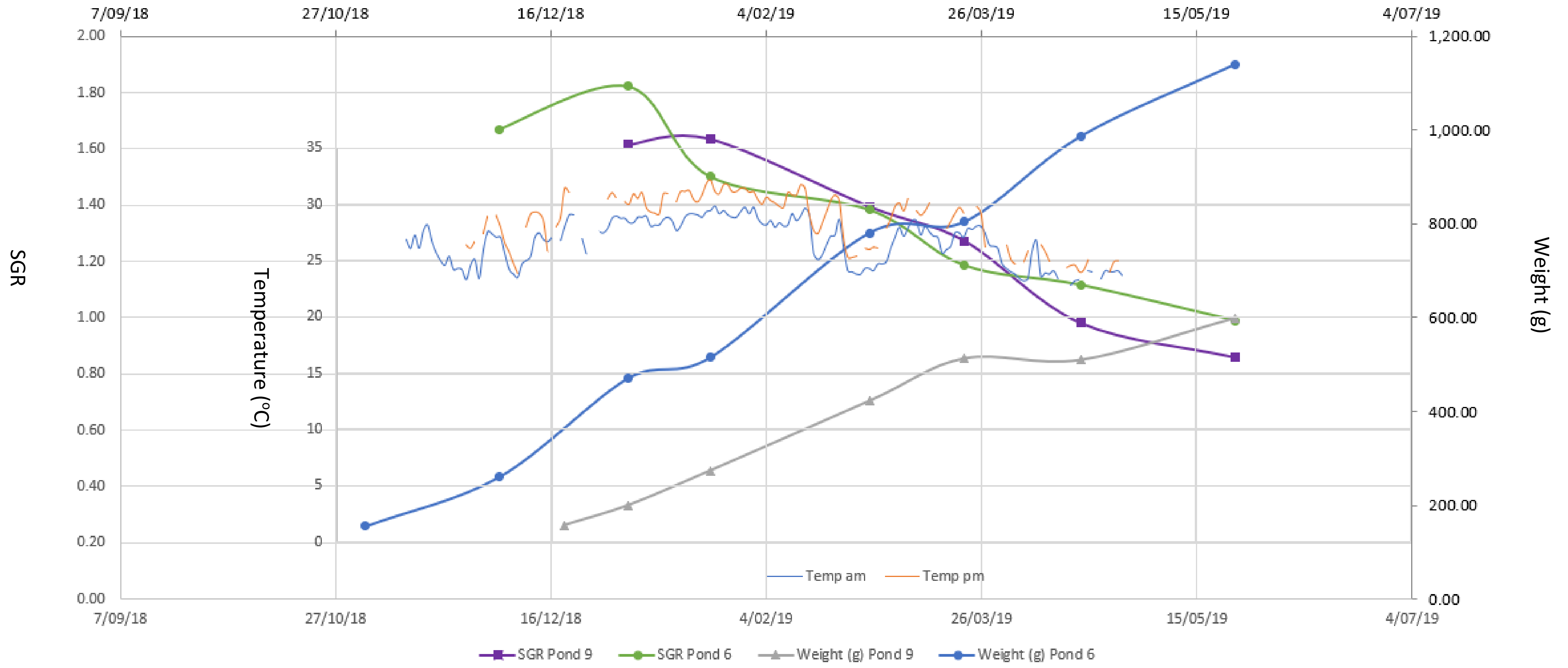
Appendix 4. Average monthly weights of grouper in various production systems



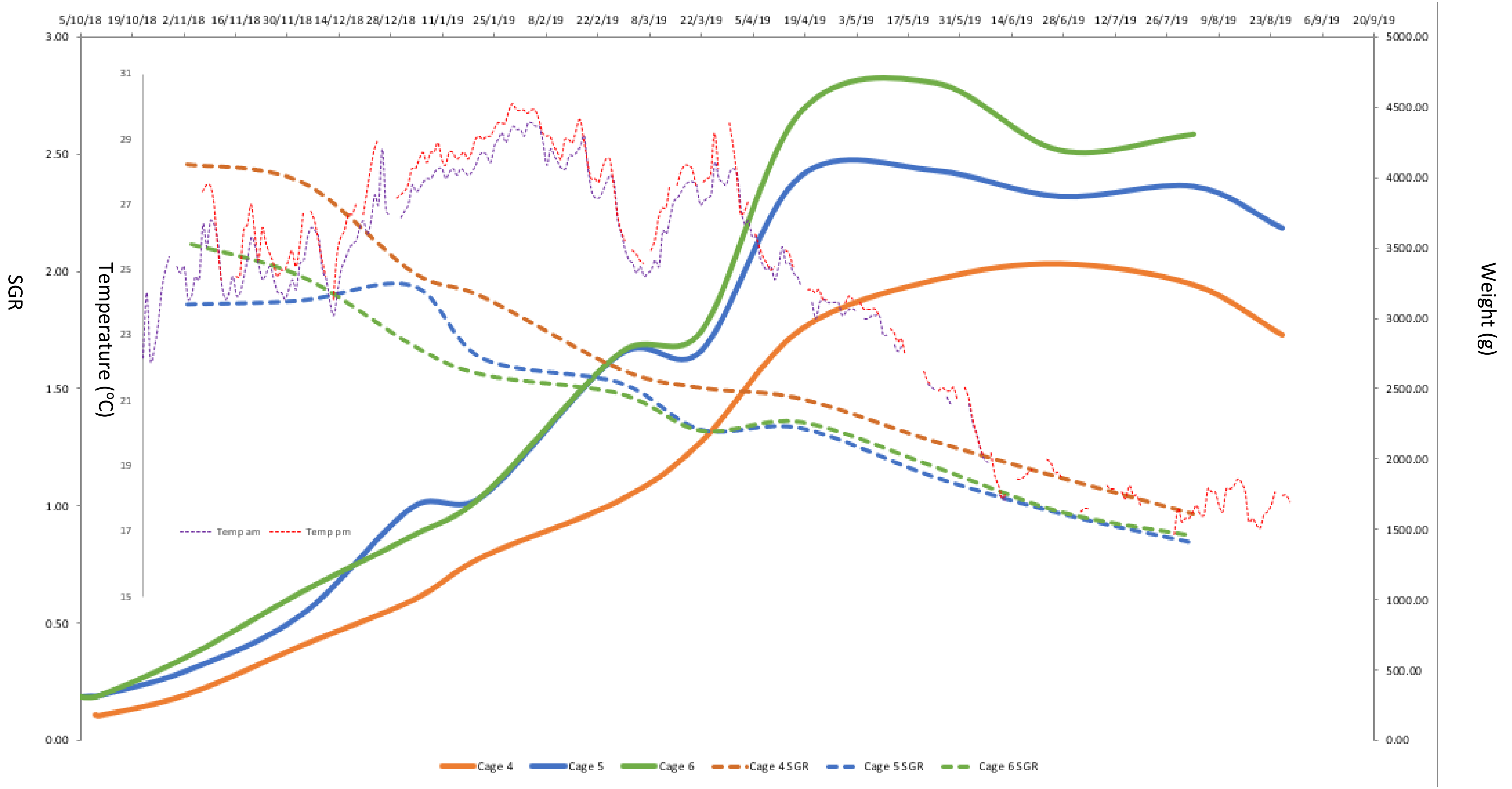
Appendix 5. Growth performance of giant grouper in tanks between 24-28oC



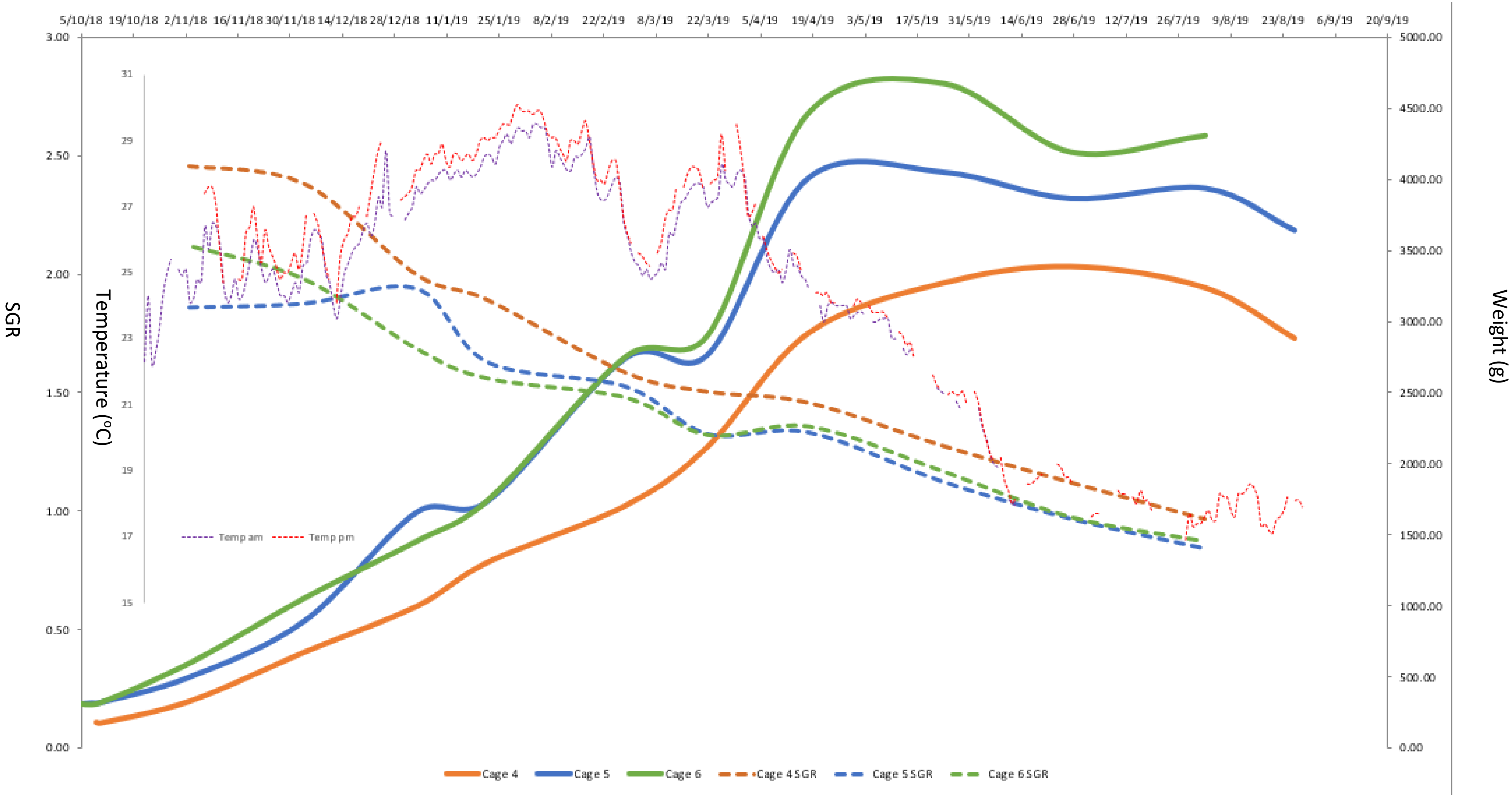
Appendix 6. Growth performance of giant grouper in ponds



Appendix 7. Growth performance of cobia in cages



Appendix 7. Growth performance of cobia in cages



Appendix 9. Summary of Health reports

Report	Health	Growth
1	- Outbreak of nodavirus occurred on 8/01/2019 in vaccinated giant grouper populations. - Hepatomegaly detected in giant groupers - Leeches detected in pond giant groupers. - Trichodina detected in giant groupers. - Gill fluke detected in giant groupers. - Cataracts detected in cage cobia. - Gas bubble disease detected in cage cobia.	- Early growth performance data presented for giant groupers and cobia. See Figure 14.1, Figure 14.2, Figure 14.3, Figure 14.4, Figure 14.5 for more details.
2	- Potential control strategies for leeches include: net change, copper sulphate - Hepatomegaly detected in giant groupers - Leeches detected in pond giant groupers. - Trichodina detected in giant groupers. - Cobia examined had normal gill architecture and an absence of ectoparasites.	- Best SGR, seen in cage giant grouper populations with higher feed rates compared to ponds. - FCR deteriorates with fish size in giant groupers. - There appears to be no negative effect of vaccination on growth performance in pond giant groupers. - Growth performance of the non-poor grade cobia were similar to one another. - Growth performance of the poor grade cobia was significantly lower than higher grade cobia. The performance different is likely due to differences in fish factors
3	- Leeches detected in pond giant groupers. - Hepatomegaly detected in giant groupers - Trichodina detected in giant groupers. - Tank giant groupers appear to be in good health - Trichodina detected in cage cobia.	- Best SGR (highest value) seen in caged giant groupers. - Significantly lower feed rate in vaccinated pond Giant Groupers compared to unvaccinated pond Giant Groupers. - Growth rate is lower in vaccinated pond Giant Groupers compared to unvaccinated pond Giant Groupers. - **Less** variation in weight for cage Giant Groupers compared to pond Giant Groupers. - **No significant** variation in length of cage and pond Giant Groupers. - **More** variation in condition factor for cage Giant Groupers compared to pond Giant Groupers - Less variation in condition factor seen in cage Giant Grouper in January and February. - Size difference in lower grade cobia is still apparent.

4	- An outbreak of nodavirus occurred on 28/3/2019 in vaccinated caged groupers. Two days prior to the outbreak, an accidental fire and was extinguished with foam next to the cages. Affected grouper appeared to be blind and displayed abnormal movements when removed from water. - Hepatomegaly detected in giant groupers - Low numbers of stalked protozoa (*Vorticella spp*.) were identified in the tank grouper. - Low to moderate levels of trichodina parasites were detected in grouper. - Leeches suspected were identified in pond grouper populations. - Cobia examined had normal gill architecture and an absence of ectoparasites.	- Better average production performance (SGR, SFR, eFCR, bFCR) in vaccinated giant groupers compared to unvaccinated giant groupers. - Very low to no mortalities in pond giant groupers. - Significantly higher feed rate in vaccinated giant grouper compared to unvaccinated giant grouper. - Growth rate is higher in vaccinated giant grouper compared to unvaccinated giant grouper - The cause of decreased feed intake is unknown. - Size difference in lower grade cobia is still apparent.
5	- Leeches suspected were identified in pond grouper populations. - Hepatomegaly detected in giant groupers - The vaccination appears to have a positive effect on survivability of Giant Groupers against nodavirus during outbreaks - Large concentrations of suspended solids in the tanks also promote elevated bacterial loads in the tanks and can challenge the skin defences - Cobia examined had normal gill architecture and an absence of ectoparasites.	- Secchi readings appear to have no correlation to feed consumption rates in pond giant groupers. - SGR decreased and eFCR and bFCR increased with increased fish weight in pond and cage giant groupers - The growth appears to decrease with temperature in the caged cobia population. - Size difference in lower grade cobia is still apparent.
6	- *Vorticella* stalked protozoa was observed in tank giant grouper - Hepatomegaly detected in giant groupers - During winter, healing and immune response of cobia is slower. Repeated harvest of fish may predispose them to skin trauma and subsequently to secondary bacterial skin infections. - Cataracts detected in cage cobia - No obvious pathology to explain why slow growing cobia are performing worse than higher graded counterparts.	- Pond performance of giant groupers has demonstrated considerable variation - Growth performance (lower SGR, high FCR with similar feed rates) in the unvaccinated fish were lower than that of the vaccinated fish during the early stages. Growth performance (lower SGR, high FCR with similar feed rates) was lower in unvaccinated and mixed vaccination status tanks, compared to the vaccinated tanks - SGR was better on average in the cage populations compared to the pond populations in giant groupers. - Bloom density (secchi) did not appear to consistently affect feed rate. - The growth appears to decrease with temperature in the caged cobia population. - Size difference in lower grade cobia is still apparent.

7	- Hepatomegaly detected in giant groupers - *Vorticella* stalked protozoa was observed in tank giant grouper - Low numbers of mortalities occurred in cobia populations at temperatures ranging between 16.5-18.0^{o}C - During winter, repeated harvest of fish may predispose cobia to skin trauma and subsequently to secondary bacterial skin infections. - Low to moderate levels of trichodina parasites were detected in cobia. - *Vorticella* stalked protozoa was observed in tank cobia - *Brookylnella* protozoa was observed in tank cobia. Elevated mortalities was observed in recently transported cobia.	- The growth appears to decrease with temperature in the caged cobia population. - Vaccinated giant groupers tend to have a higher coefficient of variance (CV) compared to the unvaccinated cohorts - Variance in growth rates was not clearly attributable to vaccination status.
8	- *Vorticella* stalked protozoa was observed in tank giant grouper - Hepatomegaly detected in giant groupers - Mortalities can be expected at temperatures ranging between 17.0-18.5^{o}C in cage cobia populations. - Little to negative growth was observed during winter when water temperatures fell below 20^{o}C in cage cobia. - During winter, repeated harvest of fish may predispose cobia to skin trauma and subsequently to secondary bacterial skin infections. - Epizoic diatoms detected in cage cobia (water temperature 17.0-18.5^{o}C). - Gill fouling detected in cage cobia. Epizoic diatoms did not appear to be correlated to gill fouling. - Low to moderate levels of trichodina parasites were detected in cobia.	- Vaccinated giant groupers tend to have a higher coefficient of variance (CV) compared to the unvaccinated cohorts - CV in the tank Giant Groupers appear to be higher than the other culture systems (cage and pond). The tank fish have not been graded since stocking. Grading will likely have an impact on reducing the CV among the tank cohorts and may improve overall cohort production. - Similar CV values have been observed between cages and tanks in the cobia populations.
9	- Hepatomegaly detected in giant groupers - It appears feasible to hold giant groupers in cages through winter. - Gastric dilation detected in cage cobia	- The feed rate of giant groupers appears to be higher in tanks than in cages or ponds (approx. 0.8% in ponds and cages vs approx. 1.0 – 1.7% in tanks prior to transfer). There may be factors such as shading, hiding areas and fish visibility that may have an influence on feed rate. - Unvaccinated giant groupers from Batch 4 escaped through hole in cage. - The feeding activity in cage cobia increased significantly as temperatures increased from 18°C to 20-23°C. Mortalities also ceased. - Vaccinated giant groupers tend to have a higher coefficient of variance (CV) compared to the unvaccinated cohorts - Variance in growth rates was not clearly attributable to vaccination status.

10	- Hepatomegaly detected in giant groupers - Gill arch deformity detected in a giant grouper - *Trichodina* was observed in cage giant grouper	- There appeared to be an effect of stocking density on appetite and food conversion efficiency, whereby the lowest stocking density tank (~13kg/m^3) ate 2.23 times more food compared to fish of same size/age in higher stocked tank (>40kg/m^3). However, there was no additional growth achieved. The food conversion efficiency of the lowly stocked group was ~2.5 times worse, than the high density tanks. - Early pond and cage data from the recent stockings suggest that the feed rate of Giant Groupers in tanks is higher than that of cages which is higher than that of ponds.
11	- Increased mucus on gills of cage giant groupers in more turbid waters - Hepatomegaly detected in giant groupers - *Trichodina* was observed in cage giant grouper - *Trichodina* was observed in cage cobia - Slow growth syndrome cobia may be due to multifactorial causes. Potential risk factors include the presence of granulomas, gastric dilation and genetics.	- There appeared to be a behavioural impact from transport of Giant Groupers from the hatchery to the cages. Initially, feed rate in the cages decreased after transport. The feed rate in the cages has since increased from the time of stocking from the hatchery. - The feed rate of cobia increased after transfer from the hatchery to growout cages.
12	- Leeches were observed in pond giant grouper - Giant groupers do not appear to actively predate on tiger prawns. - *P.monodon* may assist to keep leech numbers low, however do not appear to be able to completely eradicate leeches from a pond - The livers of Giant Groupers appear to accumulate glycogen not fat - Elevated dietary carbohydrates are one of the major factors that have been associated with GHH - *Trichodina* was observed in cage cobia	- The decrease in dissolved oxygen in the lake system may be a result of increasing BOD (biological oxygen demand) through the input of nutrient not being assimilated by the lake benthos and pelagic biota. The decline in groundwater level may be exacerbating this impact if there is reduced functional exchange now occurring. The net effect of lowered ambient oxygen is commonly reduced fish growth and elevated disease risk. - The CV of the weight of cobia do not appear to be differing significantly between months. After stocking into cages, the CV did not appear to increase significantly. When comparing the cages and tanks, the CV of the weight, length and condition factor appear to be similar between the groups. - In the previous season, there appears to be a trend that vaccinated Giant Groupers have a higher coefficient of variance (CV) compared to the unvaccinated cohorts (see previous reports for details). This difference appears to be again present in the cage population, however this is not present in the pond and tank populations. The CV of length of Giant Groupers did not appear to be influenced by vaccination status.