Transitioning cobia aquaculture research and development in Queensland to industry

Evan Goulden, Luke Dutney, Brad Cherrie, Richard Dickson, Trevor Borchert, Tom Gallagher,

Hazra Thaggard, Philip Brady, Serena Zipf

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Researcher Contact Details

Name: Dr Evan Goulden
Address: PO Box 2066
Woorim QLD 4507
Phone: 07 3471 0962
Fax:
Email: evan.goulden@daf.qld.gov.au

FRDC Contact Details

Address: 25 Geils Court
Deakin ACT 2600
Phone: 02 6122 2100
Email: frdc@frdc.com.au
Web: www.frdc.com.au

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Abbreviations

AR	Aspect ratio
BIRC	Bribie Island Research Centre
BSL	DAF Biosecurity Sciences Laboratory
BW	Body weight
COVID-19	SARS-CoV-2 coronavirus disease
CV	Coefficient of variation
DAF	Queensland Department of Agriculture and Fisheries
DO	Dissolved oxygen
dph	Days post hatch
EDTA	Ethylenediaminetetraacetic acid
FCR	Feed conversion ratio
FCR_{adj}	Adjusted feed conversion ratio
FI	Feed intake
H3	Histone H3 gene
inds	Individuals
M_H	Myotome height
PCR	Polymerase chain reaction
RAS	Recirculating aquaculture system
RD&E	Research, Development and Extension
RPA	Rocky Point Aquaculture Pty Ltd
SE	Standard error
SGR	Specific growth rate
S_L	Standard length
W_f	Final weight
WSD	White spot disease
WSSV	White spot syndrome virus
TAN	Total ammonia nitrogen

Executive Summary

What the report is about

This report details a collaboration between the Queensland Department of Agriculture and Fisheries (DAF) and Rocky Point Aquaculture Pty Ltd (RPA), who sought to transition cobia (*Rachycentron canadum*) aquaculture research, development, and extension (RD&E) to commercial production. RPA successfully adopted cobia breeding and fingerling production processes across 2019-2021, producing commercial volumes of fingerlings (~65,000 inds). Fundamental to this success were refinements made to larval rearing systems including taurine-enriched live feeds, microalgae, water flow fields, and tank photic condition. Tank studies on harvestable-sized cobia conducted at DAF's Bribie Island Research Centre (BIRC) showed adjustments in feeding frequency could reduce feed input without affecting growth performance. Fingerling production gains of more than 200% throughout this project have heralded the revitalisation and expansion of the cobia sector and validated a pivotal business decision by RPA to invest in finfish aquaculture at their operations in southeast Queensland. A cobia aquaculture information webpage was developed within the Queensland Government website (Business Queensland) to provide business owners or intenders key information on cobia aquaculture, including links to current and historical reports that detail enabling strategies for the production of cobia at commercial scale.

Background

The Queensland aquaculture industry was valued at a record $165 million in 2019/20, with significant potential for expansion. A series of present-day shocks such as disease and market disruption have challenged this notion, bringing into focus the role of species diversification in shaping future industry resilience. For more than a decade, DAF and collaborators have evaluated cobia aquaculture as a farm diversification option, developing an evolving 'pond to plate' framework for industry uptake. Cobia was recently identified as a feasible alternative to prawn farming in southeast Queensland following the white spot disease shock in 2016. However, cobia aquaculture production has plateaued at both the domestic (100 t per year) and global (40-50,000 t per year) level, primarily due to the high cost of maintaining the nutritional and environmental conditions needed to support the rapid growth of this species. Key to the reinvigoration and expansion of the domestic cobia sector will be the adoption of breeding and fingerling production processes at commercial scale. Further gains will need to be found in seedstock supplies, disease management, and in feeding efficiencies of fish approaching harvestable-size that typically account for ~80% of the total feed costs (Benetti *et al.*, 2021).

Aims/objectives

The overarching aim of this project was to support the uptake and expansion of cobia production processes at commercial scale. This involved the biosecure supply of domesticated broodfish and seedstock to RPA, conditional access to seedstock by future project entrants, and exchange of reproductive knowledge between BIRC and RPA. Success was measured by the quantities of fingerlings produced at RPA against commercially-relevant targets (20,000 in 2019/20; and 40,000 in 2020/21). Another component of the project was aimed at optimising feeding strategies (feed frequency) for harvestable-sized cobia using a commercially available finfish diet at BIRC. The final objective was to extend outcomes of this report and that of previous cobia RD&E projects to end users via a web portal.

Methodology

At the beginning of the spawning season 2019/20, broodfish lines domesticated at BIRC were tested for notifiable pathogens white spot syndrome virus (WSSV) and betanodavirus. Selected fish were retained at BIRC or transported by road to RPA. Broodfish populations at both sites were conditioned

in recirculating aquaculture systems (RAS) by photoperiod and water temperature (i.e., photothermal) manipulation and induced to spawn using hormonal therapy. Fertilised eggs produced at BIRC were tested for notifiable pathogens and transported to RPA for fingerling production.

Larval rearing systems were stocked with 2 days post hatch (dph) larvae originating from spawns at either BIRC or RPA. In the first season (2019/20), larvae were intensively cultured in clearwater systems (10,000 L parabolic tanks) and fed successions of live rotifers (*Brachionus plicatilis*), *Artemia*, and graded manufactured diets. Larval rearing approaches were refined for season 2020/21, including intensive culture in 5000 L circular tanks with live microalgae (*Nannochloropsis oculata*) until 10 dph, and feeding of taurine-enriched rotifers and *Artemia*. A replicated commercial-scale experiment was performed to better elucidate the effect of live feed diet (with or without taurine or copepod supplementation) and site (BIRC or RPA) on larval performance to 12 dph. Larvae from the same seedstock were used at both sites and reared according to the refined protocols for 2020/21. Larval performance was measured by analyses of size, development, gut content, and survival. Fingerlings (35 – 105 dph) were counted and transferred to overwintering systems.

A replicated feed trial was performed to examine the effect of feed frequency (1, 2 or 3 times daily) of a commercial diet (Pelagica Float, Ridley Aquafeeds Ltd) on harvestable-sized cobia (2.55 kg). Growth performance (SGR, W_f, WG, CV, FI, FCR_{adj}) was measured at the end of the 57-day experiment. Suspected *Neobenedenia* sp. parasites affecting feed trial fish were phylogenetically resolved by sequencing of the H3 gene (Brazenor *et al.*, 2018).

Results/key findings

RPA successfully adopted commercial-scale cobia breeding and fingerling production processes. A biosecure breeding population was established at RPA in season 2019/20 that was induced to spawn successfully on three occasions during the project. This proved conceptually a capability to produce seedstock. Commercial volumes of fertilised eggs (6.84 million) were supplied under biosecure conditions from BIRC and used to support fingerling production activities at RPA. Regrettably, surplus seedstock was unable to be supplied to a potential project entrant due to the COVID-19 disruption.

RPA produced commercial quantities of fingerlings (~65,000) across two growing seasons (15,454 in 2019/20; 49,111 in 2020/21). The second season was exemplified by a >200% increase in productivity over the first season, and a >20% yield over the season target. Total fingerling output in this project also surpassed that of the previous two seasons by almost 300%. Record annual production for this species (>150 t) is forecast for 2021/22. A comparative analysis of commercial-scale larval rearing systems at BIRC and RPA indicated larval performance was significantly enhanced by site characteristics at RPA. Growth, development, and survival of RPA larvae was comparable or greater than reported benchmarks. Fundamental to the RPA success was a faculty to stimulate effective first feeding. This was likely enabled through a range of favourable and interactive conditions including taurine-enriched live feeds, microalgal density, water flow fields, and tank photic condition.

Optimised feed frequency at once per day for harvestable-sized cobia (~2.5-3.6 kg) was found also to be a practical strategy for reducing feed input (by 14.1-18.4%) without affecting growth performance. Phylogenetic analyses of partial H3 gene sequences confirmed parasites affecting harvestable-sized fish as *Neobenedenia girellae*. Several knowledge gaps were identified in this project through frequent communication and review of management practices. Possible drivers of poor larval quality produced during first and late season spawns, and exclusively at RPA, include maternal nutrient deficiencies and stocking stress. Mortality and growth-suppressive factors including malformations, cannibalism, epitheliocystis, and *Neobenedenia girellae* were found to pose further risks to cobia productivity.

Implications for relevant stakeholders

This project report provides detailed instructions for the successful uptake of cobia breeding and fingerling production processes at commercial scale in Queensland. Refinements to cobia broodstock husbandry practices and larval rearing systems with microalgae and taurine-enriched prey are set to yield performance benefits. The implementation of data-driven planning and control over larval rearing processes is primed to enhance productivity also and has broad applicability to the management of other species. Manipulation of feeding frequency for harvestable-sized fish is another strategy farmers could use to control input costs and minimise potential environmental impact.

Connecting end users to this published report and historical cobia aquaculture RD&E via the Business Queensland website will be central to future industry uptake of cobia production processes. Overall, the successful adoption of commercial-scale production processes in this project will instil confidence in operators wishing to explore new finfish opportunities within a dynamic industry landscape. Continued expansion of cobia production will contribute to resilience at the farm and industry level, and open economic opportunities in regional centres.

Recommendations

Variable reproductive performance of cobia broodfish found in this project represents a short-term challenge to the self-sufficiency of the cobia aquaculture sector. Several management levers including reducing stocking density, regimented ectoparasite treatments, and service agreements with BIRC to provide contingency seedstock supplies are recommended for the upcoming production season. Longer term objectives of the sector may include the implementation of genomic selection tools for seedstock quality assurance, monosex production, and determination of stressors that affect reproductive performance.

Increased data-directed planning and control of larval rearing parameters will help mitigate disease risks associated with larvae. For example, increased storage of quality water and the use of remote sensors to report poor quality water prior to farm intake is set to enhance operator responsiveness. Future research into flow field requirements, photic conditions, and disruptors of cannibalism will be necessary to further enhance survival outcomes of early larvae.

It is important also for cobia farmers to continue to evaluate feed frequency strategies and maintain a high level of connectedness with aquafeed manufacturers. This will ensure nutritional challenges are overcome and formulations evolve sustainably. There is likely value also in developing practical and welfare-friendly methods to suppress ectoparasites such as *Neobenedenia* sp. Ground-truthing the efficiencies and commercial outcomes of cleaner organisms for grow-out phases of cobia may open new research opportunities.

Keywords

Cobia; *Rachycentron canadum*; aquaculture; commercialisation; diversification; reproduction; larvae; fingerling; epitheliocystis; *Neobenedenia*; feed frequency; growth

Introduction

The Queensland aquaculture industry was valued at a record $165 million in 2019/20, for the first time exceeding 50% of total fisheries and aquaculture production value (Schofield, 2021). The industry has significant potential for expansion on the back of large investment in the marine prawn (*Penaeus monodon* and *Fenneropenaeus merguiensis*) and barramundi (*Lates calcarifer*) sectors and the release of over 7000 hectares of aquaculture development areas (ADAs). However, aquaculture industries are predisposed to frequent and high magnitudes of shocks (Gephart *et al.*, 2017, Cottrell *et al.*, 2019) with several impacting on the Queensland industry in recent years. In 2016/17, white spot disease (WSD) caused by WSSV, triggered an estimated $50 million in production and associated losses to the prawn sector (Ridge Partners, 2017). Imported barramundi products pose ongoing biosecurity risks to local production and dominate domestic market share (~60%) by sidestepping country of origin labelling governance (Hernandez-Jover *et al.*, 2017, Thyer, 2018). The industry dynamic is also being redefined by large, corporatized operations set to triple production volumes of prawns and barramundi in north Queensland (Norwood, 2019). This is likely to trigger resource-constrained small to medium enterprises to find new market niches (Stephens, In press). The COVID-19 disruption has had a substantial impact also on local and international food supply chains. Shifting consumer behaviours are likely to have a significant role in shaping the future of seafood industries (Fan *et al.*, 2021, Mobsby *et al.*, 2021, Ogier *et al.*, 2021), including the types of species cultured and the systems used to produce them (Hasan *et al.*, 2021, Love *et al.*, 2021).

Increasing aquaculture species diversity at the farm, industry, and national level is one strategy stakeholders and policymakers can employ to build resilience against shocks (Troell *et al.*, 2014, Metian *et al.*, 2020). Cobia, *Rachycentron canadum* (Linnaeus, 1776; Rachycentridae), was first evaluated as an aquaculture diversification option for Queensland in 2007 (Palmer, 2020). The species was recently identified along with giant grouper (*Epinephelus lanceolatus*) as feasible alternatives to prawn farming in southeast Queensland following the WSD shock (Cherrie *et al.*, 2020). Cobia is an important cosmopolitan marine fish that supports aquaculture and fisheries activities throughout its subtropical and tropical range (Shaffer & Nakamura, 1989). The species is extraordinarily fast growing, reaching 4-6 kg within the first 12 months of life and is adaptable to a range of aquaculture systems, including sea cages, tank-based recirculating aquaculture systems (RAS), coastal marine ponds (Liao *et al.*, 2004, Benetti *et al.*, 2008a, Benetti *et al.*, 2010a, Benetti *et al.*, 2010b, Dutney *et al.*, 2010a, Palmer *et al.*, 2010, Lee *et al.*, 2015, Sakthivel *et al.*, 2019a, Benetti *et al.*, 2021), and inland areas fed by amended saline groundwaters (Antony *et al.*, 2020). Wild cobia are typically solitary with limited commercial fisheries potential. However, cobia are targeted by recreational fishers for their fighting abilities and superb eating qualities (van der Velde *et al.*, 2010). Cobia is the only extant member of the family Rachycentridae that includes *R. stremphaencus* (Godfrey & Carnevale, 2021). Rising sea temperatures are projected to impact migratory and recruitment dynamics of contemporary populations of cobia (Crear *et al.*, 2020a, Crear *et al.*, 2020b).

DAF, along with multiple industry collaborators and primary support by the FRDC, has developed an evolving 'pond to plate' framework for industry uptake of cobia aquaculture (Lee *et al.*, 2015, Dutney, 2016, Dutney *et al.*, 2017, Lee *et al.*, 2018, Palmer, 2020, Lee *et al.*, In press). Advances have been made in reproductive control of broodfish and the first quantifications of sexual dimorphism and intersex individuals (Dutney *et al.*, 2010b, Dutney *et al.*, 2010c, Lee *et al.*, 2015, Dutney, 2016, Dutney *et al.*, 2017). Several larval rearing and grow-out approaches were further developed with the view to cost-effectively repurpose available farm infrastructures. A biphasic larval rearing approach was firstly described where larvae were transitioned from tank-based greenwater cultures at 12-14 days post hatch (dph) to low-input nursery pond systems (Borchert *et al.*, 2010, Palmer *et al.*, 2010, Wang *et al.*, 2010). Later development of indoor, intensive clearwater larval rearing systems permitted more stringent control over environment and biosecurity risks triggered by the WSD movement restriction

area in southeast Queensland (Lee *et al.*, 2018, Lee *et al.*, In press). Crucially, grow-out of cobia in converted prawn production ponds was found to be technically feasible, particularly in tropical northern Queensland (Dutney *et al.*, 2010a). Collaborating prawn farmers demonstrated growth of 4-5 kg in the first year of life and annual yields of more than 30 t ha^{-1} (Dutney *et al.*, 2010a, Lee *et al.*, 2015, Lee *et al.*, 2018). The bulk of farmed cobia in Australia is sent to market as whole fish 2-6 kg (Cherrie *et al.*, 2020) with a fillet recovery rate of 46% (Kidluff, 2001). Market penetration and awareness of cobia products was greatly aided by post-harvest research. Eating qualities were found to be equivalent to Atlantic salmon, and value-streaming (e.g., smoking) enhanced sensory attributes and shelf-life (Lee *et al.*, 2015). By 2017, the state government-owned Bribie Island Research Centre (BIRC) had become a nucleus for cobia seedstock supply, technology transfer, and extension of husbandry training activities (Lee *et al.*, 2015, Lee *et al.*, 2018, Lee *et al.*, In press). However, farmers reported challenges associated with grow-out in ponds, particularly disease and the high cost of maintaining water quality (i.e., oxygen demand and nitrogen removal) to support the rapid growth of this species. This decelerated plans for the sector to adopt broodfish and hatchery facilities needed to drive independent seedstock production. As a result, BIRC was burdened with the task of producing commercial volumes of seedstock and domestic production stagnated at 100 t per year.

This pause in sentiment was echoed at the global level, where cobia production plateaued at 40-50,000 t per year (FAO, 2020). Many commercial cobia hatcheries have disbanded due to the high cost of culture (Benetti *et al.*, 2021). The future viability of cobia aquaculture depends on the availability of quality seedstock and fingerlings, disease management, and feeding efficiencies throughout the farming cycle (Benetti *et al.*, 2021). Cobia seed supply can be affected by frequent mass mortality of early larvae (Franks *et al*., 2001, Hitzfelder *et al*., 2006, Gopakumar *et al*., 2012, Lee *et al*., In press). However, some hatcheries have produced reliable, commercial quantities of fingerlings for several years (Benetti *et al.*, 2008a, Benetti *et al.*, 2008b). Further gains in cobia larval performance have been demonstrated experimentally by the administration of live microalgae (Faulk & Holt, 2005), taurine-enriched live feed (Salze *et al.*, 2011, Salze *et al.*, 2012b, Salze *et al.*, 2012a), and copepods (Nguyen, 2009). Live microalgae benefit developing fish larvae through backlighting for enhanced prey pursuit and capture, and the production of chemicals that modulate immune, digestive, and gut microbiome function (Van der Meeren, 1991, Palmer *et al*., 2007, Rocha *et al*., 2008, Hemaiswarya *et al*., 2011, Yarnold *et al*., 2019). Dietary taurine has numerous functional roles in lipid metabolism, osmoregulation, and bone development (Salze & Davis, 2015) and is considered an essential nutrient for early development and survival of fish larvae including yellowtail kingfish *Seriola lalandi* (Rotman *et al*., 2017), amberjack *S. dumerili* (Matsunari *et al*., 2013), red sea bream *Pagrus major* (Chen *et al*., 2004), rock sole *Lepidopsetta polyxystra* (Hawkyard *et al*., 2014), Japanese flounder *Paralichthys olivaceous* (Chen *et al*., 2005), and cobia (Salze *et al.*, 2011, Salze *et al.*, 2012b). Copepods are an essential prey for the planktivorous first feeding stages of wild fish larvae, and have been investigated as a first feed for cultured fish larvae due to the range of benefits they provide, including small size and movement triggering feeding responses (Buskey, 2005), providing a rich source of essential polyunsaturated fatty acids (PUFAs), proteins, amino acids, antioxidants (e.g. astaxanthin), carotenoids, and digestive enzymes (Lee *et al.*, 2008, Drillet *et al.*, 2011, Nielsen *et al.*, 2017, Santhosh *et al.*, 2018, Radhakrishnan *et al.*, 2020).

Parasites and microbial diseases have restricted the broader industry uptake and expansion of cobia aquaculture (McLean, 2008). Cosmopolitan and broad-host ectoparasitic flukes such as *Neobenedenia* sp. may cause blindness, suppression of feeding and growth, septicaemia and death and have been reported for cobia cultured under a variety of conditions (Lopez *et al.*, 2002, Liao *et al.*, 2004, Ogawa *et al.*, 2006, Dutney *et al.*, 2010c, Kerber *et al.*, 2011, Moreira *et al.*, 2013, Hurley-Sanders *et al.*, 2016). Amyloodiniosis, caused by the broad-host parasitic dinoflagellate *Amyloodinium ocellatum*, is a rapidly transmissible disease often resulting in respiratory stress (Francis-Floyd & Floyd, 2011) and linked to mortality of early (Liao *et al.*, 2004, Benetti *et al.*, 2007, Benetti *et al.*, 2008a) and grow-out (Lee *et al.*, 2015, Cherrie *et al.*, 2020) stages of cobia. Epitheliocystis, another respiratory disease, is caused by a

range of host-specific intracellular bacteria (Nowak & LaPatra, 2006, Blandford *et al.*, 2018) for which *Endozoicomonas elysicola* (Mendoza *et al.*, 2013) and an uncharacterised non-*Chlamydiae* bacterium (Lee *et al.*, 2018) have been implicated in mass mortality in cobia hatcheries. Symptomatic gill lamellae impair gas transfer and osmoregulatory function, signified by flared opercula and gasping, inappetence, and lethargy (Nowak & LaPatra, 2006). Cobia cultured at seasonally low (<20^{o}C) and high (>30^{o}C) temperatures also appear susceptible to photobacteriosis (pasteurellosis) and vibriosis, bacterial diseases associated with *Photobacterium* sp. and *Vibrio* sp., respectively (Liao *et al.*, 2004, Dutney *et al.*, 2010a, Ramachandra *et al.*, 2020, Shimada *et al.*, 2020). Moreover, sustained exposure to hypoxic conditions is known to mediate shifts to the intestinal microbiome and enrich *Photobacterium* populations in juvenile cobia, increasing the risk of metabolic disorders and disease (Wang *et al.*, 2021). Preliminary trials using inactivated bacterial vaccines for *Vibrio* sp. and *Photobacterium* sp. infections for farmed cobia have shown promise as preventative control measures (Lin *et al.*, 2006, Rameshkumar *et al.*, 2020).

Better management of feeding efficiency can improve the profitability and environmental outcomes of cobia farming (Benetti *et al*., 2021) as feed is considered one of its highest operational expenditures (Miao *et al.*, 2009). Most of the feed and nutritional research to date has been performed on small juvenile cobia (<100 g), and in doing so has neglected harvestable-sized fish (>2 kg) that are responsible for around 80% of feed costs during production (Benetti *et al.*, 2021). Still, a rich and progressive nutritional research field continues to support the development of optimal nutrient profiles, feed utilisation and digestibility, and social licence of feed ingredients for cobia (Fraser & Davies, 2009, Suarez *et al.*, 2013, Raggi *et al.*, 2019). Specifically, dietary requirements for the species have been investigated for amino acids (Zhou *et al.*, 2006, Zhou *et al.*, 2007, Ren *et al.*, 2014, Watson *et al.*, 2014a), lipids (Chou *et al.*, 2001, Wang *et al.*, 2005), carbohydrates (Webb Jr *et al.*, 2010, Ren *et al.*, 2011, Zhao *et al.*, 2020), and vitamins and minerals (Wang *et al.*, 2006, Xu *et al.*, 2007, Mai *et al.*, 2009, Liu *et al.*, 2010, Yang *et al.*, 2010, Liu *et al.*, 2013, Zhou *et al.*, 2013). From an environmental sustainability standpoint, good health and growth outcomes have been demonstrated for cobia diets containing partial fishmeal replacements, including soybean products (Chou *et al.*, 2004, Zhou *et al.*, 2004, Trushenski *et al.*, 2011, Watson *et al.*, 2014b), shrimp waste (Lu & Ku, 2013), and combinations of ingredients derived from plants and marine organisms (Salze *et al.*, 2010). However, emerging sectors with low production volume like the Queensland cobia sector are unlikely to invest in research needed to develop species specific feeds.

Optimising feeding frequency of available aquafeeds may be a more practical and cost-effective strategy for improving the growth, environmental performance, and profitability of farmed cobia. Increasing feed frequency over a diurnal period was shown to positively effect growth in Atlantic halibut *Hippoglossus hippoglossus* (Schnaittacher *et al.*, 2005), Japanese flounder *Paralichthys olivaceus* (Lee *et al.*, 2000a), yellowtail flounder *Limanda ferruginea* (Dwyer *et al.*, 2002), mullet *Mugil liza* (Calixto da Silva *et al.*, 2020), snapper *Pagrus auratus* (Booth *et al.*, 2008) and pirarucu *Arapaima gigas* (Rodrigues *et al.*, 2019). Conversely, feeding more frequently than necessary could lead to additional operational cost, deteriorated environmental quality (Lee *et al.*, 2000a, Booth *et al.*, 2008), and skewed weight gain due to lipid deposition (Lee *et al.*, 2000b, Trushenski *et al.*, 2012, Sun *et al.*, 2016) that affects the quality and shelf-life of the final product (Calixto da Silva *et al.*, 2020). So far, there have only been two cobia feed frequency studies on small (16-110 g) juveniles (Costa-Bomfim *et al.*, 2014, Moreira *et al.*, 2015) and work on cobia approaching harvestable-sized is lacking.

Key to the reinvigoration and expansion of the Queensland cobia sector will be the adoption of breeding and fingerling production processes at commercial scale. This project seeks to support the commercial uptake of these processes and extend RD&E outcomes to end users through web-based media.

Objectives

The overarching aim of this project was to support the uptake and expansion of cobia production processes at commercial scale. This was distilled into three main objectives:

1) Expand cobia production:
    a) Supply of biosecure broodfish from the Bribie Island Research Centre (BIRC) to Rocky Point Aquaculture (RPA).
    b) Bilateral exchange of broodfish management, reproductive knowledge, and technical support between BIRC and RPA.
    c) On-demand supply of quality fertilised eggs and/or 1 - 2 day old larvae from BIRC to RPA.
    d) Optional fee for service ($10,000 per year) entry to future project participants, enabling access to BIRC seedstock through pro rata arrangement.

2) Optimise feeding strategies for farmed cobia using commercially available finfish diets:
    a) Conduct a feed trial at BIRC in collaboration with RPA to test the effect of feed frequency of a commercially available fish diet on cobia growth performance (FCR, SGR, biomass).
    b) Conduct a feed trial at BIRC in collaboration with RPA to fast-track winter growth of cobia juveniles using a commercially available fish diet. Note: Objective 2b was removed from the project following the cease of manufacture of the commercial diet Pelagica (Ridley Aquafeeds Ltd).

3) Contribute project deliverables to the DAF cobia aquaculture Research Development & Extension (RD&E) web portal.

Method

Animal ethics statement

All fish culture activities, manipulations, and experiments at BIRC were conducted under DAF Animal Ethics Committee (AEC) approvals. Manipulations (e.g., handling, weight checking, tissue collection, cannulation, hormonal inductions) were carried out while fish were sedated with AQUI-S® (AQUI-S New Zealand Ltd.). Unless otherwise stated, this was achieved initially by lowering the water level of holding tanks to a working depth with aeration and administering 10 mg L^{-1} AQUI-S® for light sedation. Fish were subsequently transferred to a smaller tub to achieve heavy sedation (15 - 25 mg L^{-1} AQUI-S®) for manipulations.

Expanding cobia production

Broodfish supply and management

Founding stocks of wild cobia caught in 2012 were used to establish second (F2) and third (F3) filial generation broodfish lines maintained at BIRC. At the beginning of growing season 2019/20, twenty-six (26) and twenty (20) broodfish were selected from these lines for breeding programs at BIRC and RPA, respectively. Selections were based on age, condition, and level of genetic relatedness. Individual fish were marked with a passive integrated transponder (PIT) tag in the dorsal musculature for identification.

Tissues were collected non-destructively for screening of notifiable pathogens. Briefly, gill filaments were excised using sterile scissors and fixed in either 10% formalin or 70% ethanol. Blood (1 mL) was extracted from the caudal vein into 10% EDTA using a 21G x 38 mm needle and 1 ml syringe. Tissue samples were submitted to DAF Biosecurity Sciences Laboratory (BSL) for PCR testing of betanodavirus (blood) and WSSV (gills), and for histopathological signs of gill infections (e.g., epitheliocystis/amyloodiniosis). Following clearance by Biosecurity Queensland, broodfish selected for RPA were prepared for transportation by purging for 48 h and bathing in formalin (200 mg L^{-1} for 60 min) with oxygenation to remove ectoparasites. Broodfish were loaded at a maximum density of 50 kg m^{-3} into closed transport carriers (1 m^3) that contained filtered and ozonated seawater (temperature 25.4 – 26.2°C; pH 8.2 – 8.3; dissolved oxygen [DO] 7.1 - 8.8 mg L^{-1}; and salinity 36.3 ppt), oxygen diffusers, and an air pump. Broodfish were transported approximately 2 h by road to RPA where they were unloaded into quarantine receiving tanks (10,000 L) supplied with flow through seawater (temperature 26°C; DO > 5 mg L^{-1}) at an exchange of 300% per day. Additional female broodfish (F2) captured from production lakes at RPA were also quarantined. Broodfish were quarantined for seven days and fed squid and pilchards *ad libitum*. Formalin (200 mg L^{-1} for 60 min) and freshwater (5 min) bathes were performed on days 1, 3 and 7 before relocation to maturation tanks.

Broodfish maturation tanks at RPA or BIRC consisted of multiple, independent recirculating aquaculture systems (RAS) under photothermal control (Table 1). New, treated seawater was provided to the RAS daily at ~10% of total system volumes. The treatment sequence for new seawater at RPA included disinfection and flocculation by chlorine in three 1 ML intake settlement ponds; 1 µm filtration; ozonation; activated carbon filtration; and UV irradiation. At BIRC water treatment included spin disc filtration (40 µm); multimedia filtration; ozonation; activated carbon filtration; and 10 µm cartridge filtration. Maturation tank water outflows were circulated through a sequence of RAS treatment modules before returning to the tank. For RPA this RAS sequence included a drum filter (48 µm); circulation pump; protein skimmer; biofilter; UV steriliser; and heat transfer pump. For BIRC this sequence included a coarse screen filter (500 μm); circulation pump; zeolite filter; UV steriliser; fine particulate screen (80 µm); heat/chill unit; foam fractionator; and moving bed bioreactor.

Broodfish were stocked in maturation tanks between 1.0 – 6.2 kg m^{-3} in female to male ratios of 0.4:1 – 2.5:1 (Table 1). In the off-season, broodfish at RPA were fed four times per week, alternating days of prawns, crabs, pilchards, and squid at 1.6 – 6.7% of tank biomass (Table 1). Following the discovery of suspected taurine deficiency in broodfish (septic interstitial nephritis), encapsulated taurine was implanted into pilchard feeds at 0.5% the daily ration once per week from May 2020 at RPA only. At BIRC, broodfish were fed three times per week, alternating days of pilchards and cephalopods (cuttlefish, squid, and octopus) at 0.5 – 10.8% of tank biomass (Table 1). During spawning season (October – April) feeding frequency was increased to 6-7 times per week at RPA and to four times per week at BIRC. At this time pilchard rations at BIRC only were supplemented with a vitamin mixture once weekly according to Dutney *et al.* (2010c) and Dutney (2016). At RPA, taurine supplementation was increased to 1.0% of daily ration once per week. General observations of fish health, behaviour and feeding responses were performed daily. Formalin bathing (150 – 200 mg L^{-1}) was carried out prophylactically at BIRC in monthly intervals, and reactively as required at RPA. Water quality measurements (e.g., temperature, dissolved oxygen, salinity, and pH) were recorded daily (Table 1). Water temperature was managed by heater setpoint adjustments and water flow rate; dissolved oxygen was maintained by aeration and water exchange rates; and pH was stabilised through the application of soda ash (Na_2CO_3) as required.

Table 1. Specifications of cobia broodfish maturation tanks at BIRC and RPA

Parameter	**Season 2019/20 (Nov-Jun)**		**Season 2020/21 (Jul-Jun)**	
Site	RPA	BIRC	RPA	BIRC
Tank volume (L) and number (#) of tanks	50,000 (1)	30,000 (4)	50,000 (2)	30,000 (4)
No. fish per tank	5 - 20	5 - 15	7 - 24	3 - 11
Stocking density (kg m^{-3})	1.0 - 4.7	2.1 - 6.1	2.2 - 6.0	2.4 - 6.2
Sex ratio (♀:♂)	1:1	1:2 - 1.5:1	0.8:1 - 2.6:1	0.4:1 - 2.5:1
Feed ration (% biomass)	2.0 - 5.0	0.5 - 10.8	1.6 - 6.7	0.6 - 8.0
Temperature (°C)	23.2 - 28.5	21.0 - 29.2	22.7 - 28.3	20.9 - 27.9
Dissolved oxygen (mg L^{-1})	3.7 - 8.0	4.1 - 6.4	3.5 - 7.2	3.9 - 7.6
Salinity (ppt)	16.0 - 38.0	32.4 - 36.8	25.0 - 37.0	32.3 - 36.5
pH	5.9 - 8.8	7.3 - 8.1	7.1 - 8.4	7.2 - 8.2

Biosecure seedstock supply

Seedstock (fertilised eggs or early larvae) produced from cobia broodfish at BIRC was made available on-demand to RPA and conditionally to new project entrants. A formal expression of interest for seedstock supply was lodged in early March 2020 by Indian Ocean Fresh Australia (IOFA) in association with the Western Australia Department of Primary Industries and Regional Development (DPIRD). Regrettably, the parties were unable to arrange contractual, logistical and biosecurity agreements before national public health directives were implemented in response to the COVID-19 emergency. With IOFA pausing operations from September 2020 due to the COVID-19 disruption, there was no

further interest lodged for seedstock by prospective project entrants. As such, all seedstock produced at BIRC during the project was supplied exclusively to RPA for fingerling production.

Broodfish at RPA and BIRC were conditioned to spawn in maturation tanks during the austral summer through environmental and hormonal manipulations. Commencing in August, the phototherm was progressively increased by 0.5 units to a photoperiod of 14 h and water temperature 27.5^{o}C. The phototherm was sustained between October and April. Gonadal biopsies were performed (Lee *et al.*, 2015, Dutney, 2016, Lee *et al.*, 2018) on female broodfish to determine the state of ovarian maturation up to two weeks before hormonal inductions. Micrographs of the biopsy samples were taken using either an inverted or stereomicroscope fitted with a digital camera (Nikon Digital Sight DS-Fi2). Oocyte size-frequency distribution was mapped (Lee *et al.*, 2015, Dutney, 2016) within an image area of 29 mm^2 using Nikon NIS Elements BR 5.11 software. Where possible, only females with a predominance of quality matured oocyte stages (>685 µm Ø) were selected for hormonal inductions. Males were checked for spermiation by abdominal massage and were hormonally induced if milt was not expressed. Broodfish were implanted between 0600 and 0800 h with luteinising hormone releasing hormone analogue (LHRHa) in sustained release cholesterol pellets (Lee *et al.*, 1986) using dosages of 40 µg LHRHa kg^{-1} for females or 20 µg LHRHa kg^{-1} for males. Four to five implanted spawners in female to male ratios of 1-1.5:1 were transferred to a clean and vacant spawning tank (BIRC/RPA), or when vacant tanks were unavailable, returned to the general population for spawning (RPA).

Eggs spawned within broodstock tanks were harvested into collection baskets (20 mm PVC frame, 500 µm mesh) positioned within reservoirs of tank side-overflow drains. At RPA, eggs were gently harvested and stocked into conical-bottomed hatch tanks (1000 L) supplied with flow through seawater (temperature 25.8 – 29.1 ^{o}C; DO 5.1 – 6.5 mg L^{-1}; pH 7.6 – 8.1; salinity 29.8 – 36.5 ppt) at 2 L min^{-1}. At BIRC, seedstock (fertilised eggs or early larvae) were prepared for on-demand supply to RPA. At harvest eggs were surface disinfected with 5 mg L^{-1} povidone-iodine (Ovadine, Syndel, USA) for 60 s at the gastrulation stage (Fig. 1a) approximately 12 – 13 h post fertilisation (Lefebvre & Denson, 2012, Sakthivel *et al.*, 2012). Eggs were stocked (up to 5000 eggs L^{-1}) in aerated conical-bottomed hatch tanks (450 L or 1000 L) supplied with flow through seawater (temperature 26.6 – 28.7^{o}C; DO 4.7 – 5.7 mg L^{-1}; pH 7.6 – 8.1; salinity 32.6 – 36.2 ppt) at 2 L min^{-1}. Fecundity (total eggs), fertilisation rate (%), and embryo development were determined from repeated volumetric samples and examined microscopically. Unviable eggs were periodically removed by siphoning settled negatively buoyant eggs following cycles of discontinued aeration. Viable, fertilised eggs were readied for transport at the segmentation/organogenesis stage (Fig. 1b) approximately 16 – 18 h post-fertilisation (Lefebvre & Denson, 2012, Sakthivel *et al.*, 2012) or allowed to hatch in incubators for transportation as early larvae (1 - 3 dph). A sample of eggs (*n* = ~50) was preserved in >70% ethanol and submitted to DAF BSL for pathogen testing (betanodavirus and WSSV). Seedstock was transported in oxygen-pressurised aquarium bags (150 µm thickness, 30 L total volume) in 15 L of seawater at 650 - 8000 inds. L^{-1} containerised in airline approved 40 L polystyrene boxes. At receival, eggs and larvae were acclimated in quarantine hatch tanks or larval rearing tanks. Tank effluent water was batch treated with 100 mg L^{-1} chlorine for 24 h and neutralised before release. Movement of larvae was constrained to receival quarantine areas until pathogen test results were declared and clearance was authorised by Biosecurity Queensland.

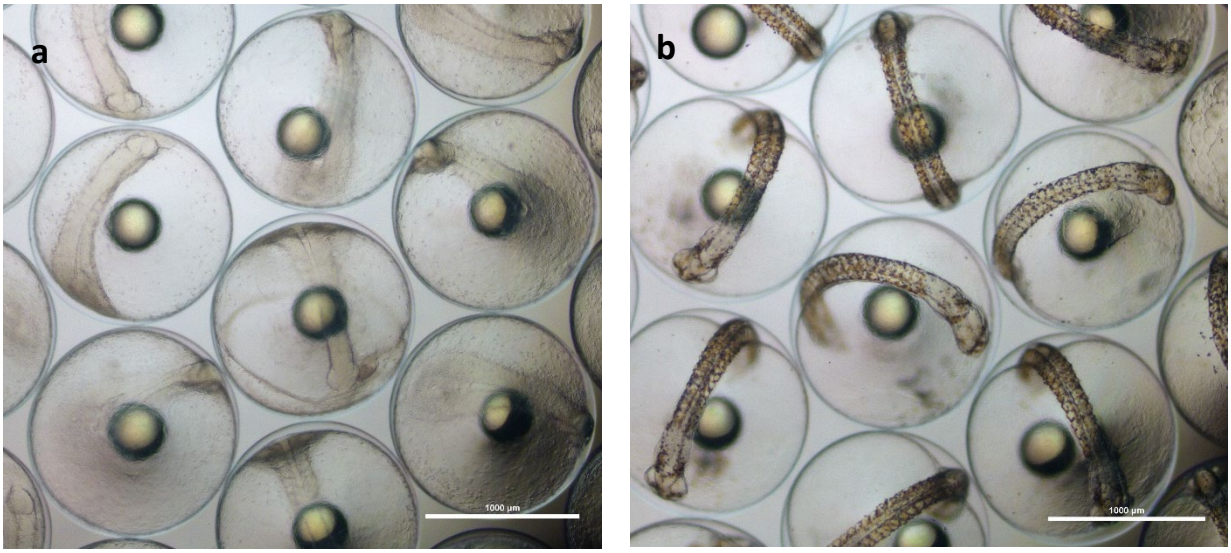

Figure 1. Cobia embryo development
(a) Gastrulation, approximately 12-13 h post fertilisation. Note the presence of tail buds, optical cups and free melanophores. Eggs were surface disinfected with 5 mg L^{-1} povidone-iodine at this stage. (b) Segmentation/organogenesis, approximately 15 - 16 h post fertilisation. Note distinct somites, eye rudiment, migration of melanophores to oil globule. First flexion (movement) was detected. Eggs were prepared for transport at this stage. All scale bars = 1000 µm.

Fingerling production at RPA

For season 2019/20, fingerlings were produced at RPA using an intensive clearwater larval rearing approach developed in accordance with stringent biosecurity requirements for aquaculture production in the Moreton Bay region (Lee *et al.*, In press). Five fingerling production trials (Trials 1 - 5) were performed in a closed facility with controlled photoperiod. Early larvae (2 dph) were stocked (30 - 52 larva L^{-1}) into parabolic rearing tanks (10,000 L capacity, final volume 7000 L) supplied with flow-through seawater pre-treated as previously described. Tanks were fitted with an internal drainpipe with interchangeable screens (300 – 1000 µm) that enabled daily exchange rates to be progressively increased from 50% to 200% with larval development. During early larviculture (e.g., 2 - 12 dph) water exchange was suspended for 8 h d^{-1} to maximise contact time with live prey. Constant aeration was provided by droppers positioned along the centreline of the tanks. Water quality parameters were measured daily and maintained over the five trials at temperature 26.1 - 29.1 °C; DO 4.6 - 5.9 mg L^{-1}; pH 8.1 - 8.3; salinity 15 - 35 ppt; and total ammonia nitrogen (TAN) < 0.5 mg L^{-1}. Larvae were fed an overlapping sequence of rotifers, *Artemia*, and formulated weaning diets until fully weaned and translocated to overwintering tanks (Fig. 2). Large strain (LS) rotifers *Brachionus plicatilis* (MainStream Aquaculture, Pty Ltd) were cultured in semi-continuous culture systems using Rotigrow® Plus (Reed Mariculture, Campbell USA). Rotifer cultures were harvested every 2 - 3 days and fed unenriched at 5 inds mL^{-1} to 2 dph larvae. From 3 – 11 dph, larvae were fed unenriched rotifers (14 inds mL^{-1}) between 0700 – 0800 h. This was followed by rotifers (6 inds mL^{-1}) enriched for 6 h in Super Selco® (INVE Aquaculture, Inc.) between 1400 – 1500 h. *Artemia* sp. were cultured and harvested using the INVE SepArt platform (INVE, Belgium). Un-enriched *Artemia* nauplii were fed at 2 inds mL^{-1} day^{-1} between 7 - 8 dph. Second instar *Artemia* enriched for 24 h in Super Selco® were fed at 3 – 5 inds mL^{-1} day^{-1} from 8 - 32 dph. Residual counts of live feeds were performed twice daily to ensure prey availability. A succession of artificial diets was provided 4 timers per day commencing with Start-L (0.2 – 0.3 mm; INVE Aquaculture, Inc) 12 – 16 dph; Wean-L (0.3-0.5 mm; INVE Aquaculture, Inc.) 17 – 21 dph; Grow-L (0.5 – 0.8 mm; INVE Aquaculture, Inc.) 22 – 25 dph; Nurse-L (0.8 – 1.2 mm; INVE Aquaculture Inc.) 26 – 32 dph; and NRD G12 (1.2 mm; INVE Aquaculture, Inc.) from 33 dph. Grading was performed during weaning (16 – 25 dph) to mitigate the effects of cannibalism. Once fully weaned, fingerlings (35 – 44

dph) were restocked into 20,000 L parabolic tanks supplied by RAS for overwintering. Health and developmental checks were performed intensively during early larval stages (1 - 12 dph) and weekly thereafter. Animals displaying health conditions were sampled and sent to DAF BSL for histological examination and/or pathogen testing.

Following repeated catastrophic early larval mortality events in season 2019/20, a major revision of intensive larviculture practice was adopted by RPA for season 2020/21. Changes included the introduction of live microalgae to larval rearing systems, taurine enrichment of live prey, and replacement of parabolic larval rearing tanks with circular designs. Three fingerling production trials (Trials 1 - 3) were conducted in a semi-open facility with transparent roofing, supplemental shade cloth and ambient photoperiod. Early larvae (2 dph) were collected from hatch tanks and stocked (22.2 – 55.5 larva L^{-1}) into circular rearing tanks (5000 L capacity, final volume 4500 L). Pre-treated flow-through water and tank water exchange was managed as described for Season 2019/20[1]. Aeration was provided by a single diffuser ring encircling the drainpipe. Tanks were inoculated daily until 10 dph with *Nannochloropsis oculata* to provide a microalgal density of ~22,000 cells mL^{-1}. Water quality parameters were measured daily and maintained over the three trials at temperature 23.5 – 30.0 °C; DO 3.7 – 7.0 mg L^{-1}; pH 7.2 – 8.2; salinity 23 – 37 ppt; and TAN < 0.5 mg L^{-1}. Larvae were fed the same quantities and overlapping sequences of feeds as described for season 2019/20 (Fig. 2), with some exception. This included 6 h enrichment of the second daily addition of rotifers (6 inds mL^{-1}) with Red Pepper (BernAqua NV, Olgen, Belgium) and taurine (4 g L^{-1}); and 24 h enrichment of second instar *Artemia* in Red Pepper and taurine (4 g L^{-1}). Taurine enrichment of rotifers and *Artemia* was not performed for half the population of Trial 2 larvae as part of an experiment described in the next section (Comparative analysis of larval performance at BIRC and RPA). Fully weaned fingerlings (35 – 37 dph) were restocked into an overwintering facility as described for season 2019/20. Health and development were monitored as previously described.

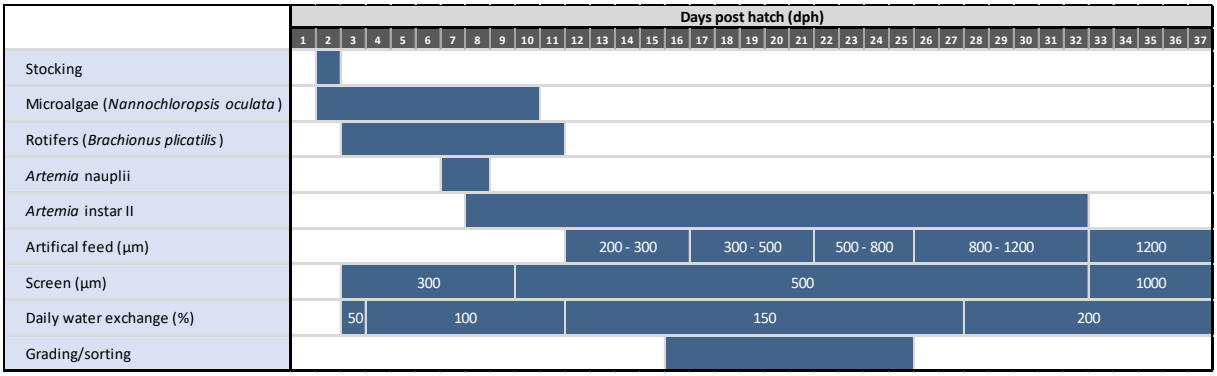

Figure 2. Management of cobia larval rearing systems at RPA
Note: Microalgae was added to systems in season 2020/21 only.

Comparative analysis of larval performance at BIRC and RPA

Revisions to fingerling production processes in 2020/21 yielded substantial performance gains over the previous season. A commercial-scale experiment was subsequently designed to validate the refinements and to delineate the effects of diet (with or without taurine or copepod supplementation) and site (BIRC or RPA) on cobia larval performance until 12 dph.

[1] Except fingerling production Trial 1, where chlorine was not used to disinfect and flocculate intake settlement ponds.

Larvae and culture systems

Cobia larvae (standard length, S_L: 4622.4 ± 28.7 µm, *n* = 80) used in the experiment were derived from the same batch of eggs (Trial 2, season 2020/21). Larvae (2 dph) were stocked at approximately 44 larva L^{-1} into three and four commercial-scale larval rearing tanks at RPA and BIRC, respectively. A fourth tank at RPA was later stocked with 3 dph larvae from the same batch following a volumetric counting discrepancy. Tanks at BIRC consisted of blue 7000 L circular (2900 mm diameter; 630 mm water depth; aspect ratio [AR] = 0.43) flat bottomed fibreglass designs housed in an indoor, photothermally-controlled facility. Tanks at RPA consisted of white (opaque) 5000 L circular (2570 mm diameter; 780 mm water depth; AR = 0.61) sloping-bottom plastic designs housed in a semi-open facility. Tank colour and dimensions were unable to be equalised at each site due to resource constraints and the availabilities of existing infrastructure. Operating characteristics such as tank water volume (4500 L), water exchange rates, drain screens, and ring aeration were standardised across sites and were as described in the previous section (Fingerling production). Live microalgal cultures of *N. oculata* were produced independently at each site and due to operational differences were provided to tanks daily at final concentrations of ~22,000 cells mL^{-1} at RPA and 1600 – 6100 cells mL^{-1} at BIRC. Photoperiod was maintained at 14:10 h light:dark using fluorescent lighting (500 lux) at BIRC or ambient for RPA, equivalent to 14.5:9.5 h. Water quality parameters were measured daily (temperature, DO, salinity, pH) or every second day (TAN) targeting optimal ranges for temperature 27 - 28 ^oC; DO > 5 mg L^{-1}; salinity 35 - 37 ppt; pH ~8.0; and TAN < 0.5 mg L^{-1}.

Live feed treatments

The four larval rearing tanks at each site were randomly allocated two dietary treatments in duplicate. At BIRC, this included a control that was fed the standard succession of rotifers and *Artemia* (Control-B) and a treatment that was fed copepods, rotifers, and *Artemia* (Copepod-B). At RPA, the control (Control-R) and treatment (Taurine-R) were provided with the same prey successions as Control-B; however, in Taurine-R, all live prey were enriched with taurine. Rotifers and *Artemia* were cultured, enriched, harvested, and administered as described in the previous section (Fingerling production; Fig. 2). Copepod-B received supplementary additions of a harpacticoid copepod (*Tisbe* sp.) domesticated at BIRC for a DAF-funded Animal Science Queensland Innovations (ASQ-I) project. *Tisbe* sp. stocks were initially maintained in aerated, static systems consisting of two 1000 L conical tanks provided with a 1:1 mix of live *Chaetoceros muelleri* and *Tisochrysis lutea* microalgal cultures. Nauplii and copepodites were harvested from the stock cultures by size fractionation through a 125 µm screen and evenly distributed into six production tanks (100 L) containing 1:1 mixes of the live microalgal cultures. Seven to ten days later, production tanks were harvested, enumerated, and the nauplii/copepodite fraction was supplied to Copepod-B tanks only at densities 0.06 – 0.18 inds mL^{-1} day^{-1} between 2 - 5 dph. Stock cultures were used to supply a < 125 µm fraction to Copepod-B tanks at densities 0.11 – 0.2 inds mL^{-1} day^{-1} between 6 - 7 dph. Cobia larvae were sampled from each replicate tank at time points known to coincide with expected morphological and dietary change (3, 6, 8, 10 and 12 dph) for morphometric and gut content analysis.

Morphometric analysis

For morphometric analysis, 30 larvae were randomly collected and euthanised in 5-10 mg L^{-1} AQUI-S for 5 min. A low dose rate was used to avoid body distortions due to sudden overdoses. Euthanised larvae were transferred to 35% ethanol in freshwater and preserved at -20^oC (Gagliano *et al.*, 2006). Within 16 - 19 days of preservation, micrograph images of larvae were taken using a stereomicroscope (Nikon SMZ18) fitted with a digital camera (Nikon Digital Sight DS-Fi2) reporting to Nikon NIS Elements BR 5.11 software. Images were later processed to determine the prevalence of malformation (%) and proportions of ontogenetic stages (e.g., preflexion, flexion, postflexion, and anal fin ray development) at each data point. Image libraries for each data point were subsequently quality controlled by removing images that depicted larvae in the incorrect anterior-posterior horizontal plane; or severely

curved due to preservation; or damaged or malformed to the extent that reliable measurements could not be obtained. The ends of each image library were further trimmed sequentially until a sample size of 20 was obtained for each data point. NIS Elements BR 5.11 software was used to measure (± 0.01 µm) standard length (S_L), taken from the tip of snout to the (1) tip of the notochord in preflexion larvae; or (2) hypural crease in postflexion larvae. Measurement of myotome height (M_H) was taken immediately posterior to the anus. Compared to freshly euthanised larvae (*n* = 20), preserved cobia larvae were found to increase in S_L by 4.3 – 6.1% for early stages (3 - 8 dph) and 2.7 - 2.9% for later stages (> 10 dph), consistent with trends reported for fish larvae preserved in 30% ethanol (Korwin-Kossakowski, 2014). Size heterogeneity for S_L and M_H at 12 dph was found by the coefficient of variation (CV % = [standard deviation/mean] x 100). Larval condition was determined through the relationship between M_H and S_L (Koslow *et al.*, 1985). Survival at 12 dph was calculated by volumetric subsampling of euthanised larvae at BIRC; or by estimation at RPA due to continued use in commercial production.

Gut content analysis

For examination of gut contents, 15 larvae were randomly sampled from each tank 2 - 3 hours after live feed applications, euthanised and preserved in 95% ethanol. The whole gut (foregut to anus) was excised from the body wall onto a paraffin wax stage and viewed under stereomicroscope (Nikon SMZ18). The proportion of larvae that contained gut contents was determined for each sample. A qualitative measure of the percentage gut fullness and digestive state of gut contents (1 = fully digested; 2 = partially digested; 3 = intact) was also assigned. The proportion of prey (copepods, rotifers, *Artemia*) and unidentifiable digesta (undefined) of the gut content was further determined. We acknowledge that larval prey taxa vary in their resistance to digestion, and this could introduce bias and underestimations of gut content when the unsegmented gut is examined (Sutela & Huusko, 2000). Analysis of the unsegmented gut was chosen specifically in this study to screen for the longitudinal occurrence of undigested prey linked to mass mortality events in season 2019/20.

Optimisation of feeding strategies

The effect of feed frequency on growth performance of harvestable-sized fish

A feed trial was performed to examine the effect of feed frequency of a commercial diet (Pelagica Float 15 mm Ø, Ridley Aquafeeds Ltd) on the growth performance of harvestable-sized cobia over 57 d. Ninety-six cobia (2.55 ± 0.01 kg) were randomly distributed to 12 x 7000 L holding tanks (*n* = 8 fish per tank) supplied by a RAS (Fig. 3). Fish were hand fed to apparent satiation one (0700 h), two (0700, 1600 h), or three (0700, 1130, 1600 h) times per day, in four replicate tanks per treatment. The dry weight of both consumed and recovered uneaten feed pellets was recorded at each feeding time. Water quality was measured daily (Table 2) while TAN and nitrites (NO_2^-) were measured weekly and was maintained at < 0.25 mg L^{-1} and < 0.0 mg L^{-1}, respectively. The photoperiod incorporated both a natural diurnal cycle of sunrise (0445 – 0500 h) and sunset (1800 - 1830 h) and artificial lighting (between 0700 and 1700 h).

At the end of the experiment (57 d) fish were assessed for growth performance. Survival, health index, final weight (W_f), coefficient of variation (CV) for final weight, weight gain (WG), specific growth rate (SGR), total feed intake (FI), and feed conversion ratio adjusted for mortality (FCR_{adj}) were calculated as follows:

Survival (%) = (final no. fish / initial no. fish) x 100

Health index = mean of eye and dermal score calculated for each tank (see Parasite management and identification)

W_f (kg) = mean final weight

CV (%) = 100 x (Standard deviation W_f / W_f)

WG (%) = 100 x (W_f - W_i) / W_i

SGR (% day^{-1}) = 100 x (ln(W_f) - ln(W_i)) / time (days)

FI (kg) = Weight of feed (DW) applied – weight of uneaten pellet (DW)

FCR_{adj} = total FI / (total final biomass + weight of dead fish – total initial biomass)

Where, W_i = mean initial weight; and DW = dry weight (kg).

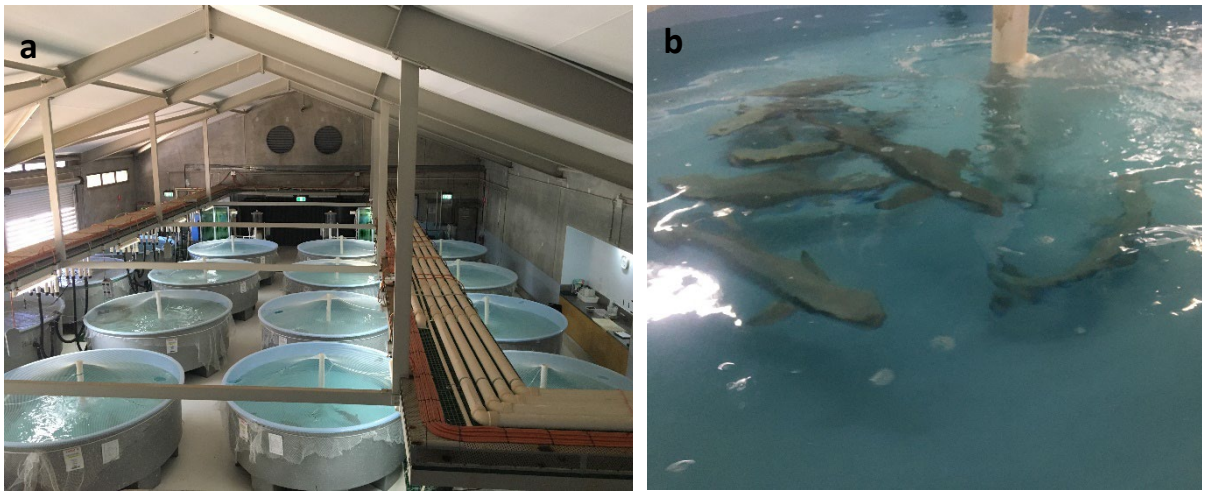

Figure 3. Feed trial for harvestable-sized cobia
(a) Tank array consisting of 7000 L circular fibreglass tanks. (b) Harvestable-sized cobia (2.55 ± 0.01 kg) stocked at 8 fish per tank.

Table 2. Water quality recorded during feed trial (57 d)
Data presented as means ± SE

Parameter	**Feed Treatment (per day)**		
	One feed	**Two feeds**	**Three feeds**
Temperature (°C)	25.98 ± 0.02	25.97 ± 0.02	26.00 ± 0.01
Dissolved Oxygen (mg L^{-1})	5.14 ± 0.02	5.04 ± 0.05	5.10 ± 0.01
pH	7.68 ± 0.01	7.67 ± 0.02	7.67 ± 0.01
Salinity (ppt)	36.56 ± 0.01	36.55 ± 0.02	36.57 ± 0.01

Parasite management and identification

During (29 d, 42 d) and at the end (57 d) of the experiment, fish were subject to aerated freshwater baths to treat suspected *Neobenedenia* sp. infestations. Detached parasites recovered from freshwater baths were preserved in 70% analytical grade ethanol. Phylogeny of four representative isolates (DAF-BIRC 1 - 4) was resolved through sequencing of the histone H3 gene (Brazenor *et al.*, 2018) alongside reference specimens of *Neobenedenia* sp. (JCU1 and JCU2) isolated from rabbitfish (*Signaus doliatus*) culture systems (donated by colleagues Katie Motson, James Cook University, Australia; and Kate Hutson, Cawthron Institute, New Zealand). Briefly, DNA was extracted from whole (or half) parasites using the Isolate II Genomic DNA kit (Bioline, London, UK) according to the manufacturers protocol. PCR amplifications of partial H3 gene was carried out in 25 µL reactions using 12.5 µL ReadyMix™ Taq PCR reaction mix (Sigma Aldrich, St. Louis, USA), 1 µL of each primer: G926 5'-GAC CGC YCG YAA AAG YAC-3' and G927 5'-AGC RTG RAT DGC RCA CAA-3' (Perkins *et al.*, 2009), 1 µL of template DNA extract, and 9.5 µL of purified water. Thermal cycling conditions consisted of: initial denaturation at 94 °C for 1 min; 30 cycles of denaturation at 94 °C for 2 min, annealing at 53 °C for 2 min, and extension at 72 °C for 3 min; and final extension at 72 °C for 10 min. Sequencing of amplification products was performed at the Australian Genome Research Facility using a BigDye™ Terminator v3.1 cycle-sequencing kit (Applied Biosystems™) on an AB3730xl capillary sequencer (Applied Biosystems™). Sequences were edited using BIOEDIT (Hall, 1999) and aligned using CLUSTAL-W v1.8 (Thompson *et al.*, 1994). Selected *Neobenedenia* sp. H3 sequences were downloaded from GenBank and aligned also to our dataset. MEGA-X (Kumar *et al.*, 2018) was utilised to determine the phylogenetic (evolutionary) relationships of taxa. The evolutionary history was inferred using the Neighbour-Joining method (Saitou & Nei, 1987). Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004).

A health index was determined for individual fish affected by parasite infestations using a categorical scoring system for the condition of each eye (0 = absent; 1 = minor; 2 = moderate; 3 = severe) and dermal ulcerations (0 = absent; 1 = present). Any dead fish were removed, weighed, and not replaced in the experiment. A group of fish from the same cohort but physically isolated from experimental fish also exhibited signs of *Neobenedenia* sp. infestations. Three of these juveniles were humanely killed by overdose (100 mg L^{-1}) in AQUI-S and immediately necropsied. Major organs (eye, gill, liver, and kidney) were fixed in 10% formalin and submitted to DAF BSL for histopathological examination.

Statistical analysis

For the comparative analysis of larval performance, water quality and biological data were analysed using general linear models (GLM; McCullagh & Nelder, 1989) followed by protected Fisher's least significant difference (LSD) testing to explore treatment effects. A Normal distribution was appropriate for most data; Binomial distribution with a logit link function was used for the binary (survival) data. A one-way model with the four site/diet combinations was initially used, followed by an incomplete factorial model of site and diet. For the effect of feed frequency on growth performance of harvestable-sized cobia, replicate tanks (n = 4) served as experimental units for statistical analysis. Spatial effects (trend and random effects for the tank row/column array) using restricted maximum likelihood (REML) models were trialled but proved to be non-significant. Analyses were subsequently simplified to a one-way analysis of covariance (ANCOVA) to determine the effect of feed treatment on W_f, WG, SGR, CV, FI, or FCR_{adj}, using health index as the covariate. Residual plots justified the assumptions of data normality and homogeneity of variance. No *post hoc* significant difference testing was required. All statistical analyses were performed in GenStat (version 19, VSN International) with the significance level set at $P < 0.05$.

Results

Biosecure supply of broodfish and seedstock

At the beginning of season 2019/20, 20 selected cobia broodfish (5.2 to 15.2 kg) were tested and declared free of notifiable diseases (WSSV and betanodavirus) and translocated from BIRC to RPA with 100% survival. Rotating breeding cohorts were subsequently established at RPA and BIRC between F3 males (hatch year 2017) and F2 (2016) or F3 (2018) females. In season 2019/20, broodfish were successfully induced to spawn at BIRC and RPA a combined total of five times from six attempts, including a repetitive spawn (Table 3). Batch fecundity of successful spawns ranged 1.10 – 4.16 million eggs, while fertilisation rate (46 - 87%) and hatch rate (65 - 95%) varied considerably. The most successful spawns occurred 37 - 40 h after hormone implantation when females presented with a high proportion (≥65%) of matured oocytes in gonadal biopsies (inductions 1,2,3 and 5). Conversely, poor spawns were typified by prolonged latency (>56 h) and release of unfertilised eggs by females that were implanted with ≤28% of matured oocytes (induction 4 and 6). A single volitional spawn (batch fecundity 0.75 million eggs; fertilisation rate 49%; hatch rate 81%) was recorded for a breeding cohort on 6 February 2020 at BIRC (data not shown). Overall, three batches of seedstock totalling 3.1 million eggs/larvae declared free of notifiable diseases were supplied by BIRC to RPA in season 2019/20 (Table 3). A further 2.07 million fertilised eggs were produced by spawns at RPA (Table 3).

In season 2020/21, female broodfish with ≥65% prevalence of matured oocytes were targeted for hormonal induction. Broodfish at BIRC and RPA were induced to spawn six times as in the previous season, but again only five of these were successful (Table 3). Further, broodfish spawned at RPA yielded only malformed larvae unsuited for larval rearing (induction 1) or unfertilised eggs (induction 4). One late season spawning attempt at BIRC (induction 5) also resulted in mass cessation of egg development around gastrulation, low hatch rate, and large numbers of malformed larvae unsuited for larval rearing. Batch fecundity of successful spawns at BIRC ranged 3.70 – 7.29 million eggs, with varying fertilisation (46 - 89%) and hatch (<1 - 99%) rates. No volitional spawns were recorded at either site for season 2020/21. A total of 3.74 million eggs/larvae declared free of notifiable diseases were supplied by BIRC to RPA in season 2020/21.

Table 3. Summary of induced spawns and cobia reproductive success

	Season 2019/20						**Season 2020/21**					
Hormonal induction attempt	**1**	**2**	**3**	**4**	**5**	**6***	**1**	**2**	**3**	**4**	**5**	**6**
Broodfish (origin)	BIRC	BIRC	RPA	BIRC	RPA	BIRC	RPA	BIRC	BIRC	RPA	BIRC	BIRC
Date of spawn	19/01/20	08/03/20	12/04/20	20/04/20	26/04/20	29/04/20 - 03/05/20	19/11/20	24/11/20	24/01/21	21/03/21	29/04/21	06/05/21
Batch fecundity (x10^6)	4.16	2.20	3.00	0.31	1.10	0.14 - 2.80	0.55	3.70	7.29	0.01	5.58	4.87
Fertilisation rate (%)	87	76	46	0	61	0 - 42	78	89	51	0	58	46
Hatch rate (%)	79	65	95	-	95	37 - 80	19	71	86	-	< 1	99
Total number of seedstock supplied (x10^6)	1.10	1.70	1.40	-	0.67	0.3	0.10	1.20	1.10	-	-	1.44

*Three repetitive spawns over 5 days. Seedstock produced from final spawn 3/5/20 (fecundity 2.8 x 10^6 eggs; fertilisation rate 42 %; hatch rate 37%) was supplied to RPA for a fingerling production trial.

Fingerling production at RPA

For season 2019/20, 3.5 million larvae were stocked across five production trials at RPA (Table 4). A total of 15,454 fingerlings were produced exclusively from Trials 1 and 2, representing a 22.7% shortfall on the project target (20,000 fingerlings). Survival rates ranged 0.8 - 1.1% after 57 - 105 dph. In Trials 1 and 2, a considerable amount of mortality (15 - 30%) occurred within the first 3 dph, followed by a second more damaging mortality event (70 - 80%) at 6 - 8 dph. Early larvae (1 dph) in Trial 2 presented with swollen yolksacs and by 5 dph demonstrated poor feeding response (48%), underutilised oil globule, underdeveloped gut, and spinal and jaw malformations (32%) that pervaded later ontogenetic development. Trial 1 larvae were further affected at 30 dph by an intake of poor-quality seawater (<15 ppt salinity) following a heavy rainfall event, resulting in >2000 mortalities by 35 – 37 dph. Epitheliocystis infection was confirmed in moribund larvae by pathological examination of gills at BSL. This was arrested by medicated feeds as prescribed by an aquatic veterinarian, however irreparable gill damage and scarring lead to persistent, chronic mortality (1 or 2 per day) until at least 80 dph. No fingerlings were produced from Trials 3 - 5 in season 2019/20. This was due to catastrophic loss of larvae during the mixed feeding stage at 8 - 9 dph (Table 4). Unlike Trials 1 - 2, these larvae were produced from late season (April - May) spawns (inductions 3 - 6) and exhibited characteristic lethargy at first and mixed feeding stages. At 5 dph, there was a low incidence (2 - 6%) of malformations such as underutilised oil globule and underdeveloped gut. Feeding responses were initiated in these populations (39 - 67%), however demonstrated little perceptible increase in size and were observed excreting undigested rotifers.

For season 2020/21, 2.4 million larvae were stocked across three production trials at RPA (Table 4). A total of 49,111 fingerlings were produced from Trials 2 and 3, signifying a 217.9% increase over the previous season and a 22.7% inflation on the project target (40,000 fingerlings). Survival rates more than doubled (2.2 - 3.2% after 35 - 37 dph) compared to the previous season. In Trial 1, larval performance was tracking in line with historical benchmarks until total loss occurred at 23 dph. This coincided with the intake of seawater high in fine suspended particulates following a heavy rainfall event. Moribund larvae exhibited gill flaring, lethargy, and inappetence. Epitheliocystis infection was confirmed by pathological examination of gills at BSL. Regrettably, larvae were unable to be rescued by formalin or oxytetracycline bath treatment as prescribed by an aquatic veterinarian. It was estimated that 100,287 larvae (survival 16.7%) populated tanks prior to the mortality event at 22 dph. Trial 2 formed part of a commercial scale experiment described elsewhere in this project (Comparative analysis of larval performance at BIRC and RPA). There was a high incidence of malformation (57%) in early larvae (2 dph) related to pericardial oedema associated with jaw deformity, yolksac swelling, and spinal curvature (Fig. 4). Size heterogeneity and high levels of mortality related to intracohort cannibalism were observed from 13 dph. Trial 3 larvae also exhibited yolksac swelling and segmentation and pericardial oedema (46%) in early stages (2 dph) and a heavy mortality event (~30%) during mixed feeding (7-8 dph). A later onset of intracohort cannibalism (~20 dph) contributed to further mortality in Trial 3. Slower growth (0.2 - 0.42 g) of Trial 3 larvae compared to Trial 2 (0.5 - 0.9 g) was associated with lower culture temperatures of the former (23.5^{o}C). An additional larval rearing attempt with seed produced from broodfish at RPA (induction 1) was abandoned after 4 dph following mass mortality and was not considered a production trial.

Table 4. Summary of fingerling production

	Season 2019/20					**Season 2020/21**		
Hormonal induction attempt	**1**	**2**	**3**	**5**	**6**	**2**	**3**	**6**
Broodfish (origin)	BIRC	BIRC	RPA	RPA	BIRC	BIRC	BIRC	BIRC
Fingerling production trial	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**
Total number of larvae stocked ($x10^6$)	0.60	1.10	1.00	0.50	0.30	0.60	0.83	1.00
Stocking density (larva L^{-1})	43	52	48	35	30	22 – 44	36 – 55	55
Length of trial (dph)	105*	57*	8	8	9	23	37*	35*
Number of fingerlings produced	6393	9061	-	-	-	-	26,898	22,213
Survival (%)	1.1	0.8	-	-	-	-	3.2	2.2
Weight (g)	75	5.5	-	-	-	-	0.5 – 0.9	0.2 – 0.4

*Earliest time of counting. Fingerlings were subsequently overwintered indoors and on-grown in production lakes to harvestable size.

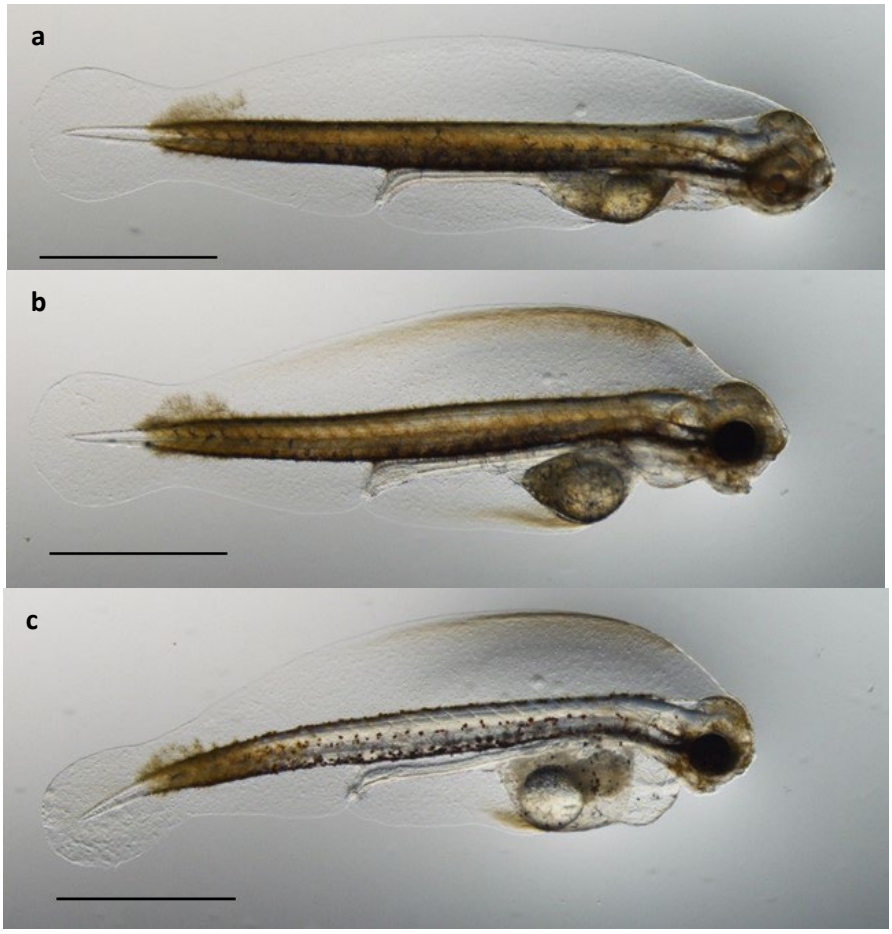

Figure 4. Cobia larvae (2 dph) euthanised in 5 mg L^{-1} AQUI-S
(a) normal phenotype; (b) pericardial oedema, locked jaw and dorsal finfold thickening; (c) severe pericardial oedema, yolksac swelling and spinal curvature. Scale bars = 1000 µm.

Comparative analysis of larval performance at BIRC and RPA

General

Factorial analysis indicated site had a significant effect on water quality parameters (Table 5). Temperature, salinity, and DO were recorded at significantly lower values at RPA compared to BIRC, while pH was significantly increased at RPA compared to BIRC. Observed larval distribution patterns in tanks were also different between sites. At RPA, a ring of uniformly dispersed larvae was maintained a distance from the sidewalls while at BIRC larvae were concentrated in three or four patches at the sidewalls. Pink bacterial biofilms on tank bottoms were observed in Control-B tanks at BIRC, however were diminished or non-existent in Copepod-R tanks and not recorded in any tank at RPA.

Table 5. Water quality parameters at BIRC and RPA

Site	Temperature (°C)	Salinity (ppt)	DO (mg L^{-1})	pH
BIRC	27.63 ± 0.11	35.53 ± 0.06	6.64 ± 0.01	7.85 ± 0.01
RPA	27.01 ± 0.11	32.23 ± 0.06	5.01 ± 0.01	7.90 ± 0.01

Survival and growth

Factorial analysis showed site and not diet had a significant effect on survival and growth of cobia larvae (Fig. 5a). Larval survival at 12 dph was approximately 10 times greater at RPA when compared to BIRC. Larval standard length (S_L) and myotome height (M_H) increased significantly and exponentially through time at both sites (Fig. 5b,c), with larvae at RPA growing significantly larger (Fig. 5b,c) compared to larvae at BIRC (Fig. 5b,c). Larval size variation at 12 dph was significantly lower at RPA (S_L: CV = 12.72 ± 0.67 %; M_H: 17.92 ± 0.82 %) when compared with BIRC (S_L: CV = 19.69 ± 0.67 %; M_H: 30.64 ± 0.82 %). Larval condition, expressed by an exponential relationship between M_H and S_L (Fig. 5d), was significantly enhanced at RPA when compared with BIRC.

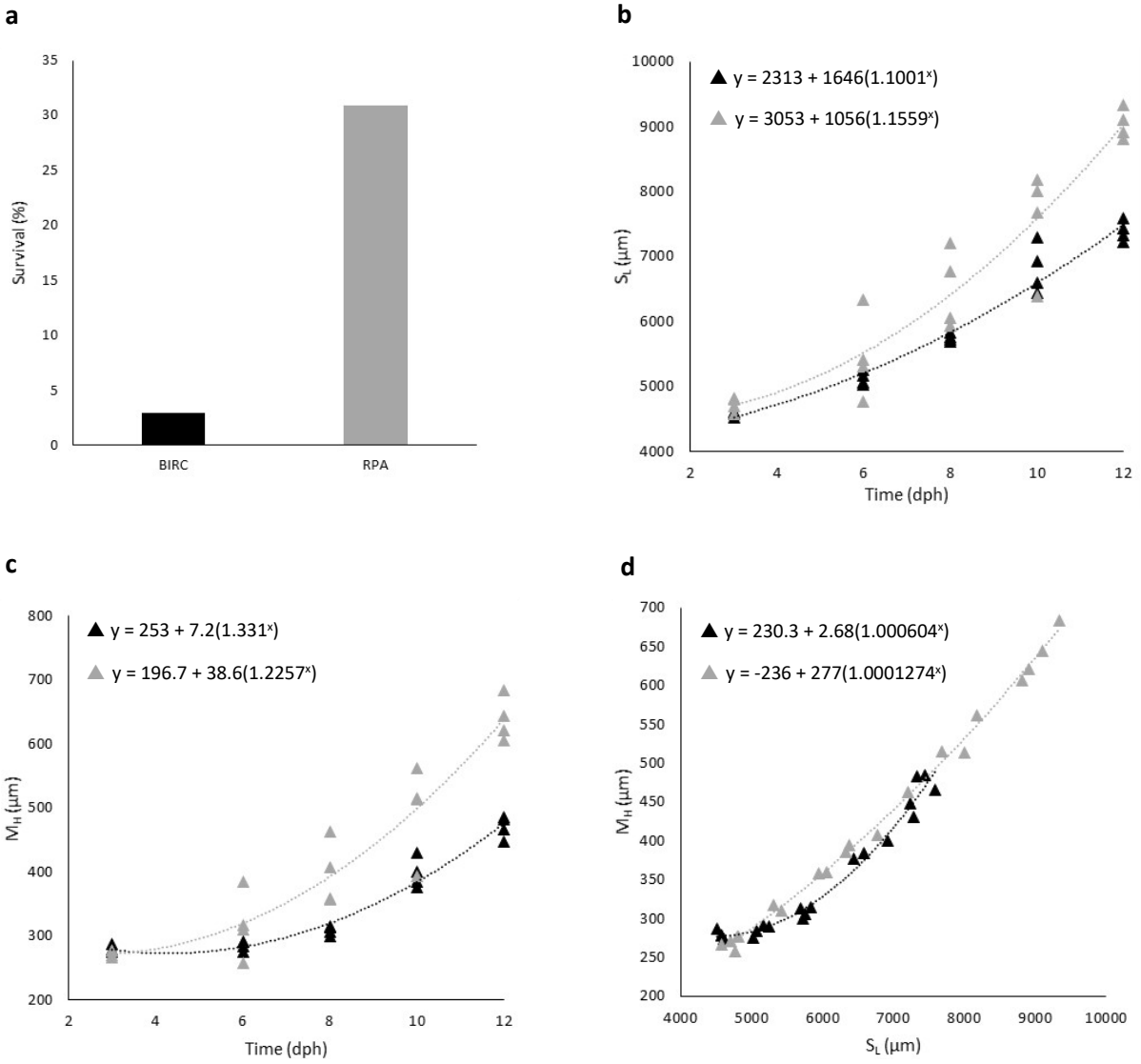

Figure 5. Survival and growth performance of cobia larvae at RPA and BIRC
(a) Larval survival at 12 dph, values expressed as adjusted means. (b) S_L (adj. R^2 = 92.1%) and (c) M_H (adj. R^2 = 91.4%) of larvae from 3 to 12 dph. (d) Relationship between M_H and S_L (adj. R^2 = 99.0%). Key: RPA ▲; BIRC ▲

Malformation and development

Larval malformations at 3 dph included pericardial oedema associated with jaw deformity, underutilised oil globule, and thickening of the dorsal finfold. These malformations in addition to a small number of spinal anomalies persisted until 6 dph (Fig. 6a-b). Factorial analysis showed that time and not site or diet affected the incidence of malformation in cobia larvae, which significantly decreased from 3 to 6 dph and continued to decline thereafter (Fig. 6c). Larval development was significantly affected by time and/or site, but not diet (Fig. 7a). Numbers of preflexion larvae (Fig. 7a, b) began to significantly diminish from 8 to 10 dph and disappear completely from populations by 12 dph, but were significantly more enumerate at 10 dph at BIRC compared to RPA (Fig. 7a). Flexion larvae (Fig. 7a, c) first appeared 6 dph at RPA, with numbers increasing significantly from 8 to 10 dph, however no significant differences between the sites were found (Fig. 7a). The incidence of postflexion larvae (Fig. 7a, d) was first recorded 10 dph and generally increased with time however no significant differences were found between sites at 10 or 12 dph (Fig. 7a). Anal fin rays (Fig. 7a, e) were exclusively observed in 12 dph larvae and were significantly more prevalent at RPA when compared with BIRC (Fig. 7a).

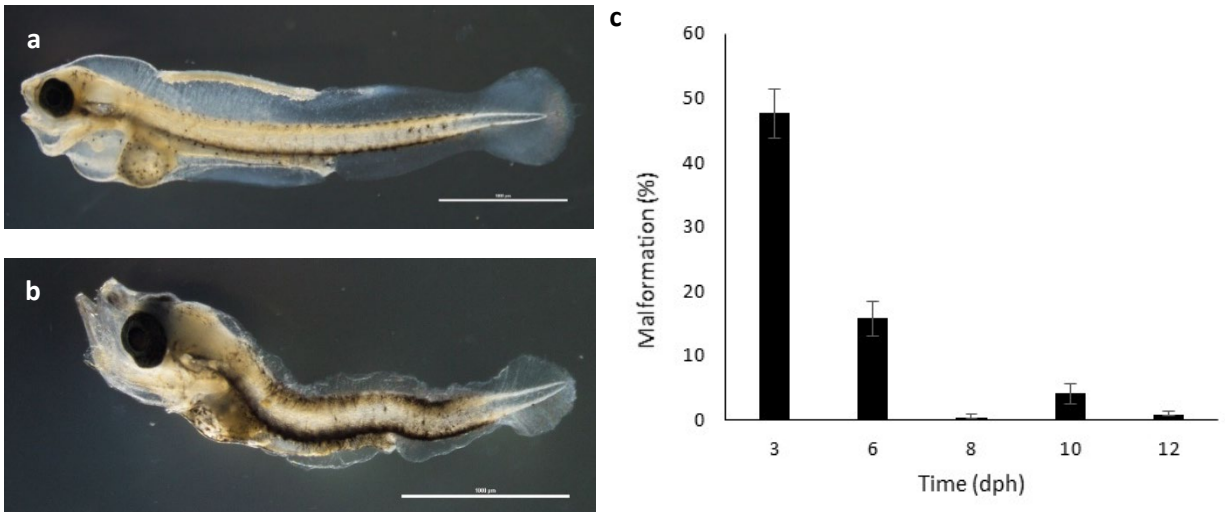

Figure 6. Malformations of cobia larvae
Larvae (6 dph) preserved in 35% ethanol showing (a) pericardial oedema, jaw deformity and dorsal finfold thickening; and (b) multiple notochordal axis abnormalities. (c) Incidence of cobia larval malformation observed in the experimental batch between 3 -12 dph. Values expressed as adjusted means ± SE. Scale bars = 1000 µm.

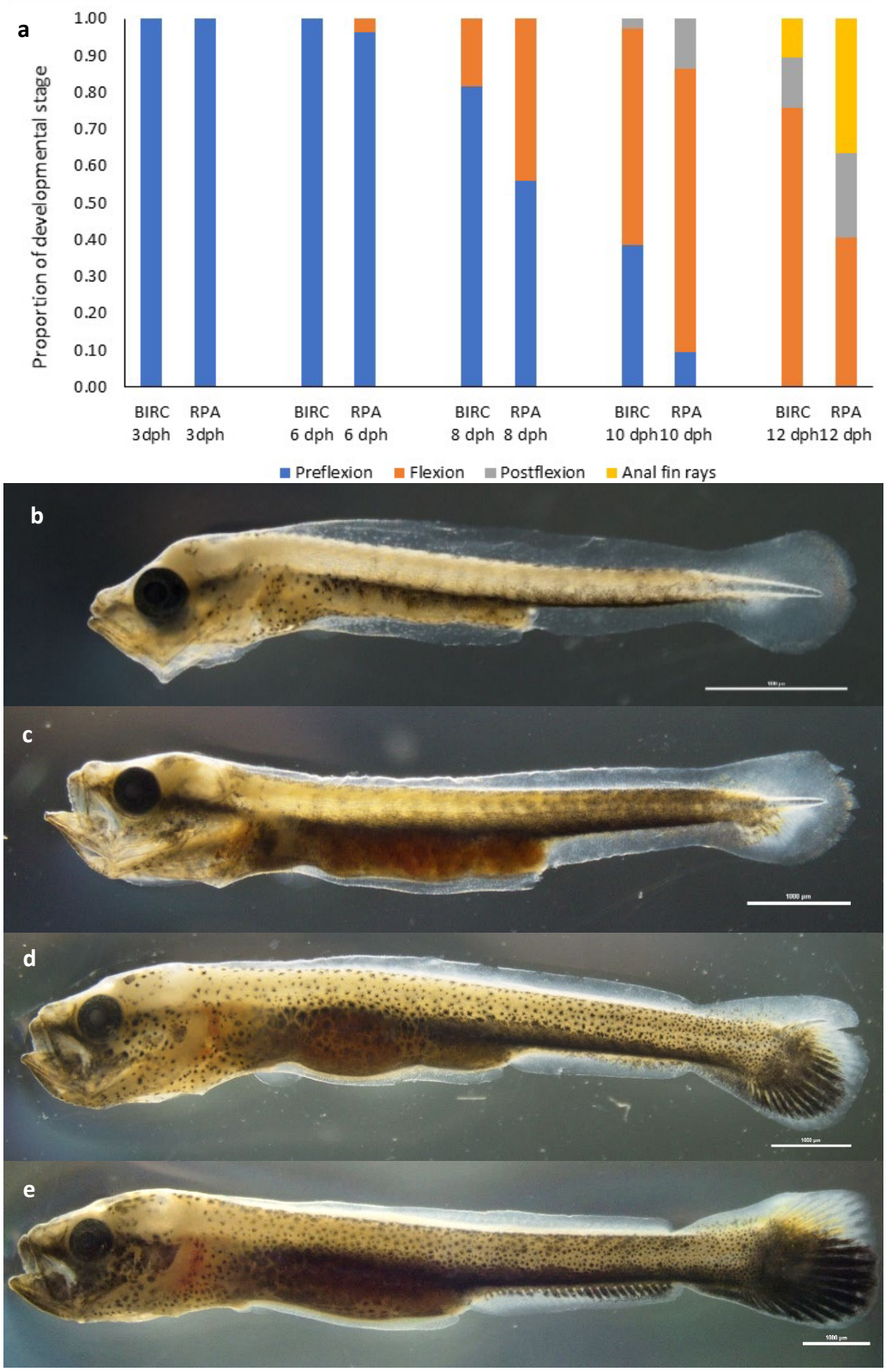

Figure 7. Cobia larval development

(a) Proportion of cobia larval developmental stages (preflexion, flexion, postflexion and anal fin rays) at RPA and BIRC between 3 to 12 dph. Values expressed as adjusted means ± SE. (b) Preflexion larva 6dph. (c) Early flexion larva 8 dph. (d) Early postflexion larva 10 dph. (e) Anal fin ray development in larva 12 dph. All larvae depicted preserved in 35% ethanol. Scale bars = 1000 µm.

Gut analysis

Factorial analysis showed that time, site, and/or diet significantly affected the proportion of larvae in tanks recorded with gut content, gut fullness, digestive state, and proportion of prey type in the gut (Fig. 8a-d). Significantly less numbers of larvae were found with gut content at 3 dph compared with 6 - 12 dph larvae. A higher proportion of larvae were recorded with gut content 3 dph at RPA when compared with BIRC; however, this was significantly enhanced only for Taurine-R larvae. Larval gut fullness significantly increased with time, and RPA larvae were more likely to have a fuller gut than BIRC larvae. Similarly, the intactness of digested gut contents significantly increased with time, with RPA larvae exhibiting significantly higher intactness of gut content at 10 dph than BIRC larvae. Copepods were not observed in the gut of Copepod-B larvae (or other treatments) at any stage during 3 – 12 dph. Rotifer numbers in the larval gut declined with time and was significantly decreased at 12 dph, while RPA larvae harboured significantly higher proportions of rotifers in the gut at 10 dph and 12 dph compared to BIRC larvae. Numbers of *Artemia* in the larval gut increased significantly at 10 dph after the onset of *Artemia* feeding. The proportion of undefined or unclassified digesta decreased significantly from 3 - 10 dph and was significantly lower in RPA larvae at 6 and 10 dph compared with BIRC larvae.

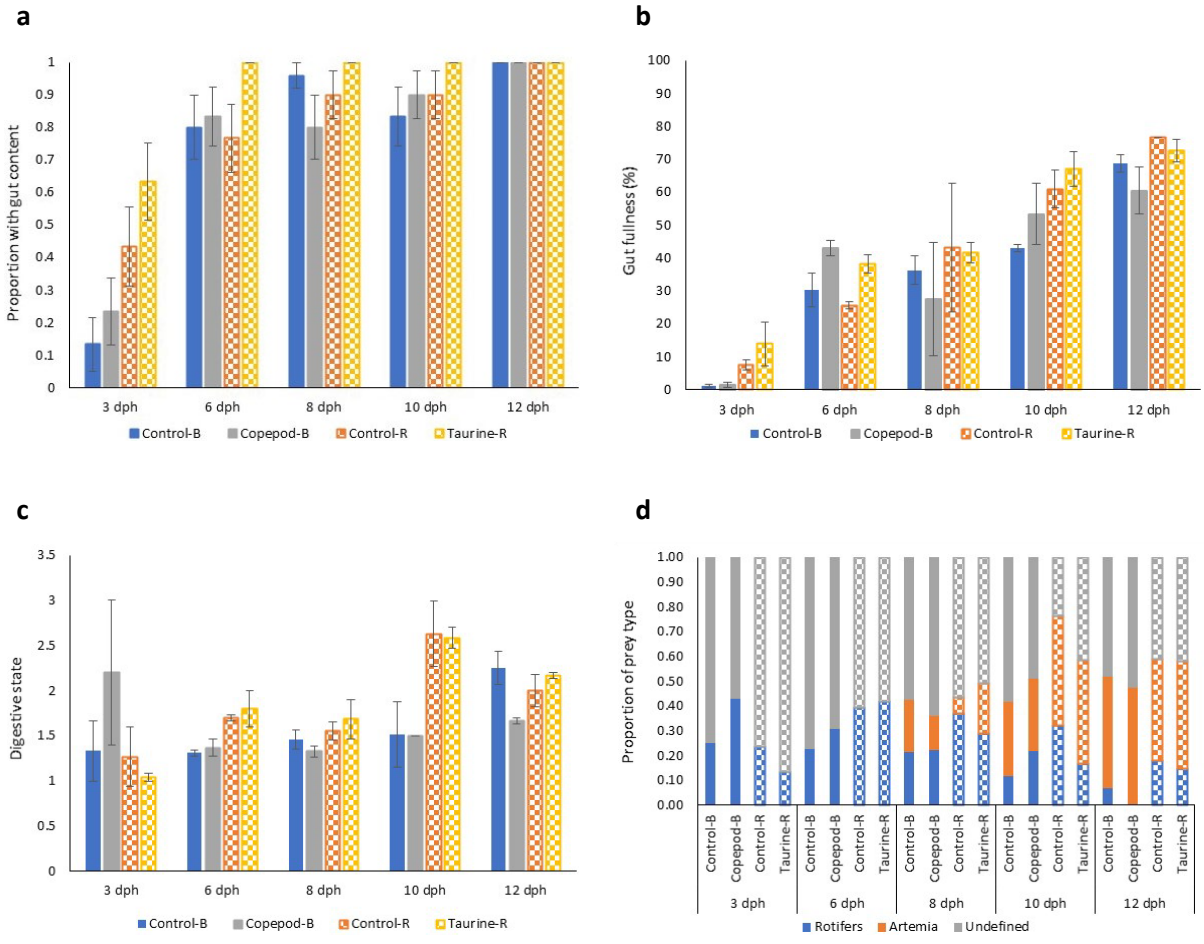

Figure 8. Cobia larval gut analysis

(a) Proportion of larvae (*n* = 15) recorded with gut content. (b) Subjective measure of gut fullness (%). (c) Digestive state of gut contents indicated by categorical assignations 1 = fully digested; 2 = partially digested; 3 = intact. (d) Proportion of prey types recovered from cobia larvae. Key: Solid fill = BIRC; Checkerboard fill = RPA. Values are expressed as adjusted means ± SE.

The effect of feed frequency on growth performance of harvestable-sized fish

Feed frequency had no significant effect ($F_{2,8} < 2$, $P > 0.05$) on any measured performance parameter (Table 6). Health index was non-significant ($P > 0.05$) when fitted as a covariate, so was omitted from the final models. There was a trend that growth rate and size uniformity increased by feeding two or three times per day. Although weight gain was similar for all treatments over the duration of the trial (57 d), fish fed twice and three times per day consumed 14.1 and 18.4% more feed respectively when compared to the fish fed once per day (Table 6). Daily feed intake equated to 1.4 – 1.6% BW d^{-1} and 1.0 – 1.1% BW d^{-1} at the start and end of the experiment, respectively. Survival ranged 93.8 - 100% across treatments.

Table 6. Growth performance of harvestable-sized cobia
Presented as ANCOVA-adjusted means ± SE, with *P*-values testing for treatment differences.

Parameter	One feed	Two feeds	Three feeds	SE	*P*-value
W_f (kg)	3.52	3.62	3.64	0.119	0.756
CV % (mean weight)	19.10	15.11	15.34	1.845	0.285
Weight gain (kg)	1.006	1.078	1.065	0.123	0.907
Biomass gain (%)	39.93	42.37	41.37	4.918	0.940
SGR (% BW day^{-1})	0.58	0.62	0.60	0.197	0.925
Total feed intake (DW kg)	14.93	17.06	17.70	1.094	0.232
FCR_{adj}	2.13	2.03	2.14	0.126	0.803

Parasite management and identification

Experimental fish first exhibited signs *of Neobenedenia* sp. infestation including flashing behaviours, inappetence, and corneal redness at 14 d. Gross examination of three non-experimental cobia representative of the *Neobenedenia* sp. infestations showed concave coelomic cavity, empty stomachs, severe corneal inflammation and dermal lesions around the head, ventral surfaces, and pectoral/anal fins. Histological examination indicated changes to the corneal epithelium consistent with fluke activity (Ogawa *et al.*, 2006). Eyes exhibited massive capillarisation at corneal edges, dilated capillaries, and infiltration of stroma by mononuclear cells, and ulceration and loss of corneal epidermis (Fig. 9a-d). Liver parenchyma contained fibrous granulomas and hepatocytes were lacking cytoplasmic vacuoles (Fig. 9c). The caudal kidney of one individual presented with fibrous granulomas harbouring a central amorphous mass with small clumps of Gram-negative, rod-shaped bacteria (Fig. 9d). No morphological abnormalities were noted in gill sections.

Parasites recovered from freshwater baths were tentatively identified by microscopy as *Neobenedenia* sp. (Capsalidae: Monogenea) based on the presence of a haptor with marginal hooks and two anterior suckers (Fig. 10a). Phylogenetic analyses of partial H3 gene sequences confirmed all isolates from the current study (JCU 1 - 2 and DAF-BIRC 1 - 4) as *Neobenedenia girellae* (Fig. 10c). A neighbour-Joining phylogenetic tree displayed similar topology to constructs from a previous study by Brazenor *et al.* (2018) showing *N. girellae* sequences are confined to a monophyletic clade (Fig. 10c).

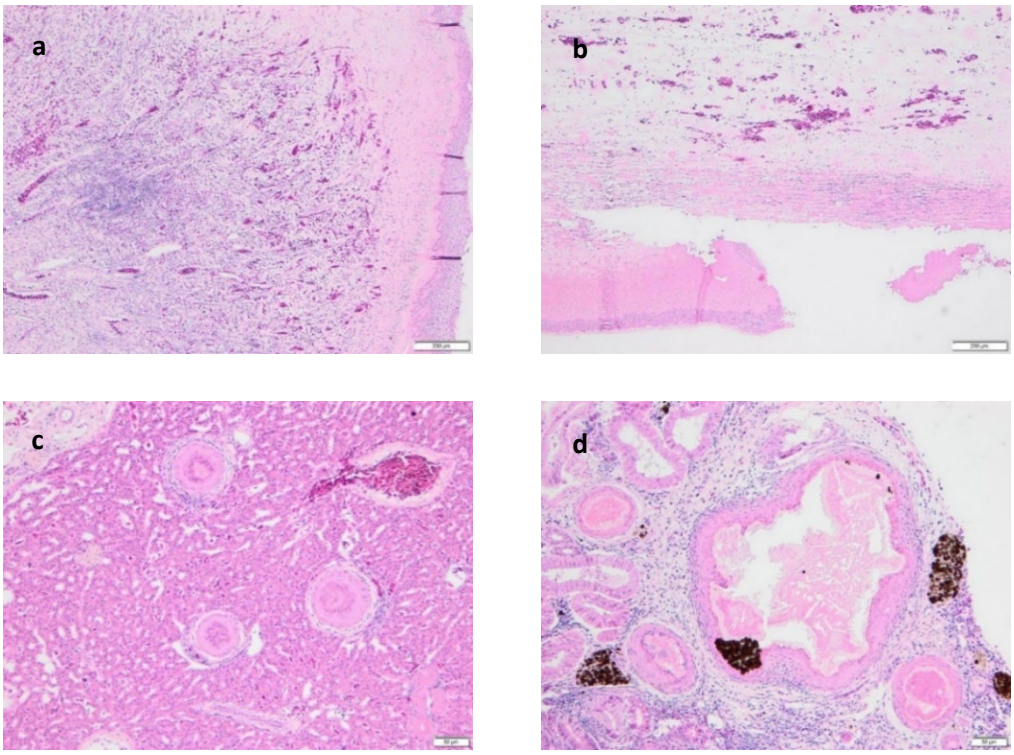

Figure 9. **Histological sections of tissues from cobia infested with *Neobenedenia* sp.**
(a) Cornea showing dilated capillaries and stromal infiltration with mononuclear cells. Scale bar = 200 µm. (b) Corneal surface, showing stromal ulceration and inflammation. Scale bar = 200 µm. (c) Liver granulomas and lack of cytoplasmic vacuolation in hepatocytes. Scale bar = 50 µm. (d). Caudal kidney granulomas, with one showing purple bacterial microcolonies in the centre. Scale bar = 50 µm. Images by Dr. Ian Anderson (BSL).

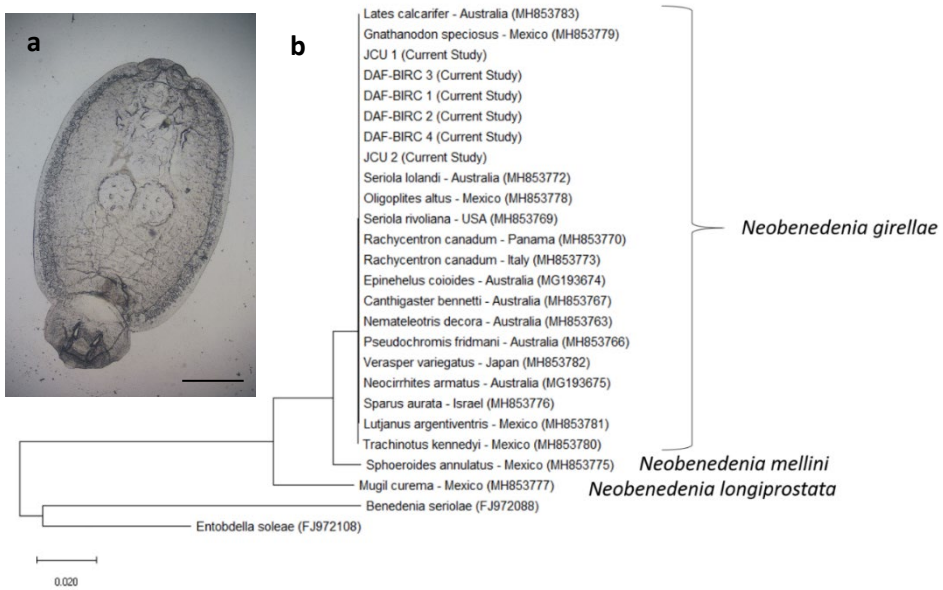

Figure 10. **Specimen and phylogeny of *Neobenedenia* sp.**
(a) Microscope image of ethanol-preserved *Neobenedenia* sp. recovered from harvestable-sized cobia. Scale bar = 500 µm. (b) Phylogenetic relationships of *Neobenedenia* isolates based on Neighbour-Joining analysis of H3 sequences collected from Genbank and the present work. *Benedenia seriolae* and *Entobdella soleae* sequences were included as outgroups. Numbers in parentheses indicate Genbank accession numbers.

Discussion

Expanding cobia production

Biosecure supply of broodfish and seedstock

The translocation of cobia broodfish and seedstock to suitable aquaculture growing regions will be crucial to the commercial expansion of this species. A high level of biosecurity practice was implemented in this project to ensure authorised movement of cobia within the WSD movement restriction area in southeast Queensland. This included adoption of farm biosecurity management practices developed in previous research (Lee *et al.*, 2018, Lee *et al.*, In press), laboratory testing, and declaration of freedom for notifiable diseases (WSSV and betanodavirus).

Broodfish translocated to RPA were successfully induced to spawn on three occasions in this project. This shows a conceptual uptake of a cobia breeding program at RPA, and a capability to produce seedstock self-sufficiently. However, a major caveat to this success was that larvae produced from these spawns were not viable beyond 8 dph. In season 2019/20 this phenomenon similarly impacted the quality of seed produced by BIRC broodfish. This was likely attributed to the use of first-time and late season (April – May) spawners. First-time spawning fish are known to release eggs deficient in nutrients (e.g., fatty acids) essential for fertilisation and survival of larvae (Evans *et al.*, 1996). In contrast, larger, older females have gonadal fatty acid profiles more supportive of higher quality offspring than smaller conspecifics (Pecoraro *et al.*, 2020). Poor larval survival is also a frequently reported downstream characteristic of late season spawners with gonad regression (Garcia, 1989, Gardes *et al.*, 2000, Carral *et al.*, 2003, Berlinsky *et al.*, 2005, Antolović *et al.*, 2013, Jerez *et al.*, 2018).

The lack of reproductive success exclusive to RPA broodfish in season 2020/21 is more difficult to delineate. Discussion among project staff identified several possible drivers within the areas of nutrition and feeding, and with broodstock husbandry and parasite management. Broodfish nutrition and feeding is an obvious area to consider given the impact of maternally-derived nutrients on seed quality. Dietary taurine was provided to RPA broodfish only in 2020/21 to arrest possible deficiencies. Taurine was found experimentally to improve reproductive performance, spawning frequency, fecundity, fertilisation, hatching rate, and larval survival in several species including tilapia *Oreochromis niloticus* (Al-Feky *et al.*, 2016), Japanese yellowtail *Seriola quinqueradiata* (Matsunari *et al.*, 2006) and Greater amberjack *S. dumerili* (Salze *et al.*, 2019). However, excessive dietary taurine may reduce body free amino acid content and retard growth (Qi *et al.*, 2012, Zhou *et al.*, 2015), possibly translating to reduced egg protein content and poor-quality larvae (Sarih *et al.*, 2019). Excessive feeding may have also diminished reproductive success of cobia broodstock at RPA, as shown for zebrafish, *Danio rerio* (Lawrence *et al.*, 2012) and Nile tilapia, *Oreochromis niloticus* (Bhujel *et al.*, 2007). In contrast, reproductive output can be improved by restricting feed rate during vitellogenesis to ensure a superior pool of maternal nutrients are mobilised to eggs (Reading *et al.*, 2018, Chatzifotis *et al.*, 2021).

Holding conditions are also known to have a large bearing on cobia reproductive outcomes. Natural spawns did not occur at RPA and only once at BIRC in this project. Volitional spawns by captive cobia broodstock are reliably attained in other parts of the world using only photothermal manipulation, low stocking density (e.g., 0.7 – 1.8 kg m^{-3}; 10 – 14 fish per 80 m^3), and female to male ratios of 2:1 (Benetti *et al.*, 2008a, Stieglitz *et al.*, 2012). Broodfish at RPA were stocked at high levels (e.g., up to 6.0 kg m^{-3} and 24 fish per 50 m^3) to improve the chances of selecting fish with matured gonads at the time of hormonal therapy. However, this strategy appears to have created stressful crowding conditions and aggressive behaviour, leading some females to become underconditioned (pers. obs. the authors). Chronic stress is known to promote oocyte atresia and decrease fecundity, gamete quality and larval survival in species such as brown (*Salmo trutta*) and rainbow (*Oncorhynchus mykiss*) trout (Campbell *et al.*, 1994), damselfish *Pomacentrus amboinensis* (McCormick, 2006), cichlid *Neolamprologus pulcher* (Mileva *et al.*, 2011), and zebrafish *Danio rerio* (Abdollahpour *et al*., 2020). Stress hormones such as cortisol can trigger the expression of gonadotrophin inhibitory hormone (GnIH) which reduces the levels of circulating luteinising hormone (LH) required for oocyte maturation and

ovulation (Choi *et al*., 2017). Further, cortisol can be maternally transmitted to developing embryos which may increase egg mortality and larval otolith asymmetry also linked to high mortality rates (Gagliano & McCormick, 2009).

While formalin treatment is effective for managing ectoparasites, it is also known to improve fish condition by reinvigorating appetite and vitality (Katharios *et al.*, 2006). Formalin treatments were conducted reactively and therefore less frequently at RPA compared with BIRC, which may have contributed, at least in part, to the variation in spawning success between the two sites. Another methodology that could be considered for ectoparasite management is the use of cleaner fish (*Gobiosoma oceanops*). Cleaner fish have been shown to control parasites in cobia broodfish maturation systems, reducing stress and improving spawning consistency (Benetti *et al.*, 2021).

The reproductive performance of cobia has been an area of concern in recent growing seasons (Lee *et al.*, In press) and may represent a short-term challenge to the self-sufficiency of the cobia aquaculture sector in Queensland. In the interim, contingency supplies of quality seedstock were made available from BIRC for this project. A total of 6.84 million fertilised eggs were supplied under biosecure conditions from BIRC to RPA, resulting in 4.43 million larvae for fingerling production. The vast quantities of seedstock surplus to project needs further shows a demand for centralised hatchery production to support a budding sector. Regrettably, this outcome did not eventuate due to logistical constraints and the business risk aversions taken during the COVID-19 disruption. However, there remains strong expressions of interest from new proponents across northern Australia wishing to explore cobia aquaculture in sea cages, ponds, and land-based recirculating systems.

Fingerling production

RPA successfully adopted and refined cobia larval rearing processes and produced commercial volumes of fingerlings. Almost 65,000 fingerlings were produced across two seasons (15,454 in 2019/20; 49,111 in 2020/21). The second season was exemplified by >200% increase in productivity over the first season, and over 20% growth on the season target. Moreover, total fingerling output of this project surpassed that of the previous two seasons (16,262) by almost 300% (Lee et al., In press). Farm yield at RPA is forecast to exceed 150 t in 2021/22, which could see it produce the largest annual volume of this species recorded in Australia.

The performance gains in season 2020/21 was likely due to several interactive biological and technical changes. This included the use of second time, larger spawning broodfish, taurine-enrichment of live feeds, addition of live microalgae to larval rearing tanks, and the transition from parabolic to circular larval rearing tanks. As discussed previously, larger, repeat spawning fish have gonadal and egg nutrient profiles that translate to higher quality larvae (Evans *et al.*, 1996, Pecoraro *et al.*, 2020). Both dietary taurine (Salze *et al.*, 2011, Salze *et al.*, 2012b) and live microalgae added to larval rearing systems (Faulk & Holt, 2005) have been reported to elicit positive growth and survival responses of cobia larvae. Moreover, the circular tanks and ring aeration likely overcame extensive larval damage and death otherwise promoted by multiple aerators and several upwelling zones (Sakakura *et al.*, 2006) in the parabolic tanks.

This project also identified several larval mortality factors such as malformations, cannibalism, and disease that could have catastrophic consequences for cobia fingerling production. The developmental and behavioural abnormalities seen at both sites in season 2019/20 were likely linked to yolk nutrient deficiencies and maternal physiological condition (Mylonas & Zohar, 2007, Bobe & Labbé, 2010, Mylonas *et al.*, 2010, Migaud *et al.*, 2013, Bobe, 2015, Reading *et al.*, 2018, Cheung *et al.*, 2019) and the observed total loss of larvae 8-9 dph. High mortality during the transition from yolksac to exogenous feeding phases (6-9 dph) is reported frequently for cobia larvae (Franks et al., 2001, Gopakumar et al., 2012, Lee et al., In press). These outcomes are predestined to occur without the supply of adequate yolk nutrients that support normal organogenesis and growth (Bobe & Labbé, 2010, Lubzens *et al.*, 2010). Early cobia larvae rapidly utilise yolk proteins, carbohydrates, and lipids to quench a high energy demand for organ development (Huang *et al.*, 2020). Specifically, yolk fatty acids (e.g., C16:1n-7 and C22:5n-3) and amino acids such as glutamic acid, leucine, arginine, and lysine appear important to organogenesis in early cobia larvae (Huang *et al.*, 2020).

In season 2019/20, the underdeveloped larval gut (e.g., Trials 2-5) and excretion of undigested rotifers (e.g., Trials 3-5) were again suggestive of yolk nutrient deficiency and an inability to synthesise functional gastrointestinal components (Rønnestad *et al.*, 1993, Sivaloganathan *et al.*, 1998, Kamler, 2007). Larval spinal curvatures (e.g., Trial 2) further have been associated with amino acid deficiencies (e.g., arginine/glycine and methionine) in cobia eggs (Nguyen *et al.*, 2012). Interestingly, eggs diminished in amino acids have been also linked to swollen yolksac aberrations and spinal/jaw malformations in larvae of Murray cod, *Maccullochella peelii* (Gunasekera *et al.*, 1998) similarly described in this project (e.g., Trial 2). Finally, lethargy at the mixed feeding stage was a common feature across trials (e.g., Trials 2-5) that may have been related to the presence of underutilised oil globules. Oil globules provide lipid fuels for swimming and foraging activities (Kamler, 2007) that may have been lacking or inaccessible to cobia larvae.

In season 2020/21, larvae produced at BIRC (e.g., Trials 2 and 3) exhibited a high incidence (46 - 50%) of pericardial oedema and notochordal axis abnormalities similar to lordosis-scoliosis-kyphosis syndrome (Afonso *et al.*, 2000). Pericardial oedema has been reported in Japanese Eel, *Anguilla japonica* (Kurokawa *et al.*, 2008, Okamoto *et al.*, 2009), gilthead sea bream *Sparus aurata* (Polo *et al.*, 1991), yellowtail kingfish *Seriola lalandi* and striped trumpeter *Latris lineata* (Cobcroft *et al.*, 2004). The swelling pushes against the sternohyoideus muscle and causes the hyoid arch of the lower jaw to be pulled downward, giving rise to jaw deformity (Cobcroft *et al.*, 2004, Kurokawa *et al.*, 2008). Possible causes for these malformations include genetic and heritable factors, suboptimal hatching temperature, and salinities, and maternal physiological deficits (Cobcroft *et al.*, 2004, Boglione *et al.*, 2013, Cobcroft & Battaglene, 2013). These malformations likely severely compromise food intake and growth and contribute significantly to high mortality rates during early finfish larval culture (Lv *et al.*, 2019).

Intracohort cannibalism was another prominent mortality factor of larvae in season 2020/21. Cannibalism in fish larval stages has been widely reported in aquaculture species, facilitated to a large degree by size heterogeneity and regulated by factors such as population density and feed frequency (Pereira *et al.*, 2017). Both size heterogeneity (Benetti *et al.*, 2008b) and premature feeding with artificial diets (Nguyen, 2009) are known to promote cannibalism in larval cobia. Precocious photosensory development is thought to be one mechanism allowing individual larvae to more effectively transition from planktivory to piscivory, and thus target conspecifics for consumption (Colchen *et al.*, 2020). Alternatively, Pham *et al.* (2020) found larval cannibalism is under genetic control with moderately low heritability in barramundi (*Lates calcarifer*). While this has not been established for cobia, low stocking density < 10 larva L^{-1} (Hitzfelder *et al.*, 2006, Benetti *et al.*, 2008b), and frequent grading from as early as 12 - 14 dph (Benetti *et al.*, 2008b, Salze *et al.*, 2008, Nguyen *et al.*, 2011a) have been suggested as management practices.

Epitheliocystis was observed twice at RPA, once in each season (35 dph season 2019/20; 23 dph season 2020/21). The disease has been identified previously as a serious threat to cobia aquaculture in Australia (Lee *et al.*, 2018). In both instances here, outbreaks were preceded by intake of hyposaline water and/or water high in fine suspended solids. Cobia exposed to low salinity (e.g., 5 - 10 ppt) are susceptible to disease (Denson *et al.*, 2003), investing a large amount of energy into maintaining osmotic function and in upregulating immune responses (Cao *et al.*, 2020, Huang *et al.*, 2021). Exposure to high turbidity and fine suspended particles was shown to significantly increase the incidence of epitheliocystis in juvenile snapper, *Pagrus auratus* (Lowe *et al.*, 2015). A range of contaminants associated with suspended particles in coastal environments could further exacerbate the condition (Au *et al.*, 2004), including herbicide drift from aerial spraying (Perschbacher *et al.*, 2012) observed near RPA during larval rearing in season 2020/21. Bacteria implicated in epitheliocystis (e.g., Endozoicomonads) have been documented as common, functional members of the gill microbiome in fish (Pratte *et al.*, 2018). This may infer that under adverse and immunocompromised conditions these organisms switch from commensal to pathogen (Thomas *et al.*, 2008, Egan & Gardiner, 2016, Longford *et al.*, 2019). This places emphasis on managing respiratory stressors such as low salinity and high turbidity through increased seawater security (e.g., reservoirs or recirculation systems) and filtration effectiveness for the duration of cobia larval rearing.

Comparative analysis of larval performance at BIRC and RPA

A comparative study of larval performance at BIRC and RPA was conducted in season 2020/21 to identify other possible variables associated with the persistent early mortality events of season 2019/20. Numerous lines of evidence indicated that culture site had a strong effect on larval performance to 12 dph. The incidence of first feeding, gut fullness, growth rate (S_L and M_H), condition, size uniformity, development, and survival were all significantly enhanced in larvae cultured at RPA compared to those at BIRC. A plausible yet very fundamental explanation for these differing performance trajectories could be linked to first feeding. The reduced incidence of first feeding at BIRC has been linked to compromised gut ontogeny, growth, and survival of fish larvae (Gisbert *et al.*, 2004, Dou *et al.*, 2005, Chen *et al.*, 2007, Koven *et al.*, 2019). Additionally, BIRC larvae exhibited signatures of starvation (e.g., reduced body condition and increased size heterogeneity) previously linked to decreased prey accessibility in larvae of rock bream *Oplegnathus fasciatus* (Shan *et al.*, 2008) and Atlantic cod *Gadus morhua* (Puvanendran & Brown, 1999). Conversely, the higher incidence of feeding demonstrated by RPA larvae may have led to faster differentiation and function of anatomical and physiological features. This, in turn, allows more efficient detection, capture, and digestive processing of prey (Yúfera & Darias, 2007, Rønnestad *et al.*, 2013). Indeed, gut development of cobia larvae is more closely aligned with size than age, suggesting that larger larvae at RPA had greater capacity to assimilate proteins and lipids (Faulk *et al.*, 2007a). The increased intactness of prey and reduced proportions of digesta (≤ 10 dph) in RPA larvae was further indicative of longer digestive times exhibited by advanced larvae (Zhang *et al.*, 2021). Survival of RPA larvae (35%) was aligned also with benchmarks (17 – 35%) reported by Holt *et al.* (2007), Benetti *et al.* (2008b) and Benetti *et al.* (2010b) providing optimal weaning and grading regimens ensued 12 dph.

We revealed significant temporal effects on growth and development of larvae, which at RPA was comparable or greater than commercial benchmarks. Larvae at both sites followed an exponential growth pattern similarly recorded for cultured cobia larvae reared to 22 – 28 dph (Faulk *et al.*, 2007a, Faulk *et al.*, 2007b). Although growth rates attained by cobia larvae in this project (~0.36 mm $^{-1}$) appears arrested compared to rates (1 mm d^{-1}) reported elsewhere (Hitzfelder *et al.*, 2006, Holt *et al.*, 2007), we did not capture data after 12 dph, a known period of highly accelerated growth (Salze *et al.*, 2011). Conversely, and notwithstanding the effects of ethanol preservation, S_L of larvae cultured at RPA did exceed those at aligning time intervals reported by Faulk *et al.* (2007a) and Kidluff (2001) *viz*. 8 dph (6.5 vs 5.7 mm), 10 dph (7.6 vs 6.8 mm), and 12 dph (9.0 vs 8.1 mm). The development of morphological landmarks including flexion (6 dph) and clearly visible anal fin rays (12 dph) occurred 1 - 4 days sooner in this study when compared to earlier reports (Salze *et al.*, 2011). Developmental anomalies such as pericardial oedema and notochordal axis abnormalities also significantly decreased with time, further lending weight to the premise that these were lethal conditions contributing to considerable batch mortality.

We propose several interactive factors (e.g., aeration, tank colour, water quality) contributed to the strong effect of site on larval performance in this project. Aeration rate is an important determinant of water turbulence and flow field known to impact prey consumption and larval survival in a species-specific manner (MacKenzie *et al.*, 1994, Mangino Jr & Watanabe, 2006, Sakakura *et al.*, 2019). In cylindrical tanks with low exchange rate (<100%) and aspect ratio (AR) <1.0 such as those used in this study, optimal aeration rates should generate single-paired upwelling vortices that create sufficient radial flow velocity to distribute and maintain larvae and prey under the water surface near the sidewalls (Sakakura *et al.*, 2006, Sumida *et al.*, 2013). For example, optimised upwelling flow velocity (8 cm s^{-1}) developed for seven banded grouper (*Epinephelus septemfasciatus*) larvae (Shiotani, 2003) was applied to industrial-scale tanks (100 kL) and was shown to keep prey densities constant, reduce surface tension-related mortality, and significantly increase survival threefold (to 61%) by 10 dph (Sakakura *et al.*, 2006). The observation of a ring of uniformly dispersed larvae near sidewalls at RPA compared to three or four concentrated patches at BIRC is suggestive of a more optimised flow field at the former. A confounding factor here is light, whereupon the artificial source used at BIRC could have promoted crowding, aggressiveness, and cannibalism (Kozłowski & Poczyczyński, 1999) although the latter was not observed. Greater efforts to measure and control upwelling flow velocity in cobia larval rearing systems could significantly improve larval performance.

Differential luminance created by tank colour is known to facilitate prey detection, uptake, and growth of fish larvae (McLean, 2020). In our project, larval performance was enhanced in white (opaque) tanks (RPA) when compared with blue tanks (BIRC). Survival, feed intake, and/or growth of early fish stages increased also in white tanks compared to blue tanks for thinlip mullet *Liza ramada* (El-Sayed & El-Ghobashy, 2011), yellowfin tuna *Thunnus albacares* (de la Serna Sabate *et al.*, 2009), and barramundi *Lates calcarifer* (Santisathitkul *et al.*, 2020). Photic condition of lighter-coloured tanks is known to stimulate the production of melanin-concentrating hormone (MCH) that has a regulatory role in feed intake and growth promotion in barfin flounder, *Verasper moseri* (Takahashi *et al.*, 2014). Further examination of the interactions between cobia larval growth and tank colour will again ameliorate future designs of larval rearing systems.

The addition of microalgae, such as *Nannochloropsis oculata* and *Isochrysis galbana*, is known to improve the performance of several fish species including cobia (Faulk & Holt, 2005). Experimental evidence suggests that larval performance is benefited more by microalgal chemicals secreted into water and assimilated by larvae than by backlighting effects that increase prey consumption (Van der Meeren, 1991, Rocha *et al.*, 2008, Koven *et al.*, 2019). The lower tank densities of *N. oculata* (1600 – 6100 cells mL^{-1}) at BIRC compared to RPA (22,000 cells mL^{-1}) were within range reported for other intensive cobia larval rearing systems (Benetti *et al.*, 2008a, Benetti *et al.*, 2008b) but much less than densities (40,000 – 150,000 cells mL^{-1}) for conventional cobia greenwater systems (Faulk & Holt, 2005, Hitzfelder *et al.*, 2006, Faulk *et al.*, 2007b, Nguyen *et al.*, 2011b). It was possible that at RPA higher titres of growth-promoting microalgal chemicals were present in tanks due to the higher inoculations of *N. oculata*.

Water quality parameters differed significantly between RPA and BIRC, and although the measuring devices will have contributed some of the variance, all values were within acceptable ranges for cobia larviculture (Faulk & Holt, 2005, Benetti *et al.*, 2008a, Benetti *et al.*, 2008b, Borchert *et al.*, 2010, Lee *et al.*, In press).

Diet was found not to affect larval performance except at 3 dph when a significantly higher number of Taurine-R larvae were recorded with gut content. Fish larval olfactory cells exhibit species-specific sensitivities and attractiveness to a range of chemical substances (Rønnestad *et al.*, 2013) including taurine (Martinez *et al.*, 2004). Taurine was previously reported to stimulate strong feeding responses in cobia larvae (Salze *et al.*, 2011). Although growth and survival were not promoted by taurine treatment at 12 dph, the effect has been documented to manifest later in cobia larval development. For example, Salze *et al.* (2012b) found that the activity of digestive enzymes (e.g., amylase, trypsin) began to significantly increase in taurine-supplemented cobia larvae at 8 -11 dph, which translated to significant increases in length from 13 dph onwards (Salze *et al.*, 2011). Uptake efficiency of taurine could be further improved through live prey hydrophobic carrier systems such as wax spray beads (Hawkyard *et al.*, 2014) or liposomes (Barr & Helland, 2007, Pinto *et al.*, 2013).

Faster growth and greater survival of cobia larvae by feeding copepods has been determined experimentally (Nguyen, 2009) and purported for pond-based nursery systems (Liao *et al*., 2004, Weirich *et al*., 2004, Holt *et al*., 2007, Nguyen, 2009, Nguyen *et al*., 2011a, Zhang *et al*., 2021). In contrast, we found no evidence of cobia larvae having consumed the pelagic nauplii stages of a harpacticoid copepod (*Tisbe* sp.). This was likely related to the limited availability of copepod nauplii in tanks that were administered appreciably lower (< 0.2 inds mL^{-1}) than targeted levels (> 1 inds mL^{-1}). In contrast, a secondary benefit of copepod provision was the bioremediation potential for cleaning tank walls and bottoms as reported previously (Hansen *et al.*, 2018). It is expected that further optimisation of copepod culture systems and the timing of production scale-up will improve applications of *Tisbe* sp. to finfish larviculture at BIRC.

Optimisation of feeding strategies

The effect of feed frequency on growth performance

Feeding marketable-sized cobia (2.5 – 3.6 kg) more than once per day in this project did not produce a significant benefit on growth, feed conversion, or size uniformity. These results are consistent with those reported in an earlier study of smaller (~110 g) cobia juveniles (Costa-Bomfim *et al.*, 2014). Cobia are capable

of stomach distention for accommodating large, whole prey items (Shaffer & Nakamura, 1989) allowing for satiety to be reached after one meal (Pillay & Kutty, 2005, Costa-Bomfim *et al.*, 2014). Excessive feeding (i.e., oversatiation) can negate feed utilisation and growth by reducing the stomach residence time required for sufficient digestion and assimilation (Lee *et al.*, 2000a, Lee *et al.*, 2000b, Dwyer *et al.*, 2002, Riche *et al.*, 2004, Schnaittacher *et al.*, 2005). In contrast, significant growth gains may be achieved in early cobia juveniles (~16 g) by feeding two or three times per day (Moreira *et al.*, 2015). Early juveniles appear to have permanently acidic stomachs with digestive activity up to 7 h following a meal (Yúfera *et al.*, 2019) and a return of appetite 8 h after satiation (Minh *et al.*, 2018). It stands to reason that correct alignment of feeding interval and appetite return is required to refine feed frequency strategies across cobia developmental stages. Although we showed that feeding once per day could reduce daily feed applications by 14.1-18.4% without affecting growth performance, the strategy will need to be benchmarked against current practices under field conditions to fully realise any growth, economic, and environmental benefits.

Feed conversion, growth and size uniformity are three of the most important variables fish farmers wish to optimise for reducing feed expenses (Schnaittacher *et al.*, 2005, Costa-Bomfim *et al.*, 2014). Recently, Cherrie *et al.* (2020) found tank-reared cobia juveniles (initial weight 28 – 100 g) fed Pelagica could attain SGR of 0.5-2.5% d^{-1} and FCR 1.04-2.58. Similar variation (SGR: 0.8-2.1% d^{-1}; FCR: 1.5 – 2.5) has been reported for cobia in commercial grow-out settings (Liao *et al.*, 2004, Benetti *et al.*, 2010b, Dutney *et al.*, 2010a, Sampaio *et al.*, 2011, Cherrie *et al.*, 2020). Our results fall within the expected range for FCR but at the lower end of the growth spectrum. Moreover, like other studies on juvenile cobia we showed low overall size variation (Costa-Bomfim *et al.*, 2014) and a trend of increased size uniformity with increased feeding frequency (Moreira *et al.*, 2015). More frequent feeding can facilitate low size variance as feeding opportunities increase and undesirable social behaviours (e.g., dominance) are minimised (Wang *et al.*, 1998, Dwyer *et al.*, 2002, Rodrigues *et al.*, 2019). From an operational standpoint, minimising size variation is important for averting excess time and money invested in maintaining smaller fish (Wang *et al.*, 1998).

Parasite management and identification

Although underlying health condition did not affect the outcomes of the feeding experiment, *Neobenedenia girellae* was identified in this work as another major threat to cobia production in Queensland. This species, together with its often-misidentified relative *N. melleni* (Brazenor *et al.*, 2018), has been described for cobia under a variety of conditions (e.g., sea cages, tanks, ponds) and over a broad geographic range (Lopez *et al.*, 2002, Liao *et al.*, 2004, Ogawa *et al.*, 2006, Kerber *et al.*, 2011, Moreira *et al.*, 2013, Hurley-Sanders *et al.*, 2016). Untreated infestations can lead to corneal opacity and blindness, compromising feeding and growth (Hurley-Sanders *et al.*, 2016) and eventually starvation, septicaemia, systemic infection, and mass mortality (Deveney *et al.*, 2001). Treatment options such as freshwater or formalin baths and praziquantel are effective against monogenean adult and juvenile stages, but less so against sclerotised eggs (Diggles *et al.*, 1993, Sharp *et al.*, 2004) that persist in culture environments (Kearn *et al.*, 1992). As such, in semi-closed systems like RAS tanks and ponds, re-infestations are inevitable during outbreaks and demand vigilant on-farm surveillance and repeat treatments. The potential negative effects associated with chemotherapeutic treatment and repeated handling may prompt operators to explore more welfare-friendly biocontrol options, such as cleaner organisms (Overton *et al.*, 2020).

Conclusion

We have shown the successful uptake of cobia breeding and fingerling production processes by a commercial operator, vindicating a pivotal business decision by RPA to invest in finfish aquaculture in southeast Queensland (Cherrie *et al.,* 2020). A biosecure breeding population was established at RPA and induced to spawn successfully on three occasions, proving conceptually a capability to produce seedstock. The commercial volumes of seedstock (6.84 million fertilised eggs) produced under biosecure conditions at BIRC further demonstrated capacity for a centralised commercial hatchery to supply a budding cobia sector. Regrettably, surplus seedstock was unable to be supplied to a potential project entrant due to the COVID-19 disruption. RPA produced commercial quantities of fingerlings (~65,000) across two seasons (15,454 in 2019/20; 49,111 in 2020/21) in this project. Fingerling production increased by more than 200% in the second season of this project, and by almost 300% when compared to the two seasons prior. Record farm yield for this species (>150 t) in Australia is forecast for 2021/22. These production outcomes herald the reinvigoration and expansion of the cobia sector.

A comparative analysis of commercial-scale larval rearing systems at BIRC and RPA indicated larval performance was significantly enhanced by site characteristics at RPA. Growth, development, and survival of larvae at RPA was comparable or greater than reported commercial benchmarks. Fundamental to the RPA success was a faculty to stimulate effective first feeding. This was likely enabled through a range of favourable and interactive conditions including taurine-enriched live feeds, microalgal density, water flow fields, tank colour and photic conditions. Optimised feed frequency at once per day for harvestable-sized cobia (2.5-3.6 kg) was found also to be a practical strategy for reducing feed input (by 14.1-18.4%) without affecting growth performance.

Several knowledge gaps were identified in this project through communication between staff and a review of broodfish management practice. Possible drivers of poor larval quality produced at RPA and during first and late season spawns, include maternal nutrient deficiencies, feeding frequency, and stocking stress that can likely be addressed in the short-term. Mortality and growth-suppressive factors including malformations, cannibalism, epitheliocystis and *Neobenedenia girellae* pose further risks to cobia productivity. With better data-directed planning and control over system parameters, cobia production appears well positioned to expand and contribute to the resilience of the Queensland aquaculture industry.

Implications

This project builds on and refines cobia aquaculture production frameworks developed previously for Queensland and Australia (Lee *et al.*, 2015, Dutney, 2016, Dutney *et al.*, 2017, Lee *et al.*, 2018, Palmer, 2020, Lee *et al.*, In press). The report provides detailed instructions for successful breeding and fingerling production of cobia at commercial scale in Queensland. Care should be exercised for feeding frequency, parasite management, and stocking density in the management of cobia broodfish. Meanwhile, applications of taurine-enriched live feeds and microalgae to larval rearing systems appear to yield performance benefits. The implementation of data-driven planning and control over parameters such as flow field and photic condition is primed to enhance productivity also and has broad applicability to the farm management of other fish species. Manipulation of feeding frequency for harvestable-sized fish is another strategy farmers could use to control input costs and minimise potential environmental impact. The 14.1-18.4% reduction of feed input achieved in this project could amount to >17 t of feed saved in a two-month grow out period, based on currently projected crop yields (150 t per year).

Connecting end users to this published report and historical cobia aquaculture RD&E via the Business Queensland website (see section Extension and Adoption) will help facilitate further industry uptake of cobia production. Interest in Australian cobia aquaculture has intensified recently, with at least four entities in various stages of business growth entering initial discussions during this project. Overall, the successful adoption of commercial-scale production processes in this project will instil confidence in farmers wishing to explore new finfish opportunities within a dynamic industry landscape. Continued expansion of cobia production will contribute to resilience at the farm and industry level, and open economic opportunities in regional centres.

Recommendations

The variable reproductive performance identified in this project represents a short-term challenge to the self-sufficiency of the cobia aquaculture sector. In the interim, the following broodfish management activities are proposed to support expansion plans at RPA:

- Maintaining stocking density at 4-5 kg/m^3 year-round.
- Maintaining female to male ratios at approximately 2:1 year-round.
- Supplementing feeds with choline and vitamin mix (Dutney *et al.*, 2010c, Dutney, 2016) during spawning season.
- Restricting feeding frequency to 4 days per week during spawning season.
- Performing regimented (monthly) preventative ectoparasite treatment.
- Entering a service agreement with BIRC for contingency seedstock supply.

Cobia larval rearing could be further improved by:

- Increased storage and security of water supplies to mitigate seasonal intake of poor-quality water.
- Supplemental installation of remote sensor platforms placed up- and downstream of farm intake systems. This will improve operator responsiveness to water parameters (e.g., high turbidity, low salinity) that trigger diseases such as epitheliocystis.
- High larval stocking density (40-50 larvae L^{-1}) when cannibalism is not widely expressed (<13 dph).
- Aggressive grading at the commencement of weaning (>13 dph) and transfer (>35 dph) to ≤5 inds L^{-1} (Hitzfelder *et al.*, 2006) in large scale tanks (i.e., 20,000 L) to mitigate cannibalism. While more frequent grading has been suggested in the management of cannibalism of cobia larvae (Benetti *et al*., 2008b, Salze *et al*., 2008, Nguyen *et al*., 2011a), it must be cautioned that this practice can also promote the establishment of new hierarchies and agonistic interactions among larval and postlarval fish (Kestemont *et al*., 2003).

Regarding grow-out and performance of harvestable sized cobia, it will be important for farmers to:

- Continue evaluating feed frequency strategies, feeding efficiencies, and digestibility of new formulations to maximise their economic, biological, and environmental outcomes.
- Maintain a high level of connectedness with R&D arms of aquafeed manufacturers[2] to ensure nutritional challenges are overcome and new formulations evolve sustainably.

[2] At the time of reporting, BioMar Pty Ltd had filled the niche vacated by Pelagica (Ridley Aquafeeds Ltd) to produce a commercial grow-out diet for cobia.

Further development

A long-term management plan needs to be put in place to address an overall decline in reproductive performance exhibited by the small pool of captive cobia broodfish in Queensland. An extensive analysis of historical spawns and reproductive outcomes at BIRC could lead to a better understanding of husbandry activities and environmental conditions associated with the decline. For example, this may guide research into the effect of crowding stress on reproductive performance and seedstock quality (McCormick, 2006, Gagliano & McCormick, 2009) to resolve optimal stocking densities. An effective breeding program will be required in the longer term to minimise the rate of inbreeding and associated diminished biological fitness (Sakthivel *et al*., 2019b). Genotyping and pedigree analyses will enable careful spawning decisions and guide the introduction of new fish from the wild to increase genetic diversity (Sakthivel *et al*., 2019b). Genomic selection models currently under development for cobia will also allow operators to establish familial lines with important traits such as progeny quality, disease resistance, and growth performance (Benetti *et al.*, 2021). Further advances in genomics and gender manipulation techniques to exploit the sexually dimorphic growth of this species could lead to significant productivity gains also (Dutney *et al*., 2017; Benetti *et al.*, 2021).

Several aspects of culture systems were posited to enhance larval performance in this project, and each of these commands experimental trial at the farm level. For example, like larval grouper *Epinephelus septemfasciatus* (Sakakura *et al.*, 2007) and devil stinger *Inimicus japonicus* (Sakakura *et al.*, 2014) it is possible that early cobia larval performance will be promoted under specific tank flow fields that can be proximally controlled by adjustments to aeration rate. This may hold true also for tank colour (Cobcroft & Battaglene, 2009, Cobcroft *et al.*, 2012), inciting an overall need for increased study on parameter control of cobia larval systems. Moreover, renewed research into larval cannibalism may lead to novel management approaches such as electroreceptor disruption, as described for larval African catfish *Clarias gariepinus* (Kawamura *et al.*, 2021).

As the cobia aquaculture sector expands, so too will opportunities to value-stream and explore alternative applications for cobia products such as collagen extracts from skin (Zeng *et al.*, 2012, Sukeri *et al.*, 2021). Biosecurity plans will need to evolve also, with increased capability to detect and manage emerging threats to cobia production, including lactococcosis (Rao *et al.* 2021). For example, the molecular approach used to resolve *Neobenedenia girellae* (Brazenor *et al.*, 2018) in this project could be adopted for routine pathogen screening. Of equal value is the development of practical and welfare-friendly methods to suppress parasite infestations. For example, there is currently large investment in the aquaculture production of cleaner fish such as ballan wrasse *Labrus bergylta* and lumpfish *Cyclopterus lumpus* for use in salmon farming across Europe and the UK (Overton *et al.*, 2020). As alluded to earlier, there is precedence for the use of a biocontrol species (*G. oceanops*) in cobia broodfish maturation systems (Benetti *et al.*, 2007, Benetti *et al.*, 2021). Other compelling biocontrol options that consume all off-host life stages of parasites (i.e., *Neobenedenia* sp.) include cleaner shrimp *Lysmata amboinensis* (Militz & Hutson, 2015). Ground-truthing the efficiencies and commercial outcomes of cleaner organisms for grow-out phases of cobia may open new research opportunities and the development of a new aquaculture sector.

Extension and Adoption

This project was extended principally to RPA who, at the time of reporting, was the only producer of farmed cobia in Australia. Project staff engaged in frequent information exchange through telephone conversations, emails, and site visits. This led to the successful adoption of cobia breeding and larval rearing practices, and commercial production of fingerlings. A series of recommendations for broodfish management was made also to ensure the cobia aquaculture sector transitions towards self-sufficiency in the short term.

DAF is dedicated to make all RD&E outcomes available for industry consideration. The third and final objective of this project was to connect these outcomes to end users via a Queensland Government website. The Business Queensland website was leveraged specifically to provide business owners or intenders key information on cobia aquaculture, including culture technologies and environments. The Cobia aquaculture landing page (Fig. 11) provides an overview of favourable attributes and challenges facing investment in cobia aquaculture. The landing page further provides links to topics including breeding, grow-out, health and market opportunities of cobia. The topic pages also contain links to current and historical project reports that give practitioners and proponents access to detailed enabling strategies for the production of cobia at commercial scale.

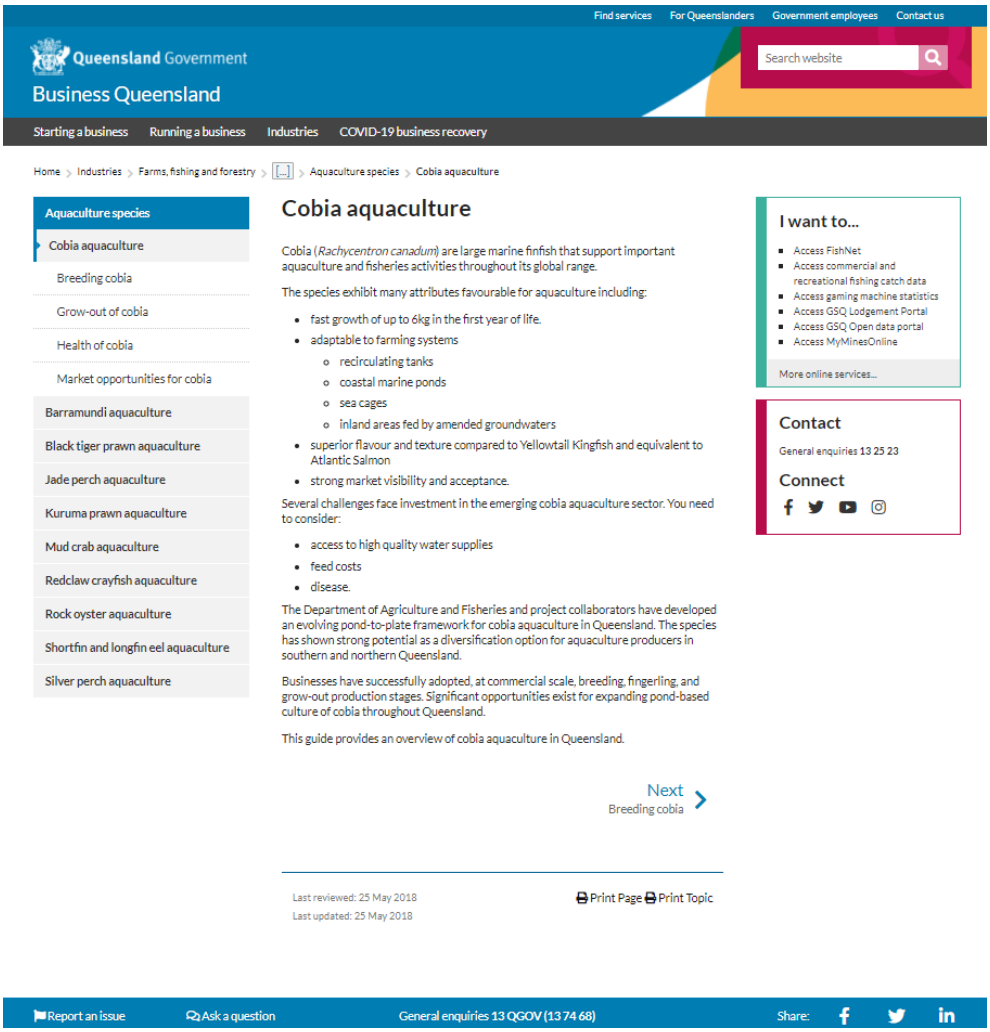

Figure 11. Business Queensland landing page for cobia aquaculture

Project coverage

The final spawning season for cobia at BIRC was promoted on social media, following routine weight measurements and gonadal assessments.
(https://www.facebook.com/QldAgriculture/videos/370081071082771)

Appendices

Appendix 1 - List of Researchers and Project Staff

Department of Agriculture and Fisheries (Bribie Island Research Centre)	
Trevor Borchert	Fisheries Technician
Dr Philip Brady	Casual Fisheries Technician (Nov 2019 – Jun 2020)
Rod Cheetham	Casual Fisheries Technician
Tom Gallagher	Fisheries Technician
Dr Evan Goulden	Principal Investigator
Dr Brian Paterson	Principal Research Scientist
Hazra Thaggard	Senior Fisheries Technician
Rocky Point Aquaculture Pty Ltd.	
Brad Cherrie	Hatchery Manager
Richard Dickson	Hatchery Technician
Murray Zipf	Owner
Serena Zipf	Owner/Co-Investigator
Ansor Consulting	
Dr Luke Dutney	Co-Investigator

Appendix 2 - References

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