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# **Commercialising the production of Cobia in Australia**

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FRDC Project No 2014/242**

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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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# Executive Summary

## What the report is about

This project is a collaboration between the Department of Agriculture and Fisheries (DAF) and the Cobia aquaculture sector, predominantly Pacific Reef Fisheries (PRF). It was undertaken to consolidate the aquaculture in Australia of Cobia, a species offering considerable potential as a diversification option for pond-based culture activities in Queensland. The project addressed key elements of the production cycle in order to move industry towards a more commercial footing and advance both the scientific knowledge and human capabilities of the sector. The project involved DAF staff at the Department's Bribie Island Research Centre (BIRC) and staff from the PRF farm in Ayr, North Queensland. From 2014-2017, research examined: health issues affecting Cobia in culture both in the hatchery and on-farm; the emerging issue of intersex in Cobia and potential for this to further impact production; capability development of PRF staff in preparation of the establishment of a commercial Cobia hatchery; and, the involvement of some new entrants to Cobia aquaculture utilising tank-based production systems.

## Background

DAF has been involved in Cobia R&D since 2007, when Cobia was identified by the prawn aquaculture sector and DAF as a suitable diversification option for pond-based farming activities in Queensland. DAF has a commitment to growing aquaculture in Queensland and working with industry to increase production. The development of both the breadth of Cobia aquaculture, by increasing production capacity, including the introduction of additional producers, and the depth of the industry, by assessing new approaches/technologies for Cobia aquaculture, are both in line with DAF industry development strategies.

Working in collaboration with PRF since 2010, DAF-led research has resulted in significant improvements to production methodologies, juvenile production, health management, post-harvest product development and marketing, summarised in Lee et al. (2015). However, several aspects of production were identified which required further research and development for Cobia production to be genuinely commercialised and vertically integrated. The identification of a significant proportion of intersex fish within breeding populations of Cobia presented a potentially serious problem for Cobia aquaculture and more information had to be generated on this issue. Other issues of on-farm husbandry, particularly relating to health and stock management, were also seen as mechanisms by which production efficiency could be improved. In addition, capacity building, particularly around all aspects of Cobia hatchery operations was identified as key to the pursuit of successful hatchery operations by PRF.

## Aims/ objectives

The current project was aimed at continuing to assist industry to progress towards commercialisation by addressing several key production areas. Health management both in hatchery facilities and on farm were a focus of the project, including the instigation of a farm-based health monitoring program. The issue of intersex individuals in Cobia, which had been identified in an earlier project was also investigated, with the aim of elucidating potential causes, impacts on production and potential management strategies. The provision of fingerlings to collaborating industry partners was continued, to enable continued market accessibility and development, as well as improved production methods and technologies. Integrated in the hatchery production was a component of the project aimed at expanding industry capability through staff training. It also enabled the expansion of the industry base through the incorporation of new industry partners utilising new production technologies.

## Methodology

Hatchery production at BIRC continued using previously developed protocols, and staff from PRF were given training in all aspects of hatchery operation. Changes to the timing of transfer and size of fingerlings were examined, with fish transported later in the season than previously, and fingerlings supplied at a

larger size than previously. In addition, a transport system supplied by PRF and operated on different principles to the system used previously was evaluated. Two additional companies seeking to produce Cobia in tank-based systems were also included in the project. Noosa Ecomarine, operating a recirculating aquaculture system (RAS), and Rocky Point Prawn Farms, using a partial recirculation system, received relatively low numbers of fingerlings to assess the performance of Cobia in their respective systems. Noosa Ecomarine and Rocky Point Prawn Farms received single, trial batches of fingerlings in March 2016 and May 2017 respectively.

Fish health on-farm at PRF continued to be monitored on a regular basis by farm staff. The company engaged a private veterinary company to assist with health management and this company also delivered training in health assessment and sampling to PRF staff. This training was conducted “in house” for PRF staff. Hatchery production at BIRC was impacted by disease events in 2015/16 and 2016/17, and hatchery protocols were modified to be entirely tank-based to help address this issue. The disease was identified as epitheliocystis due to intracellular chlamydia-like bacteria. A molecular approach to the identification of the pathogen and its epidemiology was commenced in conjunction with staff and students from the University of the Sunshine Coast.

The incidence and effects of intersex fish, previously identified at levels greater than 10% in previous cohorts of Cobia, were investigated in a detailed study of development and production parameters. Gonads from 75 individuals were dissected and examined, and gonadal development was monitored by regular non-destructive biopsies from individuals of the same cohort. Individual gonadal development was followed through to maturation at two years old when fecundity and fertility were also assessed. Growth rates of males and females were measured over a one year period and compared to those in cohorts of Cobia exhibiting significant levels of intersex.

### **Results/key findings**

The supply of a total of 59,700 fingerlings (26,300 in 2014/15; 18,100 in 2015/16 and 15,300 in 2016/17) enabled industry collaborators to maintain production at 100 T per annum, sufficient to continue to supply the market which has been developed over several years. System failures resulted in stock losses of Cobia in the RAS at Noosa Ecomarine, and no production data was produced. Preliminary observations of Cobia in a tank-based nursery system at Rocky Point Prawn Farm indicated that this approach may be viable for Cobia commercialisation in South-East Queensland. Three staff from PRF were successfully trained at BIRC in protocols around: broodstock management, including husbandry and health management; spawning induction; larval rearing; and live food production. Losses incurred during one contracted fish transportation event, highlighted the risks associated with this activity. This was successfully addressed in two later transportation activities by adapting PRF’s own prawn transport system.

Health management continues to be a key activity on-farm, with parasites identified as a significant and ongoing difficulty. Farm staff were therefore trained by a consulting veterinarian in health assessments and sampling for health monitoring. In the hatchery, catastrophic losses of larvae were diagnosed due to epitheliocystis, caused by chlamydia-like intracellular bacteria. Molecular testing however confirmed that the bacteria was not in the order Chlamydiales, unlike several other species causing epitheliocystis in fish. Management of the infection by oxytetracycline was demonstrated and this remains an effective means of control.

Intersex frequency was negligible in the cohort examined and these individuals demonstrated significantly different growth and reproductive development to intersex animals. Females grew larger than males and animals underwent normal maturation and were successfully spawned. Intersex was found to occur at negligible levels in this cohort (<1%), and sexually dimorphic growth was shown. Incidence of 0.5% was confirmed by destructive and non-destructive gonadal examinations. Growth rates were also followed and compared to cohorts known to have significant levels of intersex individuals. Animals were followed to maturity and gonadal development assessed using examination of gonadal biopsy samples. Individuals were also raised to maturity and induced to spawn with fecundity and fertilisation rates assessed.

## **Implications for relevant stakeholders**

The study has continued to demonstrate the viability of Cobia as a diversification options for prawn farms, particularly in North Queensland. While the commercial success of tank-based Cobia aquaculture is yet to be demonstrated, continued interest in this species is encouraging, particularly in relation to a potential role for Cobia as a diversification option for prawn farms in the Logan region, following the outbreak of white spot disease in 2016. The development of this sector of the industry may well occur in conjunction with new products being brought to market, potentially broadening options for industry and consumers. In providing prawn farms with diversification and therefore greater economic stability the project is contributing towards rural and remote business resilience and economic opportunities.

## **Recommendations**

Issues of production efficiency and biosecure operation, and the degree to which these are affected by hatchery and farm practices remain significant in Cobia aquaculture. Demonstration in the present study of more biosecure tank-based hatchery methods has highlighted existing products and protocols that may be evaluated to further improve hatchery efficiency, while retaining biosecure practices. Similarly, future on-farm practices which better manage fish health, and are amenable to utilising novel monitoring technologies, will further improve the efficient detection and management of disease.

The underlying cause of intersex development in Cobia was not able to be elucidated in the present study. However, negligible rates of intersex development, observed in this study are consistent with the hypothesis that incidental exposure to endocrine disrupting compounds (EDCs) may have been involved in previous cohorts. It is therefore important to continue to monitor the incidence of intersex in any future cohorts produced at BIRC. Also, investigating the genetic and/or environmental basis for sex determination in Cobia may also provide further insights into potential causes of intersex in this species.

## **Keywords**

**Cobia; *Rachycentron canadum*; aquaculture; intersex; commercialisation; health; epitheliocystis; broodstock management; capability development; pond culture;**



# Introduction

*Cobia*, *Rachycentron canadum*, is a large benthopelagic fish species, endemic to all tropical and subtropical waters across the globe with the exception of the eastern Pacific (Shaffer and Nakamura 1989). It belongs to the order Perciformes and is the only species within the family Rachycentridae. *Cobia* can attain a length of up to 2 metres and exceed 60 kilograms (Franks et al., 1999). They have an elongated fusiform body and broad, flattened heads and the body is smooth with small embedded scales. Body coloration varies from a dark brown dorsal surface grading to a prominently white ventral surface. During juvenile and adolescent stages, a prominent white stripe runs the entire length of the mid-section, which becomes less obvious as the fish ages. *Cobia* are opportunistic carnivores, feeding on cephalopods, fish and crustaceans (Salini et al., 1994). *Cobia* is a highly prized sport fish in Australia and the USA. They rarely occur in large numbers and as such are not heavily exploited by commercial fisheries. The commercial catch of *Cobia* from Australian waters is relatively small at less than 30 metric tonnes per annum (van der Velde et al., 2010)

## Cobia aquaculture

Several biological attributes make *Cobia* an exceptional candidate for aquaculture in Queensland, including: growth rates that exceed 5 kg per year; adaptability to commercially available aquafeeds; excellent palatability and culinary qualities; and, suitable temperature and salinity tolerances (Holt et al., 2007; McLean et al., 2008; Shiau, 2007; Weirich et al., 2007). Commercial *Cobia* aquaculture began in Taiwan in the late 1990s and has since been adopted by several nations through the Asia-Pacific, USA and South America (Benetti et al., 2008; Leano et al., 2008; Liao et al., 2007; Liao et al., 2004; Nhu et al., 2011; Sampaio et al., 2011).

Global production is dominated by China, which first reported production of 14,000 T in 2003 and currently produces approximately 36,900 T, or 87% of the global annual production (Table 1). Production in Taiwan peaked at approximately 4,000 T in 2007, but has since declined to less than half of that in 2015 (Table 1). *Cobia* production in Vietnam increased rapidly from its inception in 2008 to reach 11,000 T in 2012; however, production there has also since declined where several of the major farms have closed. Although China produces the majority of *Cobia* globally, the most detailed information on large-scale commercial production comes from Taiwan. This relatively modest production in Taiwan involves several different phases where fertilised eggs are collected from cage-reared broodstock. These are then transferred to extensive pond systems where they hatch and are raised for approximately 20 days. Two nursery stages are then incorporated into the production cycle before the fish are moved to nursery sea cages. Fish are moved to grow out cages when they are 600-1,000 g, and then reach 6-10 kg after a further 6-8 months in this final production stage, before being sent to market (Liao et al., 2004). The production model used in Vietnam is similar to this production model in Taiwan, although more intensive hatchery and juvenile production methods are used (Nhu et al., 2011). Pilot scale production using submersible sea cages stocked with fingerlings that are produced in a land-based hatchery has also shown promising results in the Caribbean in Panama (Benetti et al., 2010), where 1,200 T was produced in 2015 (Table 1).

Table 1 World production (T) of Cobia, 2006 to 2015 (FAO, 2017)

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
China	20,241	25,855	23,475	29,104	36,356	37,210	38,014	39,627	35,563	36,867
Colombia	0	0	0	5	112	111	145	150	150	0
Panama	0	30	30	50	150	300	230	980	1,459	1,200
Taiwan	2,914	3,998	995	2,365	2,152	1,124	1,384	1,993	1,397	1,466
Vietnam	0	0	1,500	1,800	461	622	11,743	1,873	2,664	2,961
<b>TOTAL</b>	<b>23,155</b>	<b>29,883</b>	<b>26,000</b>	<b>33,324</b>	<b>39,231</b>	<b>39,367</b>	<b>51,516</b>	<b>44,623</b>	<b>41,233</b>	<b>42,494</b>

## Cobia aquaculture in Australia

Cobia research and development began in Australia in 2007, focusing largely on introducing Cobia as a diversification option and off-season crop for prawn farms in Queensland (Dutney and Palmer, 2008). Initial research led by the Department of Agriculture and Fisheries (DAF) at the Bribie Island Research Centre (BIRC) demonstrated the feasibility of juvenile production of Cobia using hatchery infrastructure and techniques similar to those used to produce other marine and estuarine species at the site. As part of a collaborative research partnership in 2007-09, juvenile Cobia were supplied to commercial prawn farms to test the commercial viability of Cobia production in prawn grow-out ponds. These trials were the first to demonstrate the technical feasibility of Cobia grow-out production in prawn ponds and provided evidence of higher productivity yields in tropical localities (Dutney et al., 2010)

Despite the excellent potential and demonstrated feasibility of this style of Cobia production, the development of commercial aquaculture in Australia has been relatively slow. Several factors that are consistent with generally new or emerging business options appear to have contributed to this. Specifically, competition for pond space with higher-value and more well-established prawn crops have acted to slow commercial uptake. In addition, markets needed to be developed for commercial quantities of product (ABC, 2016) and low or inconsistent supply of seed stock for grow out (Dutney and Palmer, 2008) limited wider growth. In 2011, a program supported by the Australian Seafood Co-Operative Research Centre focussed research on broodstock husbandry and hatchery methods in order to improve the consistency of supply of high quality fingerlings. This saw significant breakthroughs in the control of spawning in Cobia (Dutney, 2016; Dutney et al., 2017) and the regular supply of fingerlings to industry. Research under that program also successfully addressed other early barriers to on-farm production, such as post-transfer stock management, pond management for pathogens such as *Amyloodinium*, capacity building within industry and product development. This later initiative included consumer studies which demonstrated a substantial level of consumer acceptance for Cobia (Lee et al., 2015). Collectively, these have contributed to the establishment of a successful fledgling industry of ca. 100 T per annum currently produced by a single operation, Pacific Reef Fisheries Ltd. (PRF), based in Ayr, North Queensland. However, significant barriers to industry commercialisation still exist, and these include reliance on a single government-operated hatchery, limitations to grow-out opportunities restricted to available pond space, and the occurrence of high proportions of intersex individuals in some hatchery-reared cohorts (Dutney et al., 2017).

There are currently no commercial hatcheries producing Cobia; as such there remains a need for support from DAF to supply juvenile fish. Limitations to production and the associated burden on resources has resulted in delayed development of commercial hatchery facilities by PRF. Small-scale hatchery production at PRF was successful in a previous project (Lee et al., 2015); however, PRF is yet to invest in broodstock holding and maturation facilities and is therefore still reliant on BIRC for seed supply to their hatchery. This project was therefore also focused on transitioning from research-

assisted production to vertically integrated commercial production with the overarching goal of stimulating an independent and autonomous commercial Cobia industry.

## Intersex

Cobia are considered a gonochoristic species, (Shaffer and Nakamura 1989); however, during the course of the previous production seasons (2011/12, 2012/13) at BIRC, a significant proportion of intersex individuals were identified (Dutney et al., 2017). This resulted in reduced reproductive output across the affected cohorts, and therefore the regularity and quantity of seed supply. The causative mechanism behind the incidence of intersex individuals, early identification of the anomaly, and the potential impact on reproductive output and commercial hatchery production warrant further investigation. Genetics, diet, environmental parameters and pollutants can result in impaired reproduction and gonad malformation in aquaculture and wild fish stocks (Bergman et al., 2013; Jobling et al., 2002; Purdom et al., 1994). In particular, the occurrence of intersex fish in wild fish populations has typically been associated with environmental pollutants (Jobling et al., 2002). Endocrine disrupting chemicals (EDC) originating from domestic waste water treatment facilities and other anthropogenic influences are known to interfere with the synthesis and action of endogenous hormones resulting in reproductive abnormalities (Bergman et al., 2013; Purdom et al., 1994). The possibility of EDCs and other potential mechanisms that may interfere with the reproductive development of Cobia were therefore considered in the current study.

## Health

Active management of animal health is essential in aquaculture, and regular monitoring of the health of all stock contributes to this. Our development of pond-based Cobia aquaculture has so far faced a number of challenges in this regard. Predation and fish health have had major impacts on productivity over past production seasons. The development of more effective health management strategies for farmed Cobia, both in ponds and in the hatchery, is essential for the ongoing viability of the industry. Fundamental to this is the establishment of on-farm capacity for regular monitoring of fish health, the identification of at-risk individuals or populations, and the efficient submission of samples for analysis and where appropriate, diagnosis, by a specialist laboratory.

Cobia are known to be susceptible to a range of protozoan, bacterial and viral pathogens, which affect animals at all stages of the production cycle, and in the variety of production systems used in Cobia aquaculture (Leano et al., 2008; Liao et al., 2004; McLean et al., 2008). While the type of production system used may affect the level of exposure to pathogens and the likelihood of disease outbreaks, seasonal factors are also important. Some bacterial diseases such as pasteurellosis and vibriosis are more common in colder water temperatures (Leano et al., 2008; Liao et al., 2004). Cobia in ponds, cages and tanks are also known to be susceptible to infection by the monogenean *Neobenedenia* (Leano et al., 2008; Liao et al., 2004; McLean et al., 2008). The parasitic dinoflagellate *Amyloodinium* has also caused significant levels of mortality of Cobia in ponds and cages, where fry and juveniles are particularly susceptible (Benetti et al., 2008; Liao et al., 2004). High levels of larval mortality in hatcheries have been attributed to *Amyloodinium* (Benetti et al., 2008; Leano et al., 2008) and epitheliocystis, with the pathogen identified as the gamma-proteobacterium *Endozoicomonas elysicola* (Mendoza et al., 2013).

In Australian Cobia aquaculture, on-farm mortalities have historically been due to protozoan, and less frequently, bacterial pathogens. The development of a suitable management strategy for the health of pond-reared Cobia must comprise a structured sampling program for both routine and event-responsive monitoring, diagnostic capability and suitable treatment regimens as needed. The DAF Biosecurity Laboratories provide diagnostic services to the Queensland aquaculture industry, and have considerable experience in Cobia health management. In addition, several private veterinary companies provide diagnostic and health management services to aquaculture operations in Australia.

During the course of this project, high mortality rates (up to 100%) were recorded in Cobia larvae and juveniles at BIRC. These were initially ascribed to outbreaks of *Amyloodinium*, but were subsequently found to be the result of epitheliocystis caused by a chlamydia-like intracellular bacterium. It is possible that previous outbreaks of this disease at BIRC in pond-reared fingerlings had also been misdiagnosed, because the gross symptoms are similar. Unlike the ectoparasitic *Amyloodinium*, which affects a wide range of marine fish species (Cruz-Lacierda et al., 2004; Saraiva et al., 2011; Soares et al., 2012), epitheliocystis is caused by gram negative intracellular bacteria. Epitheliocystis has been recorded in over 50 species of freshwater and marine fish (Nowak and LaPatra, 2006), including wild and farmed fish. Severe infection can result in high numbers of fish deaths, particularly in early life stages in culture (Kumar et al., 2013; Mendoza et al., 2013; Mitchell et al., 2013; Nowak and LaPatra, 2006). Typically the gill and skin epithelium is affected, with cyst-like inclusions visible in fresh and fixed tissues (Nowak and LaPatra, 2006). Based on ultrastructural studies, causative agents of epitheliocystis in fish are placed in the phylum Chlamidiae, with most species within the order Chlamydiales (Nowak and LaPatra, 2006), although this is not always the case (Mendoza et al., 2013). This study sought to determine whether epitheliocystis in Cobia at BIRC was due to Chlamydiales bacteria, as has been reported for several other cultured fish species including barramundi (Stride et al., 2013c), orange spotted grouper (Taylor-Brown et al., 2017), largemouth bass (Goodwin et al., 2005), yellowtail kingfish (Stride et al., 2013b), and striped trumpeter (Stride et al., 2013a). The study also sought to investigate the epidemiology of the outbreaks.

## **Project aim**

The current study therefore sought to advance Australian Cobia aquaculture through a combination of addressing specific bottlenecks to production, and supporting industry to make incremental improvements to new and existing production strategies.

# Objectives

1. Transitioning to independent commercial production
  - a) Provide juvenile Cobia for commercial grow out to further develop commercialisation of the species
  - b) Further develop commercial larval rearing capacity through seed supply, technology transfer and staff exchange
  - c) Technology transfer and staff exchange to develop capacity to maintain and spawn broodstock Cobia
2. Expand Cobia production to the wider industry
  - a) Engagement of new entrants with existing licences
3. Improve production efficiencies
  - a) Refine stocking rates and production densities
  - b) Improved health management through routine sampling and early diagnosis, disease investigation and health training
  - c) Implement methods to reduce predation
4. Analysis and identification of reproductive disorders in broodstock

# Methods

## Industry development

Operational and organisational constraints affected the capacity of PRF to establish autonomous Cobia hatchery facilities over the course of the project. The effort to boost industry capability was therefore focussed on an assessment of the suitability of existing prawn hatchery infrastructure for Cobia production, together with staff training and the development of Cobia-specific hatchery skills and knowledge. This was facilitated through regular contact between DAF and PRF hatchery staff, and through staff exchanges. Hatchery staff from PRF came to BIRC for a week and were trained in a range of elements of broodstock husbandry and management. In particular, this involved: hormonal induction; cannulation and oocyte assessment; spawning induction; handling eggs and newly hatched larvae; live feed production; pond-rearing of finfish larvae; larval and broodstock health monitoring; blood sampling; disease treatments; and general broodstock husbandry.

Within the constraints of DAF hatchery operations and integration within PRF on-farm activities, parameters affecting grow-out that were considered were fingerling size, time of transfer, stocking rates and production density. Production efficiency was assessed by growth rate, harvest size and survival rate for fingerlings supplied at a range of sizes and transfer times, over the course of the project (Table 2). While the design of this element of the project was not formally structured, it was planned to generate some comparative data suitable for future production modelling. Fingerlings were initially transferred to the PRF farm at Ayr, and the Noosa Ecomarine facility at Noosa, by road in 2,000 L transport tanks, using loading and transfer protocols developed previously (Lee et al., 2015). In 2016/2017 a different transport system, previously used by PRF for prawn post-larval transport, was used. Unlike the previously used system, which used only high pressure oxygen bottles, this system included the supply of low pressure air with supplementary oxygen to all tanks. Survival during transport for both systems was typically >90%.

Table 2 Details of fingerlings supplied to industry partners during the study

Collaborator	2014/15			2015/16			2016/17		
	transfer date	number	weight±SE (n*) (g)	transfer date	number	weight±SE (n*) (g)	transfer date	number	weight±SE (n*) (g)
Pacific Reef Fisheries	01/15	15,800	20.7±0.72 (6)	01/16	14,200	10.6±0.84 (5)	01/17	5,800	16.3±1.97 (5)
	04/15	10,600	13.6±1.62 (3)	05/16	2,300	45.5±1.96 (4)	05/17	7,700	10.6±1.03 (5)
Noosa Ecomarine				03/16	1,600	81.7±3.49 (3)			
Rocky Point Prawn Farm							05/17	1,800	28.3±2.62 (3)
TOTAL		26,300			18,100			15,300	

\* -mean weights were derived from samples of 30-100 fish weighed as a group. n= number of group samples used to estimate the mean

At PRF, fingerlings were stocked directly into nursery cages, 6 m x 2 m x 2 m, constructed of HDPE mesh with a hole size of 12 mm x 12 mm (Figure 1). Cages were located within ponds, and fingerlings were reared until they reached average weights of approximately 300 g. Compared with previously used systems, this facilitated both closer observation of fingerlings during the post-transfer period and capacity for medicinal treatment if necessary (Lee et al., 2015). Fish were then released from the cages into ponds that were temporarily compartmentalised with fixed nets, which again facilitated closer health and growth monitoring of stock. Fish were then released from compartments into the wider pond environment and grown up to a harvest weight of >5 kg on a floating pelletised diet (Pelagica, Ridley Aquafeeds).



Figure 1. Nursery cages used by Pacific Reef Fisheries  
A) Prior to deployment, B) Sited in a pond at Pacific Reef Fisheries' Alva Beach farm.

Barracrough and Co had well developed plans to pursue a RAS Cobia farming operation, but the company was unable to secure the necessary funding for this, and withdrew from the project in 2014. In 2015, Noosa Ecomarine contacted DAF expressing their interest in pursuing Cobia aquaculture. Noosa Ecomarine operates a recirculating aquaculture system (RAS) in the Noosa region in Queensland. The company elected to pursue Cobia for production in addition to other species and nominated to collaborate in the project in 2015/16. Noosa Ecomarine aimed to assess the potential of Cobia in their RAS at a pilot scale in 2015/16, with the goal of generating preliminary production data and producing sufficient product for a small marketing trial. Fish were initially transferred to quarantine facilities on site and later to 5-T production tanks.

## General husbandry

Cobia broodstock were held and spawned in a series of replicate 35,000 L broodstock holding systems at BIRC. Each system operated independently as a recirculating system, with approximately 90% water retention per day, allowing full photo-thermal control. Each system consisted of a holding tank, 500  $\mu\text{m}$  pre-filter screens, dual 1 kW transfer pumps, zeolite media filter, 150 W UV steriliser, 13 kW heat/chill unit, foam fractionator and moving bed bioreactor. All tanks were fitted with internal bottom drains, and overflow outlets that facilitate the collection of eggs post-spawn. Each tank was fitted with a vinyl cover to exclude natural light and prevent escape. Lighting was provided by two twin 37 W fluorescent lights. Up to 12 broodstock, in a ratio of approximately 2 females: 1 male were held in each system and subjected to environmental and hormonal regimes developed previously (Lee et al., 2015). These fish were either wild caught, from northern Moreton Bay, or captive reared animals (F1 and F2). Stocking density in the broodstock rearing systems was maintained at about  $<6 \text{ kg m}^{-3}$  through progressive grading and removal of selected fish. Fish were fed to satiety five days per week on a mixture of frozen/thawed squid, prawns and pilchards (*Sardinops* spp.). Pilchards were supplemented with a vitamin mixture, as detailed in Dutney (2016).

Water quality measurements including temperature, dissolved oxygen, salinity, and pH were recorded daily, along with general observations of fish condition, behaviour and feeding response for each broodstock tank. The pH of the system was maintained above 7.5 by adding sodium carbonate as required. Total ammonia nitrogen (TAN) was measured weekly. All water quality parameters remained stable and within normal operating levels for all trials. Water quality parameter ranges collected for three representative cohorts are presented in Table 3.

Table 3 Water quality parameters from three representative cohorts followed during the study. (DO - dissolved oxygen; TAN - total ammonia nitrogen)

Temperature( $^{\circ}\text{C}$ )	18.8-28.8	19.4-27.5	20.9-29.4
DO (ppm)	>4.5	>4.5	>4.8
Salinity (ppt)	26.0-36.1	26.5-35.3	29.2-36.5
pH	7.4-8.1	7.5-8.2	8.0-8.4
TAN (ppm)	<0.6	<0.5	<0.6

Broodstock were regularly assessed for reproductive development by ovarian sampling. Ovarian samples were taken by inserting 1-mm diameter plastic cannula through the gonopore and approximately 5-10 cm into the ovary. Oocyte samples were placed on a microscope slide with a small amount of seawater and a cover slip. Gentle pressure was applied to the cover slip to flatten the sample to provide close to a single layer of oocytes. The readiness of the female for spawning induction was assessed for each sample using a proportionate area system, developed specifically for captive Cobia which often exhibit reproductive asynchrony (Dutney, 2016; Lee et al., 2015).

Broodstock identified as being ready to spawn were sedated then injected with 30-50  $\mu\text{g kg}^{-1}$  LHRHa in a cholesterol pellet (Lee et al., 1986). Spawning typically occurred within 40 hours of induction.



Larvae were reared in semi-intensive green water tank systems for two weeks post hatch. The larvae were then transferred to extensive pond production for weaning and on-growing to a size for transfer of approximately 10 g. From March 2016, larvae were retained under semi-intensive conditions in tanks until reaching transfer size. Tanks were supplied with seawater which had been filtered to 1 $\mu$ m and UV irradiated (Smart UV E150S), to a minimum dose of 30 mJ cm<sup>-2</sup>.

## Intersex

Juvenile Cobia used in the study were produced from broodstock held and maintained in tank systems at BIRC as described above. Larvae were grown in semi-intensive green-water tank systems for two weeks post hatch and then transferred to extensive pond production for weaning and on-growing to approximately 10 g as described previously.

This study followed-up from previous observations which found a high (>6%) incidence of intersex in successive cohorts of Cobia produced at BIRC in 2011 and 2012 (Dutney et al., 2017). The cohort used in this study resulted from a spawning conducted in November 2013 and were followed through to sexual maturity in 2014/15. The spawning population comprised a single captive-reared female and three captive-reared males. The female fish contributing to the cohort was of the same origin as parental fish from a previous cohort which included a high proportion of intersex individuals. The relative contribution of each of the brood fish was not determined. All spawning events occurred following hormone induction of both male and female brood fish as described previously.

The growing conditions used in this study were similar those used previously with a maximum stocking density of 15 kg m<sup>-3</sup>. Temperature, salinity, dissolved oxygen and pH were measured and recorded daily using YSI professional plus multi-parameter meter. Total ammonia nitrogen (TAN) was measured weekly when stocking densities exceeded 5 kg/m<sup>-3</sup> in recirculating systems (Table 3). Fish were fed to satiation twice daily for five days per week, and once daily for two days per week on commercially available marine fish diets (approx. 50% protein, 14% lipid).

## Health

In recognition of high levels of mortality in larvae grown in outdoor ponds in 2015/16, protocols with higher levels of biosecurity and control were implemented for a spawning conducted in March 2016. Larvae were initially maintained in culture with approximately 10% (v/v) cultured *Nannochloropsis oculata* added per day, providing a density of approximately 0.5 x 10<sup>5</sup> cells mL<sup>-1</sup>. Approximately 42,000 apparently healthy 16-DPH larvae were transferred to three 10,000-L tanks, rather than at that stage being transferred to the outdoor ponds. These second phase culture tanks were supplied with a flowthrough (20% exchange day<sup>-1</sup>) of 1- $\mu$ m filtered and UV-treated seawater, and a probiotic product (Sanolife MIC-F) was used in line with the manufacturer's recommendations.

In addition, steps were undertaken to identify the agent(s) responsible for mortalities, and investigate its epidemiology. Samples were submitted to the DAF Biosecurity Laboratory for diagnosis in January 2016, which identified the condition as epitheliocystis, caused by a chlamydia-like organism. A project was subsequently developed in collaboration with researchers from the Centre for Animal Health Innovation, University of the Sunshine Coast and aimed to conduct a preliminary epidemiological investigation into the potential causative agent of epitheliocystis in Cobia larvae and fingerlings at BIRC. Investigations into epitheliocystis involved screening of broodstock, water and live feed cultures, as well as sampling fixed tissue from formalin-fixed specimens preserved during the epitheliocystis outbreak in early 2016. Samples were screened using primers specific to Chlamydiales bacteria which had been successfully used in the investigation of epitheliocystis in several species of farmed finfish in Australia (Stride et al., 2014; Taylor-Brown, unpubl. data).

Broodstock screening involved collection of a small amount of gill filament tissue, as well as ovarian biopsy, collected under sedation. A total of 33 captive-reared broodstock (17 male, 16 female), ranging

in age from two to five years were sampled. DNA was extracted from gill clippings (n= 33) and oocytes (n= 16) by incubating tissues overnight at 56°C in proteinase K and tissue lysis buffer, prior to DNA purification using the Qiagen Blood and Tissue kit. DNA samples were subject to conventional PCR using primers specific to the 16S rRNA gene, intergenic spacer and 23S rRNA gene of the order Chlamydiales (Table 4a), as well as two additional primers (Table 4b). A eubacterial PCR targeting the 16S rRNA gene was also conducted on all samples (Table 5).

PCR mix consisted of 1x buffer (Bioline Hotstart 2x mastermix), 0.3  $\mu$ M each primer, and 6  $\mu$ L DNA in a 40  $\mu$ L reaction. PCR conditions were: initial denaturation and Taq polymerase activation at 95°C for 10 minutes, followed by 35 cycles of 95°C for 30 seconds, and 58°C and 72°C for 45 seconds each, with a 7 minute final extension at 72°C. Positive products were purified (Roche HighPure purification kit) directly or following excision from the agarose gel, prior to Sanger dideoxy sequencing at both Macrogen (Korea). The 16-23S fragment was sequenced using four reactions instead of two, due to the longer fragment length. Resulting sequence pairs were aligned and trimmed based on quality, and analysed by BLAST. Phylogenetic relationships were constructed in Geneious®.

Table 4 Chlamydiales primers used in PCR screening of broodstock

a) 16S rRNA and 23SrRNA primers

Primer name	Primer sequence (5'-3')	Primer pair	Fragment length (bp)	Annealing temperature
16SIGF	CGGCGTGGATGAGGCAT	23SIGR		
23SIGR	GGACTACCAGGGTATCTAAT	16SIGF	~1800	56.5°C

b) Additional primers

Primer name	Primer sequence (5'-3')	Binding site
seqP2	AAABGGAATTTCMCRWGTWGC	~600
R	GGACTACCAGGGTATCTAAT	806

Table 5 Eubacterial primers used in PCR screening of broodstock

Primer name	Primer sequence (5'-3')	Primer pair	Fragment length (bp)	Annealing temperature
341F	GCCTACGGGAGGCAGCAG	EUR		
EUR	GGACTACHVGGGTWTCTAAT	341F	465	52°C

# Results

## Industry development

The project has seen further development towards the commercialisation of Cobia production by Pacific Reef Fisheries (PRF). Cobia continues to be a key element of the business and is a well-integrated element of the mixed species aquaculture operations at PRF's farm in North Queensland. The company has developed a clear, integrated, production, business and marketing plan and is committed to continuing with Cobia aquaculture.

Provision of fingerlings, together with extension and other research services from the Department of Agriculture and Fisheries (DAF), facilitated PRF's regular annual production of approximately 100 T of Cobia enabling the company to continue to pursue market development opportunities for Cobia, particularly into the restaurant sector. The company also engaged seafood consultants Fishtales (<http://thefishtale.com.au>) to further develop their marketing and sales strategy. Over the course of the project a total of 72,100 fingerlings were supplied to collaborating farms, with the bulk of these (69,000) supplied to PRF (Table 2). Transfers were undertaken later than in previous studies, with fingerlings first transferred in late January, rather late December, and additional fingerlings were supplied as late as May in some years. In general, fingerlings were transferred at 10-20 g, but in some instances, fish as large as 45 g were sent to ponds and 80 g to recirculating aquaculture system operations. Regardless of transfer time and size, fish performance was relatively consistent in pond culture, with specific growth rates in the 0.9-1.1% day<sup>-1</sup> range (Table 6). Staff training at Bribie Island Research Centre (BIRC) was undertaken by three staff from PRF. The training focussed on key elements of broodstock husbandry and larval rearing. There was also a component on broodstock health management and sample collection/submission, including conducting a necropsy.

Table 6 Production parameters of pond-reared Cobia at Pacific Reef Fisheries, Ayr, 2014-2017.

Transfer date		No. ponds	Stocking density (ha <sup>-1</sup> )	SGR (% day <sup>-1</sup> )	Av. weight (kg)	Yield (T Ha <sup>-1</sup> )	Survival (%)
2013/14	01/14	3	7110 (7110-7125)	0.90	5.1 (4.6-5.6)	21.2 (16.8-25.2)	59 (40-75)
2014/15	01/15	2	7960	0.88	6.4	13.6	55
	04/15	2	5275	0.87	5.8	28.7	45
2015/16	01/16	2	7200	1.01	6.1	31.2	70
	05/16	1	1800	1.19	6.3	9.4	83

Noosa Ecomarine was supplied with 1,600 fingerlings in March 2016 (Table 2) for production trials. These underwent a quarantine period, prior to transfer to production tanks. Regrettably, system failures soon after transfer resulted in the loss of all fingerlings and Noosa Ecomarine also elected not to continue further in the project.

Rocky Point Prawn Farms (RPPF) was one of the Logan River prawn farms affected by white spot syndrome virus (WSSV) in 2016/17. The farm was unable to continue with prawn aquaculture after the outbreak and elected to pursue investigations into Cobia and Queensland grouper (*Epinephelus lanceolatus*) aquaculture. The farm was provided with 1,800 fingerlings in March 2017 (Table 2) and these were reared in the former hatchery buildings, utilising heated water. An examination of the performance of the Cobia and grouper is the subject of a separate FRDC project led by RPPF.

## General husbandry

Broodstock management continued to be successful at BIRC with F<sub>1</sub> broodstock routinely reaching maturity and spawning, both spontaneously and via hormonal induction, in BIRC broodstock facilities. The first cohort of F<sub>2</sub> fish produced at BIRC was also produced during the project. Breakdown of the vehicle contracted to transport fingerlings in December 2014 resulted in the loss of approximately 5,000 fingerlings, and considerable disruption to production plans and subsequent transfer. As a means of both reducing the risks associated with the use of a third-party transport company and developing in-house capability, in 2016 PRF elected to use the company's own transport vehicle, which had been built for prawn post-larvae transport. This multi-tank vehicle used a system of low pressure air and pure oxygen to maintain water quality during transport. In transportation activities in January 2016 and April 2016, 1,000 L tanks were stocked with approximately 1,000 and 1,300 Cobia fingerlings, giving densities of 15 and 14 kg/m<sup>3</sup>, respectively. Water quality (DO, pH, temperature) were regularly assessed during transport and adjustments were made to the air and oxygen flow so that oxygen levels remained above 6 mg L<sup>-1</sup>. The pH remained between 6.8 and 7.5 and temperatures between 25 and 28 °C during these trips. Survival during the 20-hour journey was between 80 and 90%.

## Intersex

Individuals identified as intersex fish in cohorts produced in 2011 and 2012 were distinguished by the presence of both sperm and oocytes within individual gonadal biopsy samples. Post mortem examination of the fish confirmed the presence of both ovarian and testis tissue in these individuals (Figure 2). In affected individuals, intersex and normal gonads were first able to be distinguished macroscopically when the fish were approximately 400 g (Figure 2). Intersex gonads comprised discrete ovarian tissue located anteriorly, abutting testis tissue located posteriorly (Figures 2 and 3). The proportions of each tissue varied between intersex individuals, independent of the size of the gonad. Histological examination of the intersex gonads confirmed there was a distinct junction between ovary and testis sections containing connective tissue and a mixing of gonad tissues (Figure 3). There was no evidence of mixing of tissues beyond the junction area. Individuals showed varying stages of gonadal development, where in most cases both tissues appeared capable of producing gametes. Active sperm and developing oocytes were observed in some intersex gonads; however gamete quality was not examined. Both ovarian and testis sections of the intersex gonad were generally misshapen, being asymmetrical within and between each side of the gonad and lobulated rather than a smooth cylindrical shape (Figure 2). The gonads of several single sex fish, both male and female, were also misshapen to some extent (Figure 4).

Post mortem examination confirmed the incidence of intersex fish was 17% and 7% in the 2011 and 2012 cohorts, respectively (Table 7). In contrast only one of the 182 fish examined by dissection in the present study was identified as intersex (0.5%) (Table 7) and there was no evidence of the misshapen gonads seen in previous cohorts. The sex ratio did not differ significantly from 1:1, at 1.1:1 ( $X^2 = 0.45$ , 1d.f,  $p=0.50$ ).



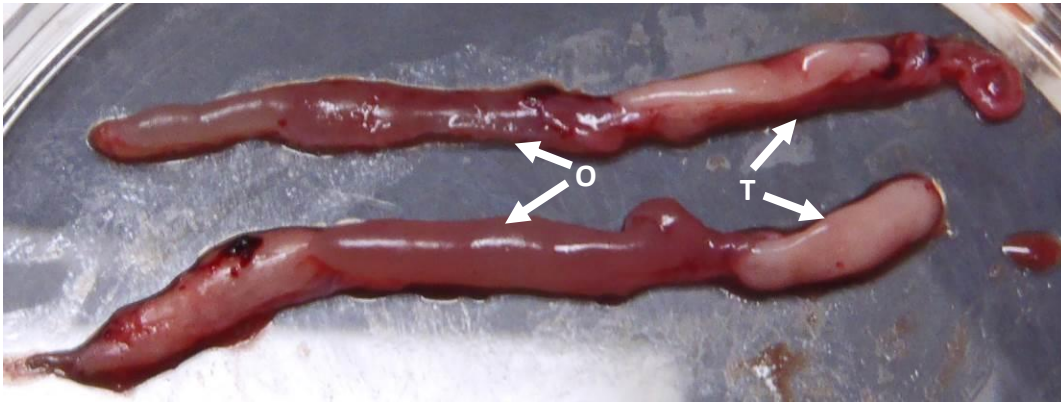


Figure 2 Intersex gonad from a 400 g Cobia showing ovarian (O) tissue located anteriorly and testis (T) tissue located posteriorly, within the gonad.

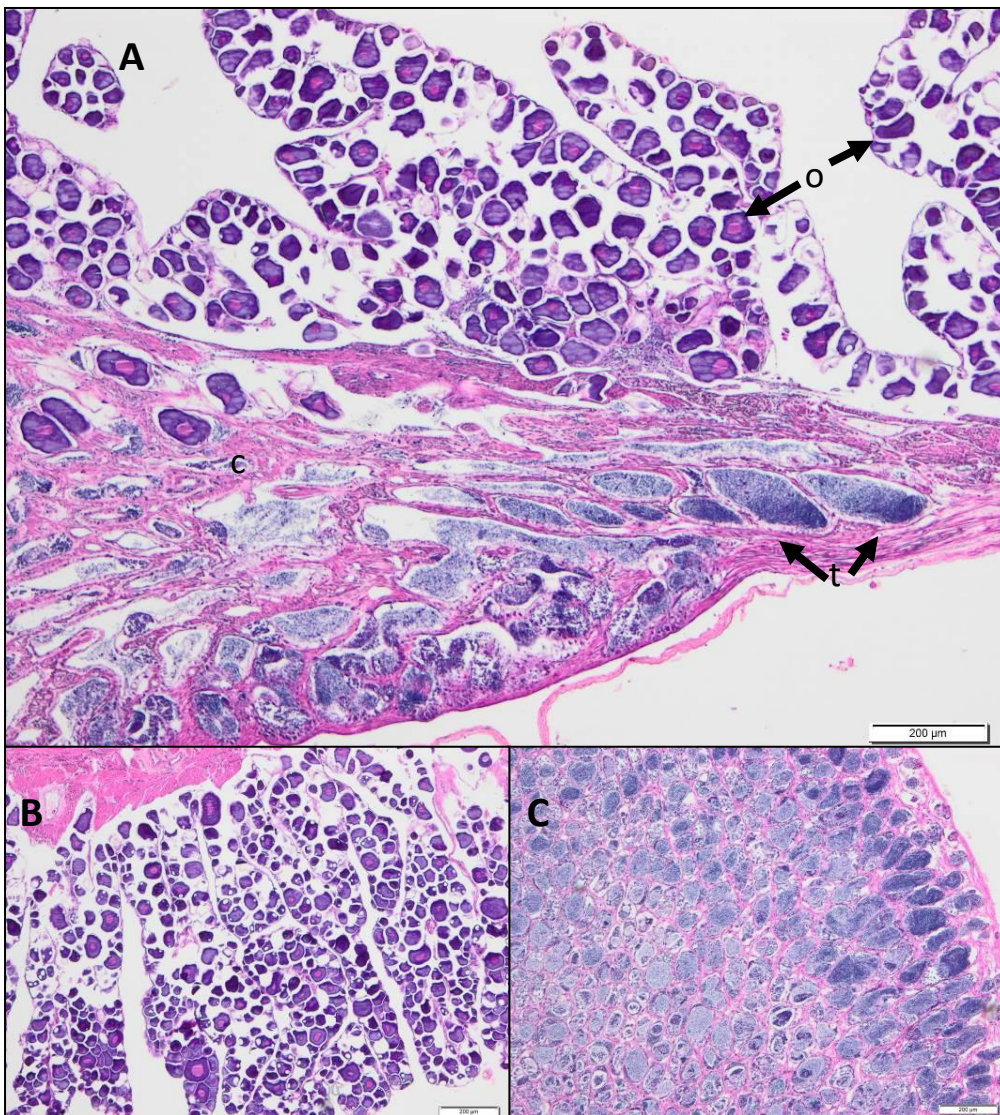


Figure 3 Histology images of the junction of the testis and ovary of an intersex gonad

**A)** Junction showing connective tissue (c) and a mixture of ovarian (o) and testis (t) material, with minimal mixing distal to the junction. **B)** A section of the anterior gonad showing ovarian tissue. **C)** A section of the posterior gonad showing testis material. Scale bars = 200  $\mu$ m.



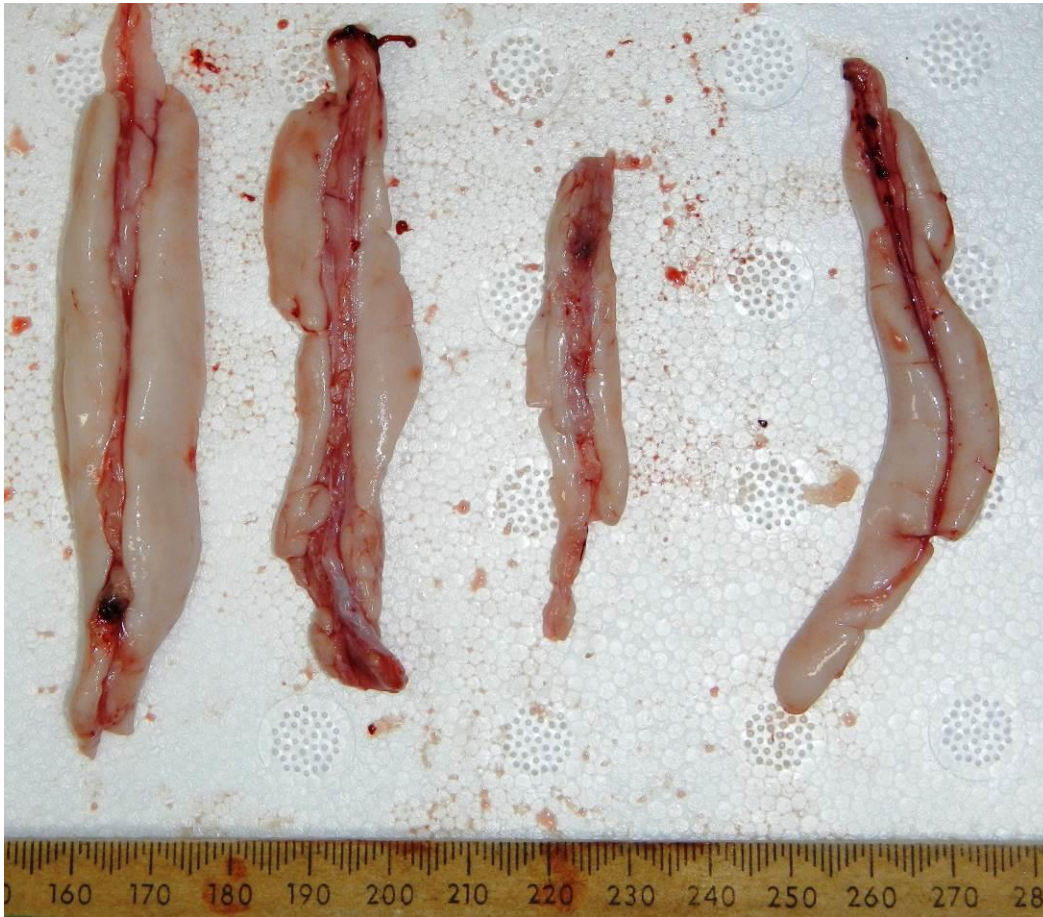


Figure 4 Gonads from male Cobia showing one regular shaped testes on the left and three misshapen testes to the right.

Table 7 Sex ratios of Cobia in three cohorts at BIRC

Year	Total	Female (%)	Male (%)	Intersex (%)
2011	71	38 (53.5)	21 (29.6)	12 (16.9%)
2012	88	58 (65.9)	24 (27.3)	6 (6.8%)
2013	182	95 (52.2)	86 (47.3)	1 (0.5%)

## Health

Training of PRF staff undertaken at BIRC included 1) aspects of broodstock health management including visual and behavioural monitoring for health status, 2) formalin bathing for the treatment of ectoparasites, and 3) necropsy sampling for histopathology and diagnosis. On-farm fish health at the PRF farm in North Queensland was managed in conjunction with consultant veterinarian Future Fisheries Veterinary Service Pty Ltd. This included site visits for sampling and investigation of health events as well as staff training in sampling, identifying and managing disease outbreaks. Disease-related mortalities during on-growing were mainly due to the parasitic dinoflagellate *Amyloodinium ocellatum*. Outbreaks affected fish at all stages of the production cycle.

At BIRC, health research focussed on issues affecting larval survival, which first emerged as a significant problem in 2015. In December 2015, 60,000 apparently healthy 13-day post-hatch (DPH) larvae were stocked into each of two outdoor ponds that had been filled with unfiltered seawater and

fertilised with a mixture of organic and inorganic fertilisers. They were noted to be in poor condition at 24 DPH, following a dense algal bloom. Increased water flow was applied to the ponds, however, moribund larvae were found at 30 DPH and following microscopic examination of gill tissue, the poor condition was ascribed to infection by *Amyloodinium*. This parasite was known to cause significant mortality in *Cobia* larvae and juveniles in ponds in both BIRC and at PRF (Unpublished data). However, a health assessment conducted by the DAF Biosecurity Sciences Laboratory for samples collected at 39 DPH indicated that the fingerlings were in good health with no *Amyloodinium* present at the time of sampling, even though gill changes were consistent with some previous diagnosed infections. More than 15,000 fingerlings were harvested and later transferred to commercial farms.

In February 2016, approximately 95,000 apparently healthy 11 DPH larvae were transferred to each of two grow-out ponds that had been filled and fertilised in a similar fashion to encourage a plankton bloom. Again, 23 DPH, some fish were observed swimming slowly on the edge and surface of both ponds. Fish were sampled from both ponds, and upon microscopic examination of the gills, *Amyloodinium* was tentatively identified through the presence of circular brown bodies on gill lamellae. Increased flow rates were applied to the ponds for the next two weeks in an attempt to manage the symptoms, however on this occasion only 3,600 fingerlings were harvested at 46 DPH.

Larvae transferred to second phase tanks at 16 DPH in March 2016 fed well up to 18 DPH, but there was reduced feeding and elevated mortality levels on 19 DPH, and upon microscopic examination of the gills, *Amyloodinium* appeared to be present in all three tanks. Water flow was increased, but on 21 DPH, large scale mortalities were observed in all three culture tanks (Figure 5). A sample of moribund larvae was fixed in 10% buffered formalin, and sent to the DAF Biosecurity Sciences Laboratory for diagnosis. Histopathology confirmed the diagnosis as epitheliocystis, caused by a chlamydia-like intracellular bacteria. The disease was characterised by cyst-like lesions in the gill epithelium (Figures 6 and 7), which were also visible in whole mounts of gill tissue as brown-coloured inclusions in the gill lamellae, which superficially resembled *Amyloodinium* (Figure 6a). All remaining larvae were subsequently euthanized with excess Aqui-S and the tanks sterilised and cleaned.

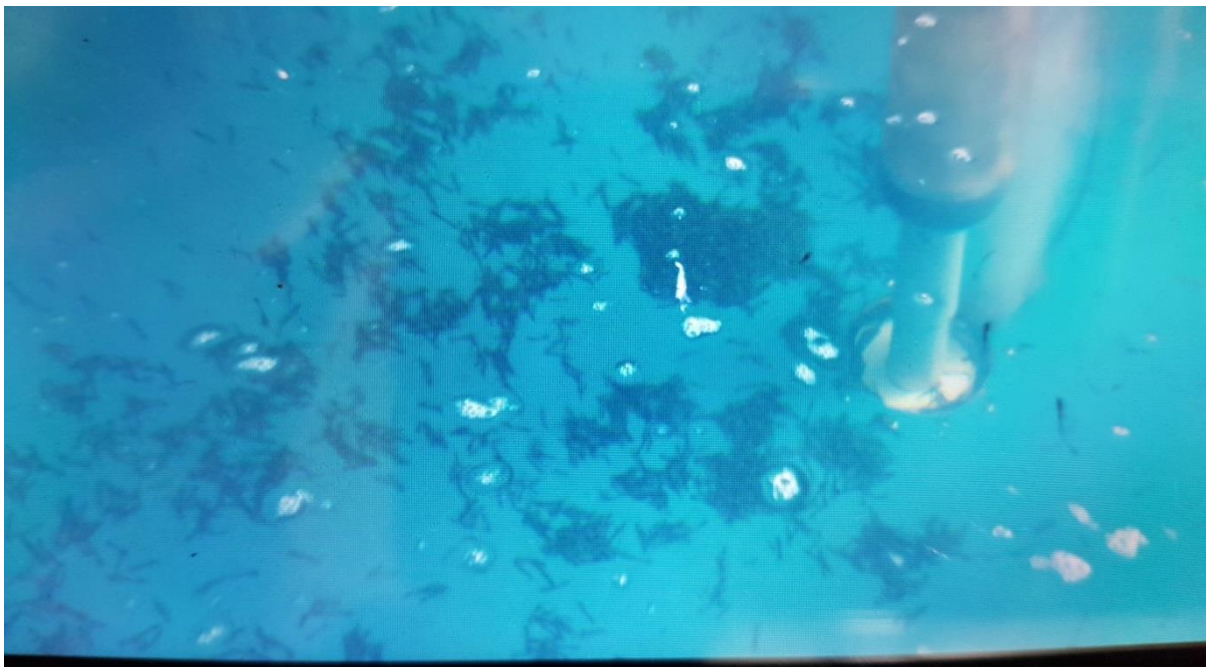


Figure 5 High mortalities of *Cobia* larvae in second phase culture tank.

A further larval rearing operation was commenced in December 2016 and samples of embryos and early stage (1 DPH) larvae were screened for the presence of Chlamydiales bacteria and found to be negative. Larvae began to exhibit symptoms consistent with epitheliocystis on day 18 (post hatch) and laboratory analysis again confirmed similar disease. Treatment was undertaken with oxytetracycline



(OTC), based on a veterinary prescription, using an immersion treatment of 100 ppm for 3 days. Water flows to the tanks were reduced to  $7 \text{ L min}^{-1}$  (equivalent to 100% exchange  $\text{day}^{-1}$ ) and OTC was added twice daily to provide a redosing of 100 ppm. Mortalities declined rapidly following antibiotic treatment, and larvae were reared through to weaning and transfer with no further significant health issues.

Subsequent to the diagnosis of epitheliocystis, a preliminary epidemiological study was undertaken to investigate potential sources or reservoirs of the infective agent. Samples were collected from all 33 broodstock over a two day period. Chlamydiales bacteria were detected in gill samples of two of the 33 broodstock fish tested (6.1%) and absent in all 16 oocyte samples, including samples from the female broodstock which gave rise to the cohorts affected by epitheliocystis (Table 8).

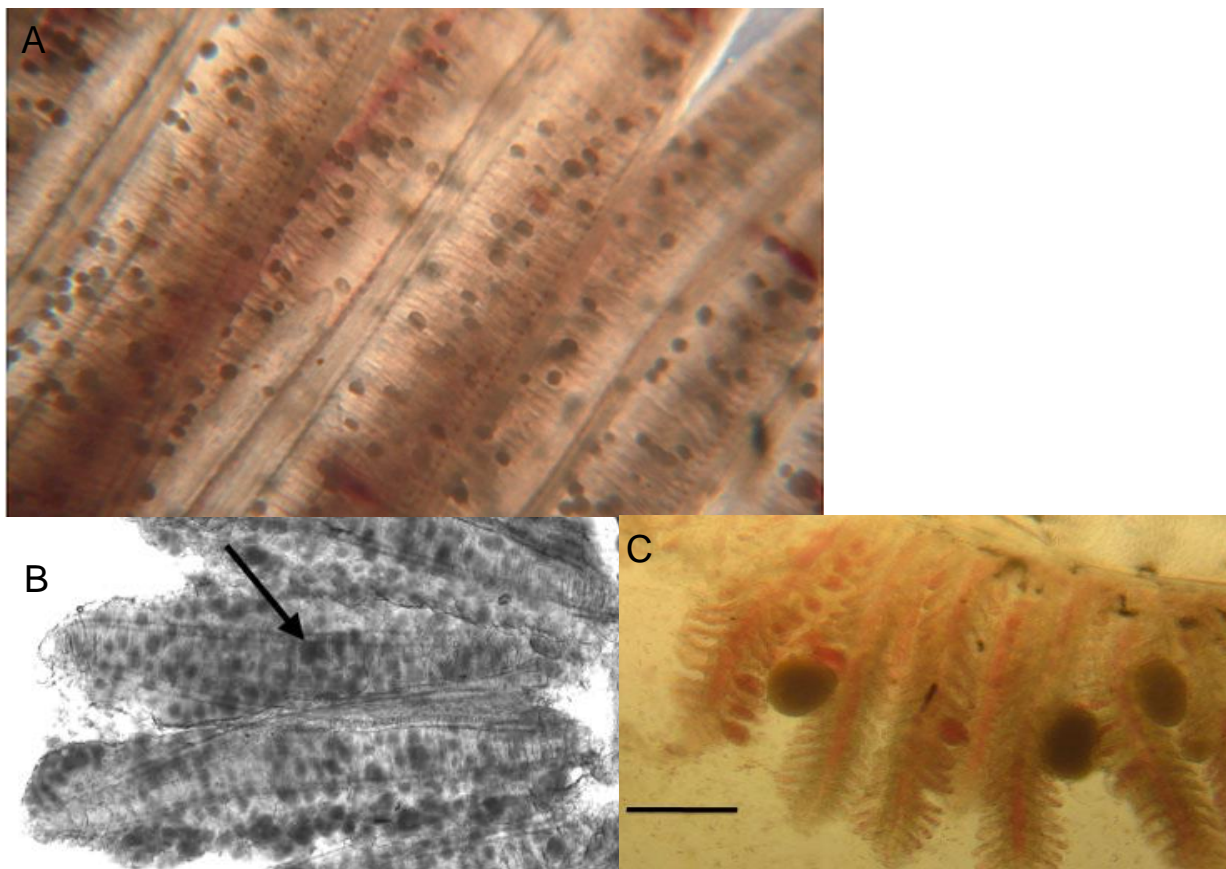


Figure 6 Comparison of the appearance of amyloodiniosis and epitheliocystis in fish gills. **A)** *Amyloodinium* on gills of infected meagre (*Argyrosomus regius*) 40X magnification (Soares et al., 2012). **B)** Epitheliocystis cysts (arrowed) on gills of infected largemouth bass (*Micropterus salmoides*) 100X magnification (Nowak and LaPatra, 2006) **C)** Epitheliocystis cysts on gills of infected Cobia larvae. Scale bar in C =  $100 \mu\text{m}$  (Mendoza et al., 2013).

Eubacterial prevalence was even higher than Chlamydiales bacteria in those Cobia broodstock examined. Positive PCR results for the eubacterial 16S sequences were recorded in six of 33 gill samples (18.2%) and 11 of 16 oocyte samples (68.8%) (Table 8). High quality DNA was not able to be extracted from formalin-fixed, epitheliocystis-positive samples from a previous spawning, and results of the analysis of these samples was equivocal, preventing any further taxonomic analysis.

BLAST analysis of the aligned and trimmed 16S-23S fragments indicated that the novel sequences belong to a bacteria related to *Similichlamydia labri*, which has previously been recorded in wrasse species in Norway (Steigen et al., 2015). A consensus sequence was produced from four sequencing fragments, and aligned with other members of the Chlamydiales (Ca. Parilichlamydiaceae family). The



alignment was trimmed to 1,091 bp and the phylogenetic tree (Appendix 1) was constructed using the neighbour-joining algorithm (bootstrap values are shown on the nodes). Sample 24G was 95.0-95.8% identical to several *Ca. S. labri* sequences and 92.2-94.4% identical to other *Similichlamydia* spp. Sample 7G was not able to be reliably analysed due to a high number of ambiguities.

The eubacterial BLAST results suggest a novel Proteobacterial sequence related to other sequences detected in various fish hosts. The *Cobia* eubacterial 16S rRNA sequence was aligned with related sequences. The alignment was trimmed to 433 bp and the phylogenetic tree (Appendix 1) was constructed using the neighbour-joining algorithm. Sequences were >90% identical to bacteria isolated from a variety of marine fish species from the Mediterranean and the eastern Pacific (Bik et al., 2016).

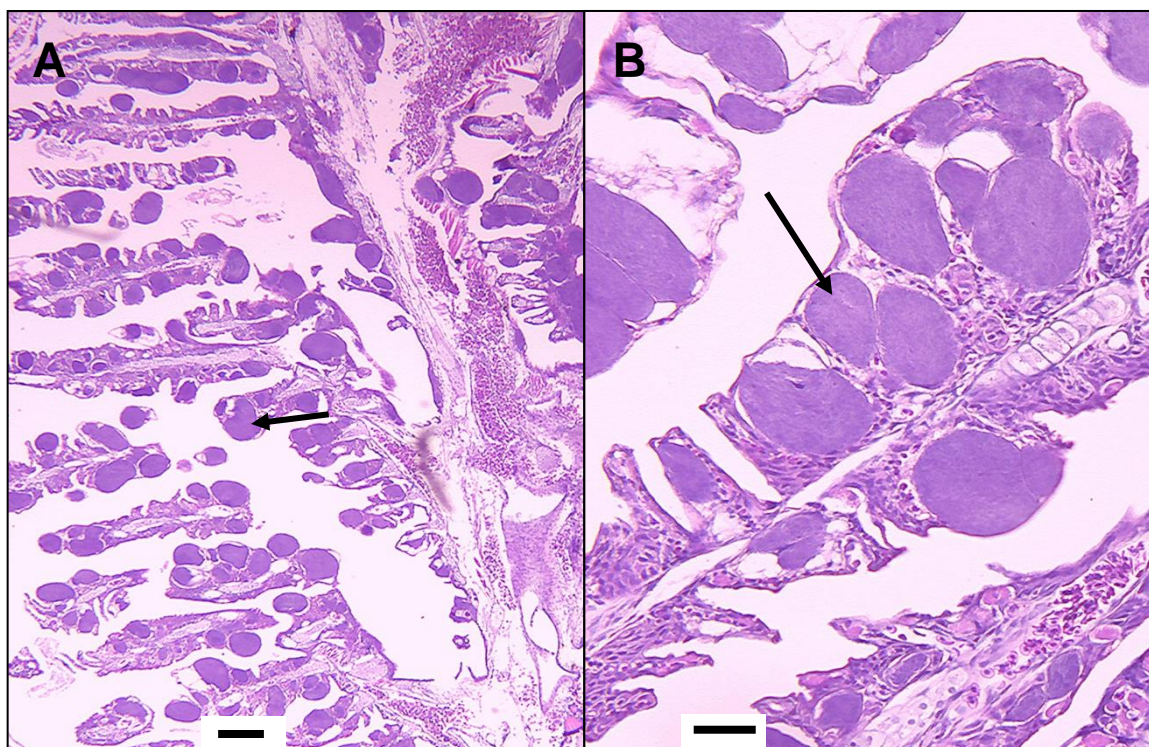


Figure 7 Sections of gills from larval *Cobia* infected with a chlamydia-like bacteria

**A)** 40X magnification, scale bar = 100  $\mu$ m and **B)** 200X magnification, scale bar = 25  $\mu$ m. Note numerous cyst-like inclusions. Photos supplied by Queensland Biosecurity Research Laboratories.

Table 8 Incidence of PCR positive (PCR +ve) reactions for Chlamydiales and eubacteria 16S sequences in *Cobia* broodstock gill (n=33) and oocyte (n=16) samples.

Sample type	No. collected	No. Chlamydiales PCR +ve	% Chlamydiales PCR +ve	No. eubacterial PCR +ve	% eubacterial PCR +ve
Oocytes	16	0	0.0	11	68.8
Gills	33	2	6.1	6	18.2
Total samples	49	2	4.1	17	34.7
Total animals	33	2	6.1	15	45.5

# Discussion

## Industry development

The project contributed to the continued development of Cobia aquaculture in Queensland by facilitating the supply of fish to market. The main industry partner, and sole market supplier, Pacific Reef Fisheries (PRF), has continued to identify and develop new market opportunities and has further developed the company's marketing strategy with the engagement of seafood industry marketing experts. Production strategies for farmed Cobia in Australia have been broadened with fingerlings supplied over a longer period. On-farm performance of fingerlings transported later in the season, assessed by growth rate throughout the production cycle, was not substantially different to earlier-transported stock. Similarly, there was no clear evidence of improved performance from larger transported and supplied fingerlings, possibly reflecting the overall rapid growth of Cobia and attainment of harvest size within one year of production. This later point is important given the greater difficulty in transporting more advanced fingerlings over large distances.

The routine use of nursery systems to control early predation and the additional cost of transporting larger fingerlings are also factors that weigh against a production strategy based on large-fingerling supplies to farms. Importantly though, extending the window for fingerling transfer allows more flexibility in production strategies and for more efficient integration of pond-reared Cobia into pond-based prawn production systems, which is likely to remain the mainstay of Australian Cobia aquaculture in the short to medium term. Although a successful tank-based Cobia production company is yet to be established in Australia, interest in this aspect of Cobia aquaculture remains high. This is likely to be driven, in part, by regulatory factors restricting the establishment of cage aquaculture on much of the Queensland coast. The renewed interest in Cobia aquaculture in South-East Queensland, resulting from losses and restrictions to prawn farming in this region due to the outbreaks of white-spot virus in 2016, also holds substantial promise for the growth of Cobia aquaculture in this region. Undoubtedly, the performance of Cobia aquaculture at Rocky Point Prawn Farm will be closely watched by prawn farms in the Logan area and elsewhere. Enquiries from potential new entrants to the Australian Cobia aquaculture sector are ongoing, with interest in pond, tank and cage production.

Although issues around the development of new farm facilities and expansion at PRF have delayed the establishment of dedicated Cobia hatchery facilities there, the company has maintained a strong commitment to specifically develop a Cobia hatchery. Due to uncertainties in the timing of this hatchery development, it was decided for the current project to focus on staff training at Bribie Island Research Centre (BIRC) rather than continued larval trials at PRF. The training and extension provided in this area to PRF's selected hatchery staff during this project ensures that the company now has a core of staff with experience across all aspects of Cobia production. The focus in this project on broodstock management and hatchery operations complements their existing knowledge in prawn hatchery operations. During the course of the project, knowledge gaps were also identified within the PRF organisation which will need to be addressed for efficient future finfish hatchery operations by a commercial entity. These include the identification of cost-effective fingerling production methods, the application of newer technologies and products, such as improved live feed enrichment and water quality management products, and enriched cultured microalgae, and more advanced forms of Cobia hatchery production, to bring hatchery methods to a more commercial footing. Based on the standards in hatcheries for other Australian finfish species, Australian Cobia hatchery production seems likely to pursue intensive production practices. Drivers for this include comparatively high labour costs, heightened biosecurity awareness and requirements for predictability in hatchery outputs. This contrasts with Cobia hatchery production practices in Taiwan and China, which utilise three different pond-based production phases prior to stocking near-shore nursery cages with 30 g fingerlings (Liao et al., 2004).

## Intersex

In the cohort that was the subject of the current study, negligible levels of intersex (0.5%) were recorded, in contrast to two preceding cohorts reared at BIRC which had intersex levels of more than 6% (Dutney et al., 2017). It is noteworthy that this reduction occurred while there was potentially shared parentage between the cohort in the current study and the affected previous cohorts. Genetic links to intersex have been proposed through inbreeding depression (Gallardo et al., 2004; Su et al., 1996). However, this seems unlikely in light of the results of the present study and the pedigree of broodstock in the current and previous cohorts, which included at least one wild caught parent.

There is compelling evidence in the literature linking endocrine disrupting compounds (EDCs), typically of anthropogenic origin, to the incidence of intersex and abnormal reproductive development in fish populations, particularly in freshwater systems (Bahamonde et al., 2013). However, there is limited evidence of impact on marine fish populations. For example, in the review by Bahamonde et al. (2013) listing 37 species in which the intersex condition was reported from wild fish, there was only one report of intersex in wild marine fish (Diaz de Cerio et al., 2012). This may be due to the diluting effect of the marine environment, as other studies demonstrating intersex in marine species have been restricted to estuaries (Bizarro et al., 2014; Tancioni et al., 2016). Notably, numerous recent studies have, however, demonstrated impacts of EDC's on marine fish at a molecular level (e.g. Dias et al., 2014; Kim et al., 2016; Kroon et al., 2015; Mills et al., 2014)

Based on the limited evidence of anthropogenic impact on marine fish and given the locality of the oceanic intake of BIRC it seems highly unlikely that the incidence of intersex observed in this study is the result of locally sourced EDCs. If the EDCs were sourced from the local environment, it would be expected that local wild stocks would also be impacted. Intersex fish have not been reported in fish from any Australian waters. Kroon et al. (2015) reported increased transcription levels of liver vitellogenin in barramundi (*Lates calcarifer*) and coral trout (*Plectropomus leopardus*) from Australian waters associated with agricultural pesticides. Whilst providing evidence of a feminising effect from EDCs there was no evidence of a phenotypic change in the fish, or resultant changes to the population dynamics.

The summers of 2011 and 2012, in which the previous cohorts with high levels of intersex individuals were spawned, saw two significant rainfall events in South East Queensland. There were no significant rainfall events recorded during the production of the cohort of fish used in the current study. Flood events have long been associated with increased levels of industrial and agricultural pollutants in local waterways, and may be associated with the intersex anomaly observed in previous cohorts. It remains difficult to explain why a similar impact has not been observed in wild fish. If the macroscopic disruption to the gonad, as seen in the current study, were to occur in wild fish in local or any Australian waters it would be reasonable to assume that it would be reported at some level.

Although the incidence of intersex in Cobia broodstock at BIRC appears to have been reduced to an acceptable level in the cohort studies, the specific cause of high levels of intersex remains unclear. Broodstock at BIRC are maintained in RAS, with minimal water exchange; however the hatchery and nursery areas, typical of most research and commercial marine fish hatcheries, rely on flow through water to a large extent, and therefore remain susceptible to a range of water-borne compounds. Additional management approaches, such as the use of additional filtration/water treatment may be considered, particularly if the risk of flood events is high. In addition, future cohorts of Cobia should be examined to confirm the incidence of intersex. Any recurrence of intersex in Cobia is likely to have an impact on commercial production of the species. It has the potential to disrupt broodstock management due to a resultant mismatch in sex ratios. It is unlikely that the intersex fish would be productive and in doing so waste the resources allocated to those individuals. It is also possible that the mechanism that induces the intersex condition could impact on the productivity of the phenotypically male and female fish.

## Health

The high mortalities of Cobia larvae due to epitheliocystis which occurred during the project demonstrate the large potential impact that this disease may have on Australian Cobia aquaculture. Epitheliocystis is recognised as an emerging significant disease affecting both larval and juvenile fish in a range of species (Kumar et al., 2013; Mendoza et al., 2013; Nowak and LaPatra, 2006; Taylor-Brown et al., 2017). While the disease itself can be confirmed by histopathology, identification of the infective agent(s) has been more difficult, due in part by an inability to successfully culture the infective agent (Nowak and LaPatra, 2006).

Epitheliocystis is caused by gram negative intracellular bacteria and evidence to date suggests that these bacteria are host-specific and that there is significant taxonomic variation within the pathogens causing disease in different fish species (Katharios et al., 2008; Kumar et al., 2013; Nowak and LaPatra, 2006; Stride, 2014; Stride et al., 2013c). In the current study, although Chlamydiales bacteria were detected in gill samples from two of 33 broodstock fish, testing fixed samples known to have contracted epitheliocystis, using primers specific for order Chlamydiales did not yield positive results. Therefore, unlike episodes of epitheliocystis in other farmed fish (Katharios et al., 2008; Kumar et al., 2013; Stride, 2014; Stride et al., 2013c), the infective agent causing epitheliocystis in farmed Cobia larvae in the current study, does not appear to be in the order Chlamydiales. This result is however supported by the results of a recently reported outbreak of epitheliocystis in Cobia larvae in Colombia, which was found to be due to the gammaproteobacteria *Endozoicomonas elysicola* (Mendoza et al., 2013). Unfortunately, due to a lack of appropriate primers for testing, this infective agent was not able to be verified as the cause of mortality in the current study.

Although the causative agent was not able to be identified, the effectiveness of means for the control and treatment of epitheliocystis were able to be evaluated to some extent. Improved hatchery practices, such as the implementation of restrictions on the movements of staff in the hatchery, use of footbaths and handwashing stations, the routine use of 1  $\mu$ m filtration together with UV treatment of incoming seawater, did not prevent the incidence of epitheliocystis. The lack of tools to detect the pathogen meant that no conclusions could be drawn regarding the epidemiology of this outbreak. The possibility of vertical transmission of bacteria, as has been proposed to be a route of infection in farmed barramundi (Stride et al., 2013c), remains a potential route of entry for the pathogen at BIRC. Egg disinfection using agents such as iodophores, hydrogen peroxide, ozone and formaldehyde have been shown to be highly effective in reducing bacterial load on the eggs of a wide range of fish species, including some prominent aquaculture species (Swaef et al., 2016). The implementation of egg disinfection procedures for Cobia eggs is therefore likely to provide some protection against infection by vertical transmission, and will be considered for future larval rearing. Live cultures of microalgae and rotifers (*Brachionus plicatilis*) are also potential sources of infection, and an association of epitheliocystis-causing bacteria with amoebae, as proposed by Nowak and LaPatra (2006), may be significant in this. The effectiveness of an immersion treatment with oxytetracycline for epitheliocystis was first demonstrated by Goodwin *et al.*, (2005) and this treatment was highly effective in reducing mortalities in the current study.

*Amyloodinium* is a major pathogen of farmed fish (Cruz-Lacierda et al., 2004; Moreira et al., 2017; Soares et al., 2012) including Cobia (Benetti et al., 2008; Liao et al., 2004). It affects both larvae (Cruz-Lacierda et al., 2004; Paperna, 1984) and juveniles (Cruz-Lacierda et al., 2004; Li et al., 2005; Liao et al., 2004), and can result in significant mortalities. *Amyloodinium* has caused mortalities in farmed Cobia in Australia both in production ponds in North Queensland, and research ponds at BIRC. Presentation of affected larvae or fingerlings in ponds at BIRC has been individuals at the surface showing signs of respiratory stress, and the presence of round or oval brown coloured bodies on the gills, in a wet mount preparation examined at low power magnification (unpubl. data). Both amyloodiniosis and epitheliocystis are diseases affecting skin and gill tissue and wet mount preparations can look similar (see Figure 6 A and B). In the present study, fish displaying very similar symptoms did not respond to treatment by freshwater bathing, experienced high mortalities and were subsequently diagnosed with epitheliocystis. This highlights the importance of obtaining an accurate

diagnosis as soon as possible, and in having staff trained at a high level to recognise potential diseases. Both diseases can be effectively treated in an aquaculture situation, through either the application of therapeutants or by changes to pond management, such as increased water flow, and provided diagnosis is made early, large losses of stock can be avoided. Accurate early detection of pathogens will also assist significantly in the management of these diseases in farmed Cobia.

# Conclusion

Commercial Cobia aquaculture in Australia has become more consolidated over the course of this project with the major commercial partner, Pacific Reef Fisheries (PRF), regularly maintaining an annual production of 100 tonnes. The company has shown considerable innovation in the development of their marketing and sales strategy, utilising their party marketing to target the high end restaurant sector. Production strategies which utilise a broader range of fingerling intake times and fingerling sizes have been demonstrated, broadening the range of options for future production seasons, and allowing for better integration between prawn and Cobia aquaculture within the company. This also provides a valuable organisational model for other prawn aquaculture companies wishing to diversify into Cobia production.

Staff from PRF have gained experience and training in various aspects of broodstock management and husbandry in preparation for the Company developing a dedicated Cobia hatchery in the near future. PRF has developed an effective approach to on-farm disease investigation, through a commercial veterinarian Future Fisheries Veterinary Services and this organisation has assisted with staff training to facilitate health management operations at PRF.

Epitheliocystis has emerged as a significant health issue for Cobia hatchery operations in Australia. While the causative agent is yet to be fully identified, accurate diagnosis is available through veterinary laboratories and it has been demonstrated in this study that outbreaks can be effectively managed through the use of oxytetracycline. The underlying cause of intersex in Cobia was not able to be elucidated in this study. However, results of this study do provide supporting evidence for the role of endocrine disrupting chemicals in causing intersex in Cobia, in that discrete cohorts were affected to a significant degree, with no apparent production-related cause. Furthermore, cohorts of fish were produced with no incidence of intersex, despite a level of relatedness to affected batches, and similar production equipment and methods. A program to monitor reproductive health and reproductive output of captive-bred Cobia should continue to ensure cultured broodstock remain viable.

# Implications

The project has contributed to the consolidation of the fledgling Cobia industry to a \$1M per annum sector. This has been achieved through the provision of production strategy options and an improved knowledge base and capability for future industry expansion, including improved knowledge for the development of commercial hatchery facilities. Health management for both the hatchery and pond-based production has been improved over the course of the project through the identification and demonstration of a treatment strategy for epitheliocystis. In addition, on-farm health management at Pacific Reef Fisheries has improved with training to improve staff capability. Intersex in Cobia appears to be a cohort-specific phenomenon, probably resulting from environmental endocrine disrupting chemicals, and possibly related to extreme rainfall events. The likelihood that intersex may have been caused by an environmentally derived agent emphasises the importance for Cobia broodstock facilities to have a high level of water treatment and environmental control. The incidence of intersex remains an issue to be monitored, in the context of overall broodstock health. The project has confirmed Cobia as a viable aquaculture option for pond aquaculture in Queensland.

# Recommendations

It is essential to continue to work with existing and new members of the Queensland aquaculture sector to develop Cobia aquaculture as a stand-alone or alternative species for Queensland, in both pond- and tank-based production. Initial interest in Cobia came 10 years ago from prawn farmers who were interested in diversification options to mitigate against catastrophic losses from disease. In a post-white spot industry, the importance of Cobia as a demonstrated viable crop for pond aquaculture, particularly in north Queensland is greater than ever. Growing interest and investment from farms in the Logan area also needs to be supported in a “pond to plate” approach as new production strategies will be needed for this area which may also require the development of new markets and/or novel products.

The hatchery sector for Cobia aquaculture should be moved to a commercial footing as soon as possible. The Department of Agriculture and Fisheries remains well placed to continue to support Cobia RD&E; however, in order to advance commercially and expand in size, a functional commercial hatchery is required. In order to facilitate this, research focussed on the assessment of appropriate commercially available products and technologies should be undertaken.

There are undoubtedly efficiencies that can be achieved in the pond-based production of Cobia, in particular the grow-out sector. This may be addressed through the development of improved feeds and/or feeding technology or strategies. Pond design and management which maximise the return on water usage are also areas of significant potential gain. Ongoing extension to support research and development will be a key element in achieving gains in this area.

Health management remains a crucial area of Cobia aquaculture, and will continue to be so, particularly as production increases and new production systems are investigated. The industry has access to high quality veterinary services from both the government and commercial sectors, and highly capable staff on-farm. It is important that health remains a focus of research and production in order to maintain these capabilities. It is essential to continue to investigate the epidemiology and aetiology of epitheliocystis with a view to control, particularly in a hatchery situation. This could include the effectiveness of egg washing to avoid vertical transmission of pathogens and analysis of potential sources or reservoirs of infection within hatchery facilities. Similarly, the development of farm-based tools to aid with early identification of pathogens, together with management strategies following detection, will be very useful for the future viability of the industry.

Although the issue of intersex has diminished in terms of the number of affected individuals in recent cohorts, it will be important to continue to monitor any impaired reproductive output and/or the occurrence of any intersex individuals, particularly in relation to further breeding from individuals from affected cohorts, or their offspring. Broodstock management, the maintenance of genetic diversity and selective breeding programs are both short and long-term goals for the industry, and research to underpin this is also a priority.



# Extension and Adoption

The project was primarily extended and communicated to industry partners through frequent meetings and telephone conversations. Staff exchanges also provided a mechanism for communication and ready adoption of learnings. This report will also form part of the communication to industry partners and the wider aquaculture sector. With growing interest in finfish aquaculture within the prawn farming industry it will be appropriate to present some of these findings at the upcoming industry forum. The project will also be featured as part of the portfolio of Cobia research which is under development for inclusion on the Department of Agriculture and Fisheries (DAF) website which will also include supporting information already in the public domain.

## Project coverage

Cobia was featured in a Landline story on ABC in March 2016. The story featured the success of Pacific Reef Fisheries and touched on the importance of research in achieving this, including DAF's role.

<http://www.abc.net.au/news/2016-04-01/Cobia-to-kick-start-multi-million-dollar-aquaculture/7288980>

# Appendices

## Appendix 1

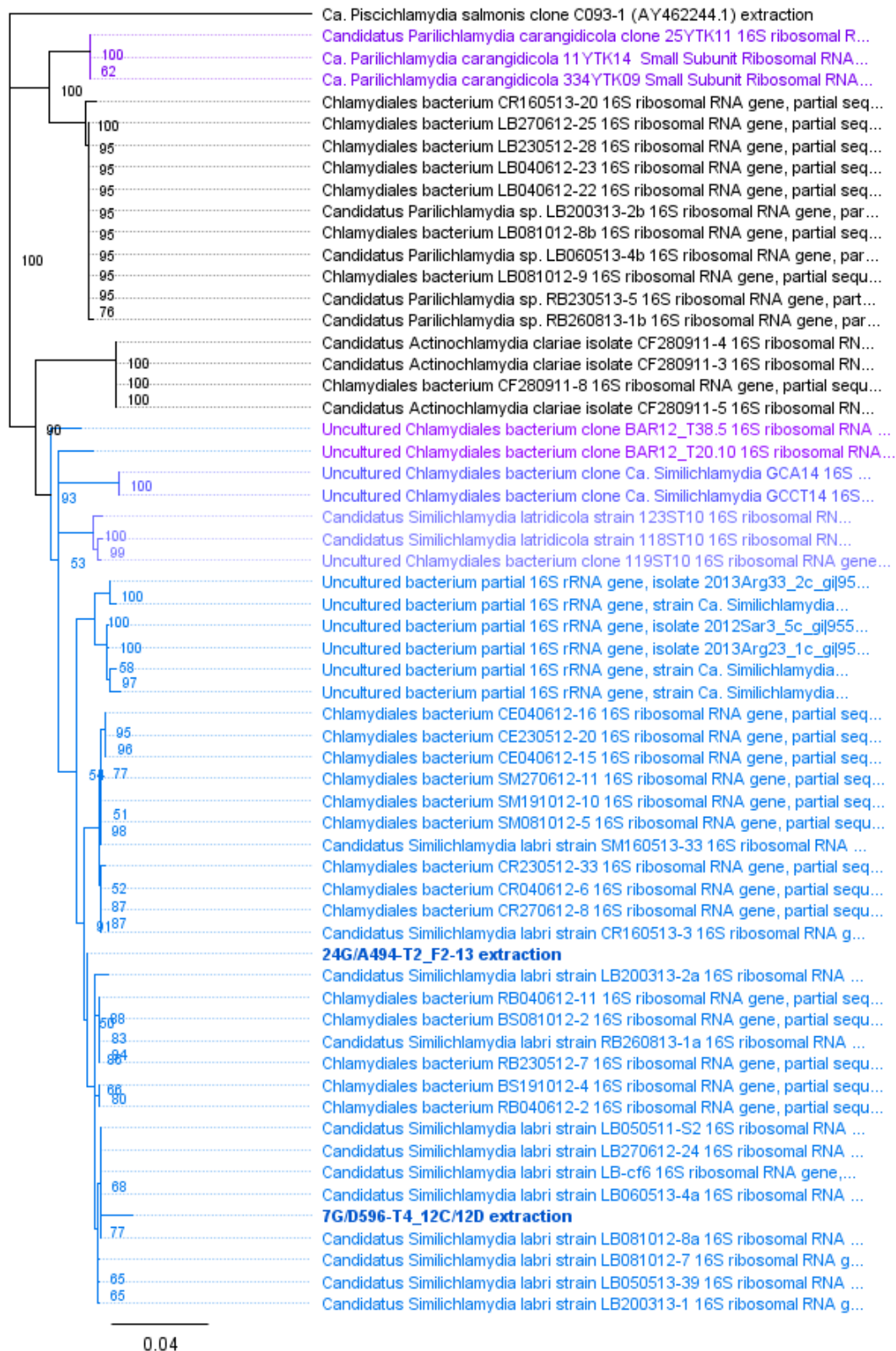
### List of researchers and project staff

<b>Department of Agriculture and Fisheries (Bribie Island Research Centre)</b>	
Trevor Borchert	Fisheries Technician
Dr Luke Dutney	Research Scientist (until Nov 2016)
Dr Jose Domingos	Research Scientist (from Feb 2017)
Dr Peter Lee	Principal Investigator
Tania Lymar	Casual Hatchery Technician
David Nixon	Fisheries Technician
Hazra Thaggard	Fisheries Technician
Ashleigh Zammit	Casual Technician
<b>University of the Sunshine Coast</b>	
Prof Abigail Elizur	Co-investigator, Principal PhD Supervisor for Luke Dutney
A/Prof Adam Polkinghorne	Co-investigator Principal PhD Supervisor for Alyce Taylor-Brown
Alyce Taylor-Brown	PhD student
<b>Pacific Reef Fisheries</b>	
Brad Callcott	Farm Manager
Bastien Finet	Hatchery Manager
Maria Mitris Honos	Chief Operating Officer
John Moloney	General Manager
Linda McIntosh	Hatchery Technician
Chris Katsaros	Hatchery Technician
Glenn Wormald	Hatchery Technician

<b>Noosa Ecomarine</b>	
Josh McNally	Owner
<b>Rocky Point Prawn Farm</b>	
Brad Cherrie	Hatchery Manager
Tony	Hatchery Technician
Murray Zipf	Owner
Serena Zipf	Owner

## Appendix 2

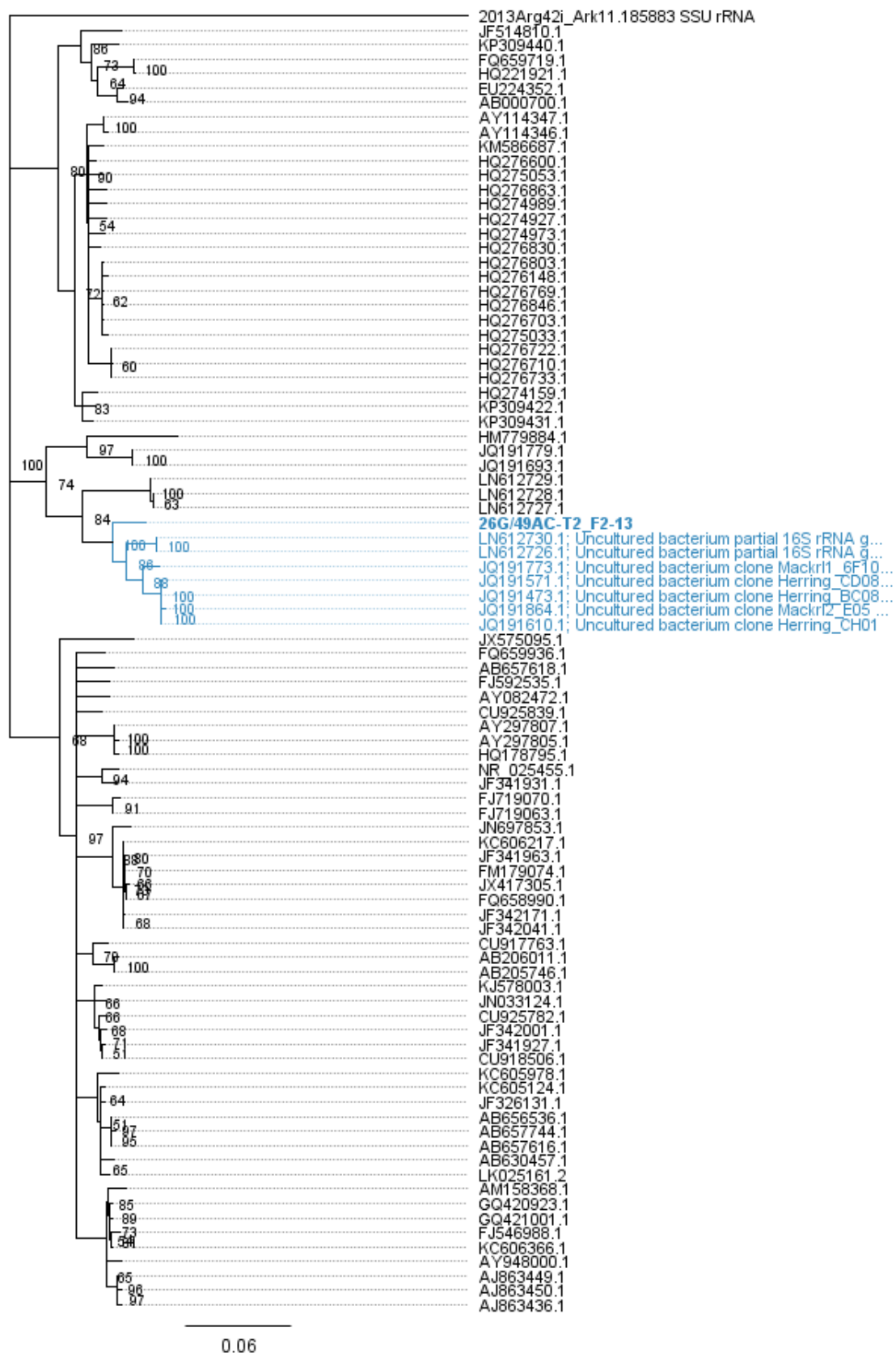
### a) Phylogenetic tree constructed for 16S-23S Chlamydiales positive samples from BIRC Cobia broodstock



KEY: Black: Ca. Piscichlamydiaceae and Ca. Parilichlamydiaceae families.  
 Blue: Similichlamydia spp. Bold: sequences from BIRC Cobia broodstock.  
 Purple: Chlamydial sequences from Australian fish.  
 Bootstrap values are shown on the nodes

## Appendix 2

### b) Phylogenetic tree constructed for eubacterial 16S RNA positive sample (26G) from BIRC Cobia broodstock



KEY: : Blue: Sequences most closely related to 26G (bold).  
 Bootstrap values are shown on the nodes

## Appendix 3

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# FRDC FINAL REPORT CHECKLIST

<b>Project Title:</b>	Commercialising the production of Cobia in Australia		
<b>Principal Investigators:</b>	Peter Lee, Luke Dutney, John Moloney, Maria Mitris, Trevor Borchert, David Nixon, Hazra Thaggard, Brad Calcott, Alyce Taylor-Brown, Adam Polkinghorne		
<b>Project Number:</b>	2014/242		
<b>Description:</b>	Brief one/two paragraph overview of what the project did and achieved.		
<b>Published Date:</b>		<b>Year:</b>	2018
<b>ISBN:</b>	978-0-7345-0458-6	<b>ISSN:</b>	
<b>Key Words:</b>	aquaculture, hatchery, Cobia, <i>Rachycentron canadum</i> , ; intersex; commercialisation; health; epitheliocystis; broodstock management; capability development; pond culture;		

Please use this checklist to self-assess your report before submitting to FRDC. Checklist should accompany the report.

	<b>Is it included (Y/N)</b>	<b>Comments</b>
<b>Foreword (optional)</b>	N	
<b>Acknowledgments</b>	Y	
<b>Abbreviations</b>	N	Few abbreviations used
<b>Executive Summary</b>	Y	
- <b>What the report is about</b>	Y	
- <b>Background – why project was undertaken</b>	Y	
- <b>Aims/objectives – what you wanted to achieve at the beginning</b>	Y	
- <b>Methodology – outline how you did the project</b>	Y	
- <b>Results/key findings – this should outline what you found or key results</b>	Y	
- <b>Implications for relevant stakeholders</b>	Y	
- <b>Recommendations</b>	Y	
<b>Introduction</b>	Y	
<b>Objectives</b>	Y	
<b>Methodology</b>	Y	
<b>Results</b>	Y	
<b>Discussion</b>	Y	
<b>Conclusion</b>	Y	
<b>Implications</b>	Y	
<b>Recommendations</b>	Y	
<b>Further development</b>	Y	
<b>Extension and Adoption</b>	Y	
<b>Project coverage</b>	Y	
<b>Glossary</b>	N	

<b>Project materials developed</b>	N	None developed
<b>Appendices</b>	Y	