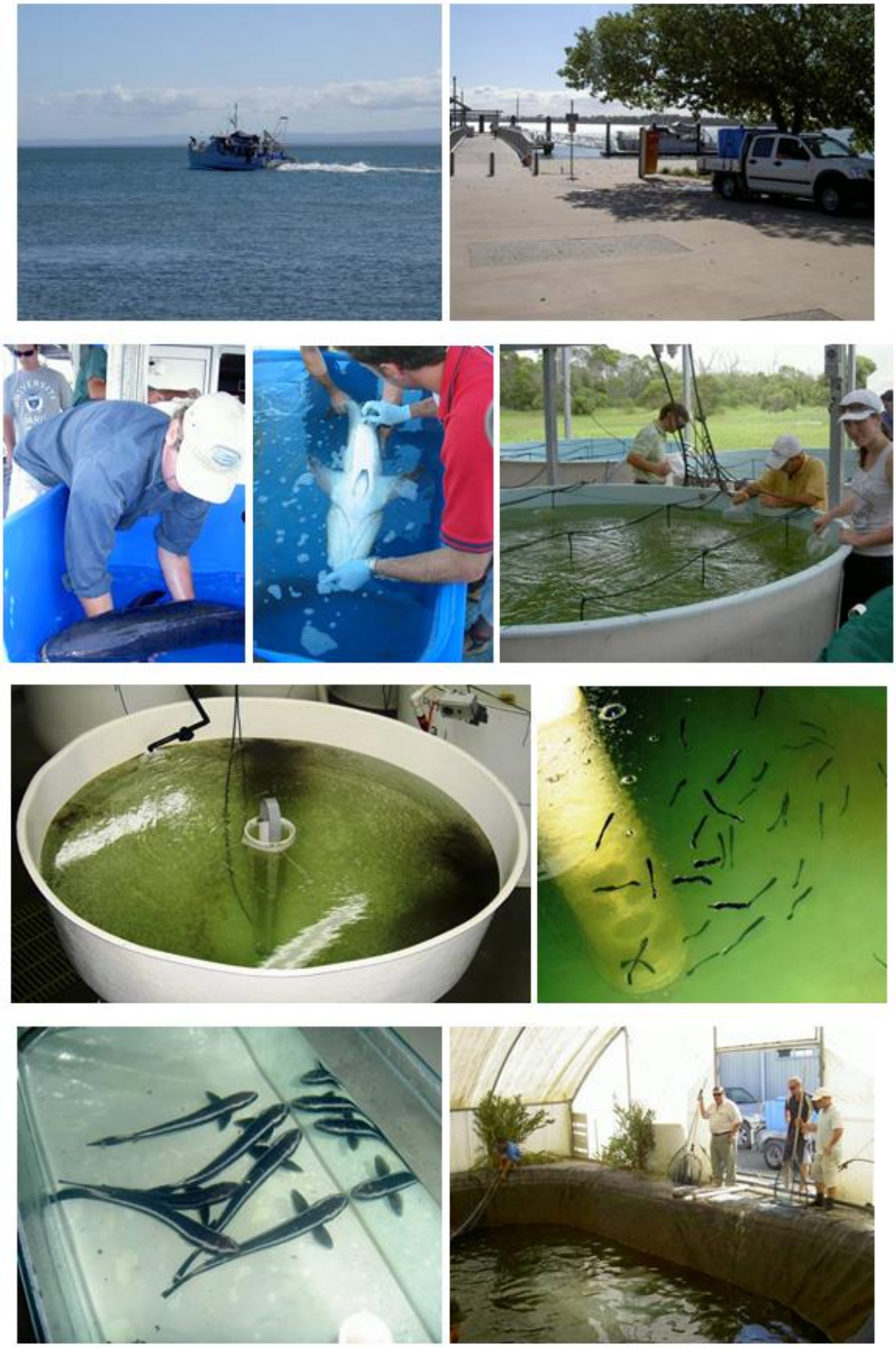
Cobia aquaculture under Queensland conditions

A project within the Queensland Aquaculture Development Initiative 2007–2009



Queensland Government

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Foreword

This report was compiled in 2010 after the first two seasons of cobia aquaculture research in Queensland. It has since been used to provide baseline methodologies, and helped guide production methods for cobia at the Bribie Island Research Centre (BIRC) from 2007 to 2014. In 2015, some disease issues experienced in the various cobia production systems stimulated the investigation of different larval rearing and nursery culture methodologies. The implementation of a white spot disease control zone in 2016 in southern Queensland (encompassing BIRC) further supported an alternative indoor culture approach for cobia fingerling production at BIRC.

As commercial operations in Queensland and Australia move towards independent cobia fingerling production, the Queensland Government is eager to make all research outcomes available for industry consideration. This early research and information may also be useful as industry moves towards further finfish diversifications in the future, because it identifies and documents methods that are applicable to many finfish species, having provided successful, scalable, biosecure and cost-effective seed stock production methodologies for several years as the virtues of this species were investigated in commercial settings.

Executive summary and overview

Background

The cobia or black kingfish (*Rachycentron canadum*) is a large pelagic species which has an extended range throughout many of the world's tropical and subtropical regions. It is widely recognised as one of the most promising aquaculture food-fish species, due to its suitability to large-scale production systems. Its rapid growth, disease resistance, and various culture amenabilities have created production interest in many countries. In 2007, annual global production was estimated at 30 000 tonnes (Schwarz and Kaiser, 2008), and this was upgraded in 2009 to a conservative figure of almost 40 000 tonnes for 2007-08 (Schwarz and Svennevig, 2009). These figures were expected to continue to rise as new ventures focussed on this species came on-line.

The culture potential of cobia was recognised in the early 1970s (Hassler and Rainville, 1975). It was reported to have many desirable attributes for commercial aquaculture. These included fast growth rate (Chou *et al*., 2001; Chou *et al*., 2004), capacity for induced and natural tank spawning with high fecundity (Franks *et al*., 2001; Arnold *et al*., 2002), efficient feed conversion and good flesh quality (Liao 2003; Liao *et al*., 2004). Additionally, the species has shown disease resistance, acclimation to tank and net pen confinement, and adaptability to commercially available aquafeeds (Schwarz *et al*., 2004). These factors led to the rapid development of cobia culture technology in Taiwan in the early 1990s (Liao *et al*., 2004) and later in other countries (Benetti et al., 2008).

At the time this report was compiled, cobia were successfully cultured throughout Asia and accounted for approximately 80% of production in marine cages in Taiwan (Liao *et al*., 2004). China, Vietnam, Indonesia, Thailand, Singapore, Malaysia, the Philippines, India, the United States, Belize, the Dominican Republic, Panama, Mexico, Brazil, Australia, and several islands in the Indian (Reunion and Mayotte) and Pacific Oceans (the Marshall Islands) were undertaking and developing commercial scale cobia aquaculture ventures (Faulk *et al*., 2007; Benetti *et al*., 2008, Schwarz and Svennevig, 2009)[1].

[1] For references cited see individual articles contained in this compilation.

The primary RD&E plan

Following approaches from several established aquaculture industry participants and research bodies in 2006, the Queensland Department of Primary Industries and Fisheries (now the Department of Agriculture and Fisheries (DAF)) began building a program designed to assist industry to investigate and develop the opportunities that this species could hold for Queensland aquaculture producers. Our charter was to investigate opportunities for this species in existing operations. Unlike many other countries which have significant offshore opportunities through investments in sea cage operations, the majority of Queensland's mariculture investments have been directed at prawn and barramundi farming, and are based in earthen ponds. Therefore we focussed our efforts on techniques that use pond-based facilities for seed production and grow-out.

Research funding provided by the Queensland Government through its Queensland Aquaculture Development Initiative (QADI 2007-09) enabled a number of technical studies into factors critical for artificial production of this species. This work was conducted in southern Queensland at the Bribie Island Research Centre (BIRC). It involved local broodstock collection from Moreton Bay, maintenance and induced spawning of captive broodstock using standardised protocols, and larval rearing procedures using generic green-water methods previously developed at the centre. Methods for producing weaned fingerlings using intensive tank-based facilities and extensive plankton-based nursery ponds were also investigated. These seed production technologies are documented in this report for industry consideration and potential uptake in the future. Fingerlings produced using these methods were provided to other research agencies (e.g. CSIRO) for preliminary growth experiments, and to industry for grow-out trials within tank and pond-based facilities. This report summarises this work and associated studies conducted between 2007 and 2009.

Objectives

Broodstock collection and maintenance

- Capture sufficient mature male and female fish to create a viable broodstock population.
- Manage wild-caught broodstock in the purpose-built finfish broodstock facility at BIRC.
- Develop high health protocols for handling and disease control.
- Condition captive fish to mature and spawn.
- Monitor the growth of captive fish and their gonadal development.
- Begin the development of domesticated first generation broodstock.

Induced spawning, egg incubation and hatching

- Assess the capacity of wild-caught broodstock to spawn in captivity.
- Collect spawned eggs and assess fecundity and fertilisation rates.
- Develop hatching protocols to provide high quality larvae for culture operations.
- Apply standardised protocols to assess hatching rates and document larval morphometrics as indicators of larval quality.

Refinements of larviculture support systems

- Operate microalgal stock culture operations that provide high quality seeds.
- Apply standardised mass culture methodologies to produce two complementary species of microalgae.
- Implement reliable methods of rotifer production to support larval fish cultures.
- Investigate the operational condition of microalgal mass cultures as they apply to use in green-water larvicultures.

Rearing larvae through to metamorphosis

- Apply standardised green-water larviculture methodologies to assess its suitability to cobia.
- Assess the metamorphosed fry production levels that are possible with existing facilities and technologies.
- Hone larviculture methods and identify the simplest methods which can produce high quality metamorphosed fry.
- Produce sufficient metamorphosed fry to supply a range of ongoing culture pathways, including tank-based and pond-based protocols.

Producing fingerlings for grow-out trials

- Assess the productivities and practicalities of tank- and pond-based approaches to fingerling production.
- Monitor growth and condition of fingerlings in these production systems.
- Investigate relevant aspects of the operating conditions of rearing environments and the behaviour of fish within them.
- Produce high quality fingerlings which can be used for experimental and industry development purposes.

Preliminary assessments of health and growth under tank and pond conditions

- Conduct regular health assessments on fish at all stages of production.
- Investigate any health issues that come to light in cultured stock.
- Monitor the growth of fish in tanks and in ponds, and document survival, growth and feed conversion under controlled conditions.

Summary of results

Broodstock collection and maintenance

In May 2007, two adult wild cobia that had been captured in 2006 were supplied to BIRC from Gold Coast Marine Aquaculture (GCMA). In October 2007, several collection trips into Moreton Bay were mounted using DAF's large research vessel, and assisted by local recreational and commercial fishers. A total of 16 wild fish (10 of which were considered mature) were captured and transferred to BIRC with very few losses in transit or soon thereafter. Captive fish quickly became accustomed to feeding and tank maintenance routines. Protocols for maintaining high health were developed and successfully applied, although difficulties were experienced in maintaining broodstock in good condition during winter. Environmental manipulations were combined with natural rhythms to successfully stimulate gonadal development in these captive wild broodstock. Domesticated lines of first generation stock were then cultured to maturity.

Induced spawning, egg incubation and hatching

A total of 10 hormone inductions were performed with the captive wild fish yielding a total of 3 successful spawnings. Female fish were cannulated, and selected for hormone induction based on oocyte size and uniformity. The spawning female in the 2008/09 season was the same fish that spawned on both occasions in the 2007/08 season. All other females that were induced did not spawn. Male fish were assessed by either gently pressing the abdomen to see if semen could be expressed from the genital pore, or via extraction of semen with a fine tube (cannulation). Total egg production on each occasion ranged from 4 to 6 million, and fertilisation rates ranged from 76% to 81.5%. Hatch rates were high, yielding ample larvae from each spawn for larval-rearing, nursery, and grow-out trials. Morphometric analyses from larval measurements suggested that there were differences in larval quality between different spawns.

Refining larviculture support systems

In support of intensive larval rearing activities, two microalgal species were supplied from laboratory starter cultures, scaled up to mass cultures, and studied in the hatchery environment. *Nannochloropsis oculata* was used as the base of controlled green-water larvicultures, and this was at times supplemented with *Isochrysis galbana*. The rotifer *Brachionus plicatilus* (large strain) was exclusively used as the first feed in green-water larvicultures. All of these live feed organisms were cultured according to standardised, documented methods. Studies focussed on providing useful practical information about these live cultures. In particular, the relationships between age of culture, cell densities, Secchi depth readings and residual ammonia levels were investigated under normal algal production conditions. The data generated can be used in the assessment of the health and vigour of cultures and their use in day-to-day hatchery operations.

Rearing of larvae through to metamorphosis

Three separate batches of larvae were reared for 13 or more days to a fry length of 8–9 mm long. At this size they proved suitable for handling and stocking into nursery ponds. The standardised green-water culture methodology previously developed for other finfish species at BIRC was successfully applied on each occasion. However, survival was lower than with other species at BIRC, and is thought to be greatly influenced by the rapid growth and highly vigorous nature of this species. Relatively high culture densities were successful in 5000 L flat bottomed tanks – starting as pre-feeding larvae at 50 L^{-1} and finishing as metamorphosing fry about two weeks later at about 6 L^{-1}. Methods were gradually improved and simplified with each batch. Over 200 000 fry were stocked into tanks and nursery ponds for on-growing to advanced fingerling sizes and for further research and industry development purposes.

Producing fingerlings for grow-out trials

Tank- and pond-based approaches to fingerling production were investigated. The most successful method and which has been implemented in continuing seed production efforts for industry development trials is a combination of intensive green-water larviculture followed by extensive rearing and weaning in prepared outdoor plankton ponds. This approach permits the production of juveniles of various particular sizes, and large quantities that appear to be limited only by the availability of pond space. Feeding studies in these plankton ponds demonstrated the tendency of cobia to hunt out the larger copepods, but fall back on smaller copepods when necessary. Fingerlings and juveniles produced in this way were in excellent condition and survived very well during transport over long distances to grow-out facilities, and in dietary research conducted by CSIRO.

Preliminary assessments of health and growth under tank and pond conditions

The health of broodstock, fingerlings and juveniles at BIRC and at industry grow-out sites was observed closely. Health and condition of animals was generally good, although a number of conditions and pathogens were identified during these extensive activities. Survival varied greatly between different facilities. Winter mortalities were observed at one farm in southern Queensland in outdoor ponds, suggesting that overwintering facilities will be necessary for cobia production in cooler regions of Queensland.

Other abnormalities observed included pancreatic malfunction possibly caused by high lipid diets, and kidney disease caused by pasteurellosis. Other pathogens positively identified during this work included cestode helminths in the intestines of fingerlings. Interestingly, one group of fish that were significantly impacted by bacterial infection recovered following prescribed antibiotic treatment and were transferred to a participating farm where they were grown-out to market size without further problems. All mortalities and welfare related issues were reported to the relevant animal ethics committee (AEC) (experiments and activities were covered by AEC approved reference CA 2007/11/230).

Growth rates achieved in ponds in northern Queensland were most encouraging. Cobia there grew to 4–5 kg in the first year of growth (specific growth rate (SGR) = 1.40–1.42) compared with 2–3 kg in southern Queensland (SGR = 1.21–1.27). Production yields ranged from 1.8 to 14.5 tonnes per hectare per year, with higher yields obtained in northern Queensland. Feed conversion ratios ranged from 1.8:1 to 2.3:1. Faster growth rates obtained in northern Queensland are attributed to water temperatures remaining consistently 5 °C higher than the southern zone through the winter.

1. The capture and management of broodstock cobia from Moreton Bay

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Abstract

To assess the potential for wild caught cobia, *Rachycentron canadum*, to spawn in captivity, fish were line-caught from Moreton Bay in South East Queensland. Captured fish were held in closely managed conditions over two years at the Bribie Island Research Centre (BIRC), a dedicated multi-functional aquaculture research facility in southern Queensland. Broodstock facilities consisted of flowthrough seawater tanks with a capacity of 35 000 L, supplied with aeration, ambient water temperature, and light control for spawning manipulation. High health protocols were developed for handling and disease control. To condition captive fish to mature and spawn, broodstock were fed a mixture of fish, squid and prawns every second day to satiation. Fish were tagged for identification, and weight checks were conducted coinciding with health checks, disease treatments and gonadal assessments. This management of wild-caught cobia led to the first generation of domesticated broodstock.

Introduction

Cobia is a marine finfish species found in tropical, subtropical and temperate waters worldwide, with the exception of the eastern Pacific (Shaffer and Nakamura, 1989). It has been reported as an excellent candidate for aquaculture, with positive culture attributes including rapid growth rates (Hassler and Rainville, 1975; Chou *et al.*, 2001; Chou *et al.*, 2004), the capacity for natural and induced tank spawning with high fecundity (Franks *et al.*, 2001; Arnold *et al.*, 2002), and good flesh quality (Liao 2003). Holt *et al.* (2007b) reviewed the emerging potential of cobia aquaculture with an overview of broodstock management and induced spawning (using hormone injections) in recirculating tank systems. Methods for the capture, transport, acclimation, sampling, health and disease management, and conditioning of cobia broodstock for natural and induced spawning in recirculating maturation systems is also well documented in Benetti *et al.* (2008). Water temperatures in the range 20 to 27 °C have mainly been used to stimulate captive spawning in wild and first generation F1 broodstock in the Americas and the Caribbean region (Benetti *et al.*, 2008). Salinities of 27 to 36 ppt have also been applied (Holt *et al.*, 2007b), with maturation diets including fish and squid, complemented with a supplementary vitamin and mineral mix (Benetti *et al.*, 2008).

The capture and management of broodstock was a primary requirement for the present study. Since it is generally accepted that wild fish that are caught when mature and subsequently held in captivity are less useful for controlled reproduction than cultured stock, a reasonable amount of redundancy was required in the number of wild fish that needed to be collected. These considerations initially guided wild collection efforts, and later justified the culture and maintenance of selected first generation broodstock. The purpose-built finfish rearing facility at BIRC was fully utilised to maintain and condition these stock.

Materials and methods

Broodstock collection

Transfer of broodstock from GCMA

Two cobia broodstock were initially transferred from Gold Coast Marine Aquaculture (GCMA) to BIRC on 15 May 2007. The fish were acclimated to the conditions of their new holding tank over 1.5 hours, then anaesthetised with 20 ppm Aqui-S, examined externally, weighed and cannulated to provide gonadal samples.

Wild fish collection

Larger scale broodstock collection attempts were initiated in October 2007, using the DAF 14.5 m research vessel “Tom Marshall” (Figure 1). Two trips were conducted to the outer reaches of Moreton Bay, to several known cobia fishing locations. The capture technique involved line fishing using a combination of lures, and live and fresh baits.

In preparation for broodstock collection the vessel was equipped with two well-secured 1000 L transport bins. Both bins were plumbed to the deck hose to allow the flow through of seawater at approximately 50 L/min. This vessel is well suited to this purpose, due to its stability when carrying tanks of seawater, and capacity to also safely carry up to 10 crew/fishers. DAF staff were assisted by a local professional fisher and an experienced recreational fisher from CSIRO.

Figure 1 – “Tom Marshall” – The DAF collection vessel

At the conclusion of the collection exercise the fish were dispatched for road transport at the public wharf on Bribie Island. The fish were anesthetised and transferred to a DAF vehicle equipped with two 1000 L transport bins, then transported to BIRC (approximately 10 minutes by road) where they were anesthetised, examined externally and cannulated, before being placed into the broodstock facility.

Once settled and feeding for several days, the captured fish were separated into two broodstock tanks according to different size classes. A further two juvenile fish (each <500 g) were supplied by a local professional fisher in early November 2007. These fish were placed with the smaller size class.

Broodstock culture system and maintenance

The broodstock facility at BIRC consists of four 35 000 L fibreglass tanks with flow through seawater available at up to 500 L/min. Flow rates were set at approximately 150 L/min and adjusted as required dependant on water quality measurements. All tanks were fitted with internal bottom drains to provide self-cleaning attributes, and overflow points that facilitated the collection of eggs after spawning. Each tank was fitted with a vinyl cover to prevent fish jumping out of the tanks, and lighting was provided by two twin-fluorescent lights.

A further four 10 000 L roto-moulded tanks (on right in Figure 2) were used for quarantine and to house immature fish selected as future broodstock.

Figure 2 – BIRC finfish broodstock facility – larger broodstock tanks on left

Water quality

Water quality measurements including temperature, dissolved oxygen, salinity, and pH were recorded daily along with general observations of fish condition, general behaviour and feeding response for each broodstock tank.

Phototherm regime

All broodstock tanks were maintained on ambient water temperatures and the light regime was coordinated to mimic ambient conditions (Appendix 1 Table 1). Based on previously published information, the captive fish were expected to be in spawning condition through summer from about mid-October. At that point egg collection baskets were installed on the tank outlets and monitored and cleaned daily.

Diet

Year 1—Fish were fed to satiety three times per week on pilchards, squid and a commercially available broodstock diet (Lansy Fish Breed M™). Amounts fed equated to approximately 7% of the body weight of broodstock per feed. Lansy Fish Breed M was used as a dietary supplement to potentially improve oocyte quality. The fish would not initially accept this artificial diet, but as a result of some innovative feeding

techniques (placing the broodstock pellet inside squid tube before feeding), the pellet was successfully added to the diet about 1–3 months after the fish were captured.

Year 2—The feeding regime remained at three feeds per week, fed to satiation. Large quantities of cultured whiting (*Sillago ciliata*) were then available following the conclusion of other experimental work conducted at BIRC. These feeder fish constituted a majority of the diet in this second year, being fed twice weekly to the cobia broodstock with the addition of vitamin supplements to the whiting gut cavity (see Appendix 1 methods and Table 2). The remainder of the diet consisted of squid and a small (approx. 5–10%) supplement of prawns. Excess food was collected soon after feeding to maintain hygiene and also to assess food consumption.

Anaesthesia and handling

To manipulate the broodstock, the broodstock tanks were lowered to a working depth (approximately 400 mm) with a volume of 4000–5000 L. About 10 ppm (50 mL) of clove oil-based anaesthetic (Aqui-S™) was added to the entire tank to initially partially sedate the fish, thus reducing stress and the potential for physical damage to the fish, as well as to ensure the safety of the staff handling these large animals. After approximately 10 min the fish were still able to maintain equilibrium yet were sufficiently sedated to improve the ease of handling. The fish were lifted via a plastic sling into a 600 L anaesthesia bath (25 ppm Aqui-S™). Full sedation of the fish facilitated various examinations including cannulation, weighing and closer health assessments.

Prophylactic disease treatment

Upon capture and transfer to BIRC, fish were routinely held for one week before administering a prophylactic parasite treatment. This involved immersing the fish in a formalin bath (200 ppm) for 60 min. Procedures involved lowering broodstock tanks to a working height containing approximately 4000 L of seawater, providing supplementary aeration in the tank, and oxygenation through two fine air stones to ensure that the dissolved oxygen levels remained at saturation for the duration of the formalin treatment. Broodstock were monitored regularly during treatment and dissolved oxygen levels were recorded every 15 min. When the treatment finished the tanks were flushed for one hour with approximately 300 L/min of seawater, and then refilled to 35 000 L within the next 90 min. At no stage was there evidence that the formalin treatments had any negative impact or caused distress to the fish. On every occasion the fish fed normally the following day.

Prophylactic formalin treatments were conducted at approx. three-monthly intervals. From January 2008, formalin treatments were adjusted to 150 ppm for one hour.

Freshwater baths were also used to control a relatively minor benedenian infection. The fish were sedated and then placed into a series of well-aerated 600 L freshwater baths for 5 min. Each bath was then flushed simultaneously with seawater at approximately 50 L/min. Then the broodstock tanks were thoroughly scrubbed clean, flushed again with clean seawater and refilled back to operational depths. As the water level in the tank rose, the previously sedated broodstock were released from the 600 L treatment tubs back into the broodstock tank. This method of treatment minimised handling and the physical lifting of the large fish between tanks.

Tagging

Passive integrated transponder (PIT) tagging of the broodstock was conducted following the completion of the first spawning season. The tag numbers were retrospectively added to the data through the tracking of fish weight, fish transfer data and particular identifying fin notches that had previously been applied to some individuals. To apply PIT tags broodstock were initially anaesthetised using the methods described above. PIT tags were inserted subcutaneously into the dorsal musculature close to the base of the first dorsal spine

on the left side of each fish. The puncture site was subsequently rinsed with iodine and a fin clip was taken and preserved for use as a genetic sample.

Growth measurement

Weight checks were conducted periodically coinciding with health checks, disease treatments and cannulation. Broodstock were anaesthetised to facilitate handling using the aforementioned methods. A sling consisting of smooth vinyl, supported by aluminium rails was used to cradle the fish. The rails were then roped together and suspended from a hanging balance to provide a weight measurement.

Specific growth rate (SGR) was calculated as follows (after Priestly *et al.*, 2006):

$$\text{SGR (\%/day)} = \frac{\ln (W_f) - \ln (W_i)}{\text{time (days)}} \times 100$$

Cannulation

Gonadal biopsies were taken using 1 mm clear plastic tubing inserted through the gonophore of both male and female fish (cannulation) to assess gonad development and viability for spawning induction. Ovarian development was measured in terms of oocyte diameters calculated by the average of the largest 10 oocytes from the biopsy sample.

Results

Broodstock collection

Transferring broodstock from GCMA

The first two fish were successfully transferred from GCMA to BIRC. Both fish settled relatively well in the 35 000 L tanks and began feeding actively four days post transfer, consuming approximately 4% of their body weight in pilchards and squid per day.

Wild fish capture

A total of 16 fish were captured from the two collection trips. All fish were hooked in the mouth parts, creating only superficial damage. One fish died during the road transport to BIRC. The remaining fish appeared healthy and in good condition upon anaesthesia recovery in the broodstock system. All fish settled well and began actively feeding within one week post capture.

Examinations of the newly captured fish for gonadal maturity found that no males were running with semen able to be exuded with abdominal pressure. Females were in a very early stage of vitellogenesis, with oocyte diameters ranging from 100 to 160 μm.

Broodstock growth

Feeding activity appeared to peak in late spring, when the broodstock were consuming up to 10% of their body weight per feed. At these times the broodstock displayed periods of rapid growth associated with increasing water temperatures and gonadal development. Growth then steadied following the peak of summer and during the spawning period, and was minimal during winter (Figure 3).

Male fish showed higher specific growth rates (SGR) in the 2007/08 season whereas females had higher growth rates in the following year (Figure 4). Growth rates were at maximum levels during the late spring and summer months and stagnated during the autumn and winter months (Figure 4).

Broodstock health

The health and condition of broodstock during these first two years was generally good, although a number of suboptimal conditions and pathogens were identified, particularly during winter. Since broodstock tanks were being supplied with ambient seawater flow-through supplies, the low water temperatures during winter (16 °C) were likely implicated.

In April 2009, there was a benedenian (parasitic worm) infection which facilitated a secondary bacterial infection causing inflammation around the eyes of some of the broodstock. One fish was considered to have a severe infection, having lost almost all eye function. Another two fish were considered to have only minor infections. Veterinarian advice was to treat the infection with broad spectrum antibiotic-medicated (oxytetracycline) food, however a few days after a preliminary freshwater bath the fish were re-examined and considered to have improved sufficiently to negate the requirement for the antibiotic treatment.

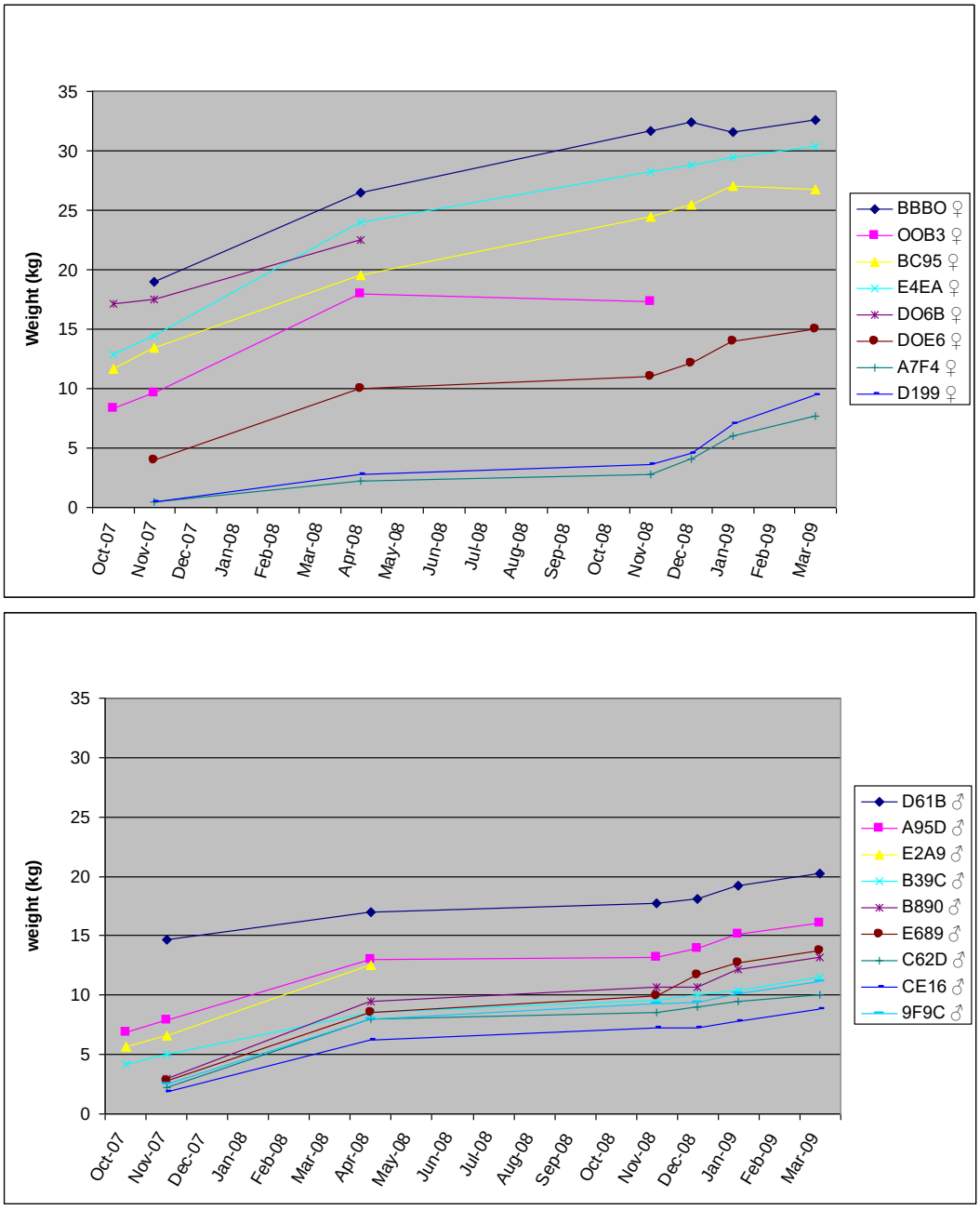

Figure 3 – Female (above) and male (below) broodstock growth rates from 2007 to 2009

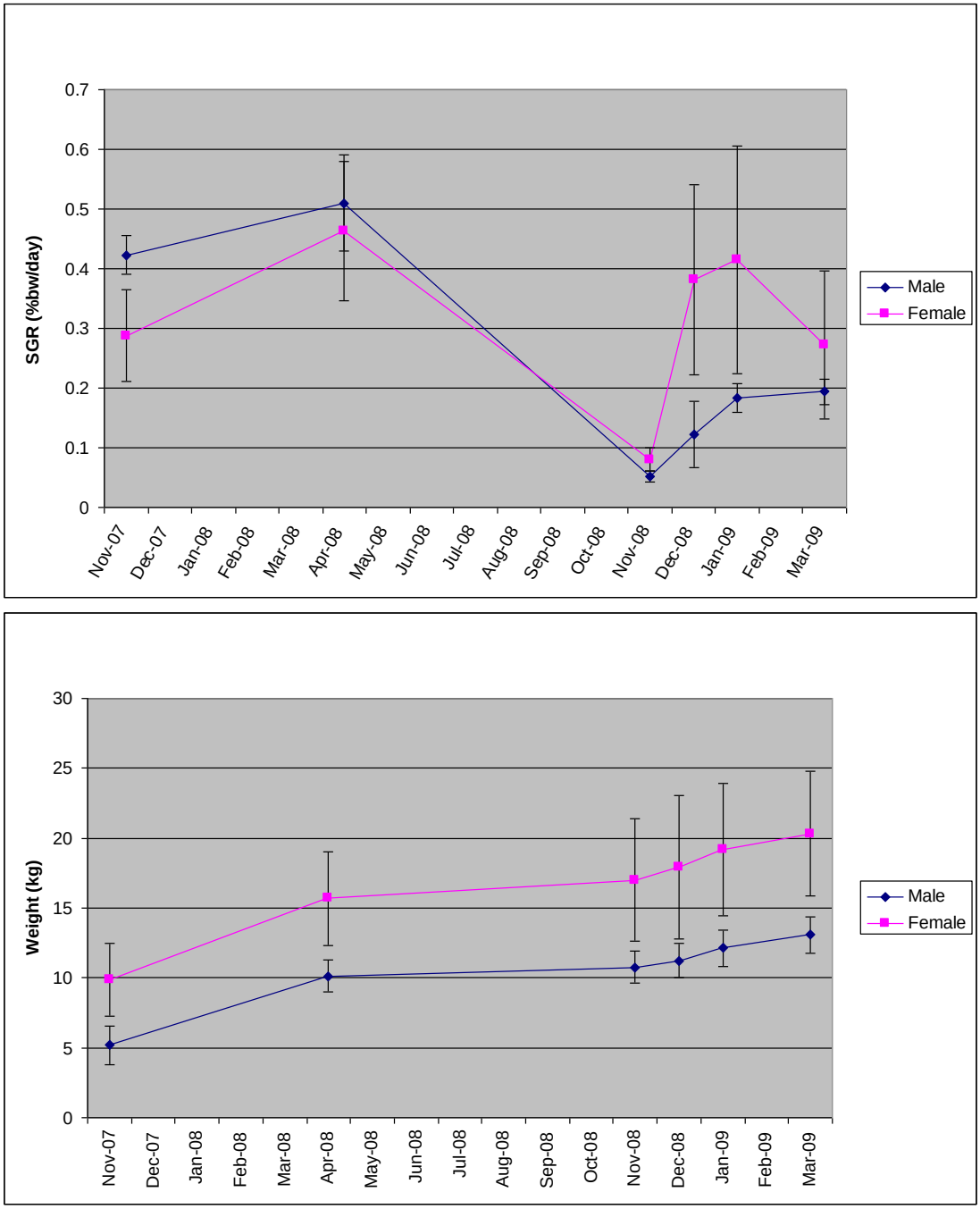

Figure 4 – Cobia broodstock specific growth rates (SGR – above) and average weights (below) showing standard errors

Discussion

The methods employed at BIRC were successful in securing and maintaining a viable population of cobia broodstock. This is evidenced by rapid adaptation of the fish to a captive environment with high survival, active feeding responses and rapid growth during the spring and summer seasons. Environmental manipulations were combined with natural rhythms to successfully stimulate gonadal development of these captive wild broodstock.

At the University of Miami Experimental Fish Hatchery (UMEH), wild and F1 cobia broodstock were conditioned to spawn through temperature manipulations, producing enough fertilised eggs for experimental

and larval-rearing trials in several hatcheries (Benetti *et al*., 2008). Cobia broodstock at the UMEH were fed a diet of frozen/fresh fish and squid, and were complemented with a vitamin and mineral mix (Benetti *et al*., 2008) that is similar to that used in the present study.

The wild broodstock at BIRC also fed very well in the broodstock tanks. They were fed to satiety three times a week and similarly fed a mixture of pilchard and squid with vitamin supplement, but also with a formulated broodstock pellet (see methods). Schwarz *et al*. (2004) stated that cobia are adaptable to commercially available aquafeeds.

Cobia are reportedly susceptible to parasitic infections and bacterial diseases such as *Amyloodinium ocellatum* and *Vibrio* spp. (Benetti et al., 2008). Regular prophylactic treatments such as formalin and freshwater baths were used at BIRC to prevent and control epizootic diseases in the broodstock facility.

There was also evidence of sexually dimorphic growth in cobia broodstock at BIRC, where females appeared to grow faster than males from a comparable starting weight. However, where the males appeared to have grown faster in the 2007/08 season, this was more likely a function of the relative weight of the fish rather than sexually dimorphic growth. Examining this data more closely, the males were much smaller than the females, with 8 of the 9 males examined being 6 kg or less. Whereas, of the females, 5 of the 8 were 8 kg or more (4 were over 10 kg). As such it would be expected that smaller, immature fish would exhibit a higher SGR. In the second season, when this differential was reduced and the males were approaching sexual maturity it appeared that the females were instead growing faster during the peak growth summer period.

To facilitate better management of the health of fish in the future, and decrease the likelihood of bacterial infections due to extended periods of sub-optimal temperatures, investment in recirculating systems at BIRC which can be more effectively heated is recommended. Such systems would also provide for greater control during the summer spawning season. Benetti *et al*. (2008) stated that their cobia broodstock were conditioned to spawn in recirculating temperature-controlled maturation tanks where temperature regimes remaining around 20–21 °C during winter, and up to 27 °C in the summer spawning season.

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Appendix 1

Table 1 – Water temperatures and photoperiods recorded between August and December during the 2007 and 2008 spawning seasons

Date	Day length (hr)	Temperature (°C) in 2007	Temperature (°C) in 2008
11 Aug	10.5	NA	16.55
18 Aug	10.5	NA	15.89
25 Aug	11	NA	16.46
1 Sep	11	NA	18.37
8 Sep	11.5	NA	18.79
15 Sep	11.5	NA	20.12
22 Sep	12	NA	21.30
29 Sep	12	NA	20.99
6 Oct	12.5*	23.11	22.30
13 Oct	12.5	23.56	22.04
20 Oct	13	22.43	22.50
27 Oct	13	23.88	22.46
3 Nov	13.5	25.01	23.95
10 Nov	13.5	23.40	24.13
17 Nov	14	23.95	24.57
24 Nov	14	25.12	23.87
1 Dec	14	25.20	24.69
NA = not available Note: spawning behaviour began at 12–13 hr light and 25–26 °C			

Broodstock vitamin supplement

Supplement is offered to compensate for suspected vitamin loss in stored frozen baitfish food. The formulation was developed by Dr K. Williams (Queensland Department of Primary Industries Nutritionist) based on known requirements of fish generally. The Northern Fisheries Centre (NFC) obtained and supplied the supplement as two separate components: (1) dry premix (NFC Vitamin Premix Code D 062/0) and choline chloride viscous liquid. These were kept separate until required to maximise the shelf life of the vitamins. Dry premix was stored in a freezer with a 3–6 month shelf life, while the liquid choline chloride which is very stable was refrigerated.

Mixing Instructions: To 980 mL of distilled water add 100 g of dry premix and 20 mL of choline chloride. Mix vigorously and store refrigerated in a dark coloured container. In use agitate frequently to keep mixture reasonably well suspended. Make up about one weeks supply at a time since shelf life of the refrigerated made up mixture is only about a week.

Administration: A 5 mL graduated plastic disposable or preferably stainless steel (Lyppards Model 74 Vaccinator or other brand equivalent) auto syringe is used to inject vitamin supplement into the broodstock food. The agitated mixture is injected at the rate of 1 mL per 50 g of baitfish when broodstock are being fed at 2% body weight per day. If feeding rate is decreased to 1% then 2 mL per 50 g of baitfish is injected.

Table 2 – Formulation of the broodstock vitamin supplement

Vitamin	Amount / kg premix	Allowance per kg brood fish per day
A	2 x 10^6 I.U.	80 I.U.
D3	0.8 x 10^6 I.U.	32 I.U.
E (DL-alpha-tocopherol)	40 g	1.6 mg (about 1.8 IU)
K3	2 g	0.08 mg
Ascorbic	40 g	1.6 mg
Thiamin	4 g	0.16 mg
Riboflavin	4 g	0.16 mg
Pyroxidine	4 g	0.16 mg
Pantothenic	10 g	0.4 mg
Biotin	100 mg	4 μg
Nicotinic	30 g	1.2 mg
Folic acid	1 g	0.04 mg
B12	4 mg	0.16 μg
Choline chloride	200 g	8 mg
Inositol	50 g	2 mg
PABA	20 g	0.8 mg
Ethoxyquin	30 g	1.2 mg
Dextrose	to 1.0 kg	–

2. Maturation, hormone induction and spawning of cobia

Luke Dutney, Steve Nicholson, Trevor Borchert and Paul J. Palmer

Abstract

A total of 18 wild caught cobia were introduced into the broodstock facility at the Bribie Island Research Centre (BIRC) in 2007. Throughout the following two years the fish were held on an ambient phototherm regime and were monitored to assess gonad development and suitability for spawning induction. There was no natural spawning during these trials. Ovarian development varied greatly between individual fish, with many females not maturing sufficiently to warrant hormone induction. Of those fish that where induced there tended to be a binary response: a highly successful spawn with excellent fertilisation and hatch rate; or alternatively there was no apparent change in behaviour or physical appearance and no spawn.

There was some correlation between ovarian development and fish weight, in which only those fish over 15 kg where found to have oocytes >500 μm. However oocytes diameters did not correlate with induction success. Of the many attempts at hormone induction only three attempts succeeded, all of which came from the same female fish. On each occasion fertilisation rates where 75% or above and hatch rate was estimated at 95%.

There remains an expectation that more reliable inductions will be forthcoming from captive bred fish in coming years.

Introduction

Cobia broodstock can be brought to maturity using a wide variety of systems and management techniques. Methods used for maturing broodstock have included capture of wild fish in spawning condition, selecting suitable fish from ocean grow out cages, holding fish in spawning ponds and maintaining fish in environmentally controlled tank systems (Holt *et al*., 2007a; Liao *et al*., 2004).

Cobia broodstock have been induced to spawn using hormone injection with HCG, LHRHa and GNRHa (Caylor, 1994; Nguyen, 2010; Weirich *et al*., 2007). Natural spawning tends to provide better quality oocytes than hormone induction, and has been accomplished using tanks and ponds influenced by ambient environmental conditions (Arnold, 2002; Liao *et al.*, 2001), and using controlled photothermal conditioning (Holt *et al.*, 2007a; Benetti *et al.*, 2008). Captive bred broodstock are generally thought to offer better performance with natural spawning, producing better quality eggs with higher fertilisation rates and hatch rates. Domesticated (F1) cobia broodstock reportedly spawn more regularly than wild fish (Holt *et al.*, 2007b; Benetti *et al.*, 2008).

As with most marine fish, the behaviour of cobia broodstock changes noticeably prior to spawning. Overall activity of the fish increases with fish pairing up, chasing and biting. The white stripe above the lateral line becomes more pronounced and the male fish tend to follow closely below the female, with their snout close to the female's vent. The abdomen of the female becomes noticeably swollen as the eggs hydrate prior to release, with most spawning events occurring shortly after dark (Arnold *et al.*, 2002; Holt *et al.*, 2007a; Benetti *et al.*, 2007).

Wild caught cobia were introduced to the broodstock facility at BIRC in 2007 (described in Section 1) to examine the requirements to produce juvenile cobia using existing infrastructure and local environmental conditions. This section describes the experiences with the assessment of gonad development and spawning induction of cobia broodstock over the first two successive spawning seasons.

Materials and methods

Gonad development

Gonad biopsies were taken using 1 mm clear plastic tubing inserted through the genital opening of both male and female fish (cannulation) to measure gonadal development and viability for spawning induction. Ovarian development was measured in terms of oocyte diameter calculated by the average of the largest 10 oocytes from the biopsy sample. Ovarian development was measured periodically from early stage winter development through to late stage maturation in the 2007/08 season. In 2008/09, analyses were restricted to later in the developmental period. Hormone induction was initiated pending appaently favourable results of gonadal development assessments.

Sperm activity assessments were conducted under a 400X microscope by scoring motility intensity and duration for semen samples collected immediately prior to injection of hormones. Samples were collected in syringes and stored on ice prior to assessments. Care was taken not to contaminate samples with urine or seawater. Three to five repeated assessments were conducted for each sperm sample. Motility intensity soon after activation with seawater was assessed subjectively according to a 0–5 scale (after Hogan and Nicholson, 1987) as follows:

0—No activity

1—Head movement only; no progressive motion

2—Slow head and tail movement; some individuals progressing slowly

3—Less energetic head and tail movement; most with forward motion

4—Very active sample; all sperm visibly progressing rapidly across the field of view

5—Most active sample observed for this species; sperm creating swirling currents obscuring the movement of individual sperm across the field of view.

Sperm duration was defined as the point in time of cessation of movement of 90% of sperm following activation with seawater. Sperm assessments were conducted immediately prior to induction in 2007/08 and during the peak of the developmental period in 2008/09.

Hormone induction

The broodstock tanks were lowered to a working depth of approximately 400 mm, with a volume of 4000–5000 L. Anaesthetic (10 ppm of Aqui-S™) was then added to the entire tank to sedate the fish. This reduced stress and the potential for physical damage to the fish, as well as ensuring staff safety while handling the animals. After approximately 10 minutes the fish were still able to maintain equilibrium yet were sufficiently sedated to improve the ease of handling. The fish were lifted using a vinyl sling and placed into a 300 L anaesthesia bath containing a higher dose of 25 ppm of Aqui-S™. Once under full anaesthesia the fish were cannulated, weighed and injected with hormones.

Hormone injections were administered using a single intra-peritoneal injection of LHRHa under the pelvic fin with the needle directed posterior at a 45° angle. The LHRHa was dissolved in a sterile saline solution then held on ice in 5 mL syringes with capped 21 g x 1.5 needles. Injections were given between 6 and 7 am to provide a 38–40 hr latency period that would coincide with dusk. The particular fish chosen for hormone induction were based on the apparent developmental stages of individuals.

Following hormone induction, broodstock were transferred to one of two 300 L recovery tanks containing aerated clean seawater without anaesthetic. The fish generally recovered well, maintaining equilibrium within 5 minutes of being placed into the recovery tanks. While the fish were held in the recovery tanks the

broodstock tanks were emptied, scrubbed and flushed clean, and then refilled to working height. Once at working height the fish were released from the recovery tanks back into the broodstock tank. The broodstock tanks were then filled completely with clean seawater over the next hour.

Activity around the tanks was then kept to an absolute minimum during the latency period, with water temperature in the tanks maintained at 26–27 °C.

Induction 1 – 7 January 2008

The first induction attempt was aligned with the new moon to allow for the incorporation of any biorhythms associated with the lunar cycle. Gentle pressure applied to the abdomen of the male fish failed to force the expression of semen. A sperm sample was alternatively extracted and examined by cannulation. The following two fish were induced.

Tag no.	Weight	Sex	Gonad	Hormone administered
BBBO	24.5 kg	♀	688 µm	25 µg/kg @ 7:00 am
D61B	16 kg	♂	Motility 4 Duration 6	25 µg/kg @ 6:50 am

Induction 2 – 21 January 2008

The second induction attempt was aligned with the full moon. Following the poor result of the first induction attempt, two fish from another broodstock tank were cannulated and hormone induced, and then introduced to the tank containing the fish from the first induction. This created an equal sex ratio of 2 males and 2 females in the spawning tank. A new batch of LHRHa was used and the dose was increased to 50 µg/kg for the female and 25 µg/kg for the male. Gentle pressure applied to the abdomen of the male prior to hormone injection caused the release of semen which was collected and analysed for sperm motility and duration. The other fish in the tank that had been induced two weeks earlier were not handled or re-examined.

Tag no.	Weight	Sex	Gonad	Hormone administered
E4EA	22 kg	♀	680 µm	50 µg/kg @ 6:30 am
E2A9	10 kg	♂	Motility 5 Duration 6	25 µg/kg @ 6:45 am
BBBO	Not weighed	♀	Not examined	Nil
D61B	Not weighed	♂	Not examined	Nil

Induction 3 – 25 February 2008

The third induction attempt in 2008 used a single paired spawning. The induction did not coincide with the peak of the lunar cycle, being five days after the full moon.

Tag no.	Weight	Sex	Gonad	Hormone administered
E4A4	23.5 kg	♀	790 µm	50 µg/kg @ 6:30 am
D61B	16.7 kg	♂	Motility 4.5 Duration 7	30 µg/kg @ 6:45 am

Induction 4 – 28 March 2008

A fourth induction was conducted, again aligned with the full moon. Both male fish were running freely with semen. One of the female fish (BBB0) appeared to have regressed with reduced oocyte diameters as well as free oil in the sample. The other female (E4EA) appeared to be still in spawning condition.

Tag no.	weight	Sex	Gonad	Hormone administered
E4EA	24 kg	♀	780 µm	50 µg/kg @ 6:30 am
D61B	17 kg	♂	Motility 4 Duration 6	25 µg/kg @ 7:00 am
BBBO	26.5 kg	♀	450 µm	50 µg/kg @ 6:45 am
A95D	13 kg	♂	Motility 4 Duration 6	Nil

In 2009, six separate hormone induction attempts were made between January and March with fish of varying sizes and with various gonad development stages. Hormone dose rates were maintained at approximately 50 µg/kg for females and 25 µg/kg for males. Full descriptions of the spawning inductions conducted in the 2008/09 season are contained in Appendix 2. Timing of these hormone inductions coincided with the full moon peaks of the lunar cycle.

Egg collection

Egg collection baskets were installed on the outlet pipes of the broodstock tanks from late spring through until early autumn. The baskets consisted of 500 µm mesh supported by a 20 mm PVC frame and were placed in a 200 L overflow sump. The 80 mm upper tank outlets were fitted with a series of elbows to minimise the splashing of water and provide a gentle water flow, thus reducing potential damage to newly released eggs (Figure 5). Following hormone inductions flow rates into the tanks were reduced and all waste water was directed through the upper tank outlet.

Figure 5 – The egg collection system used on the 35 000 L cobia broodstock tanks

Results

Gonadal development in 2007/08

Patterns for oocyte development in the two wild female fish selected for hormone induction are shown in Figure 6. In both fish there was a steady increase in oocyte diameter through the spring and early summer to 700 μm in January. In February the oocytes of one of the fish started to regress, whilst the oocytes of the other continued to develop to approximately 800 μm and then appeared to plateau at that size. The remaining five wild-caught females were either immature or failed to develop oocytes larger than 400 μm, and therefore were not induced.

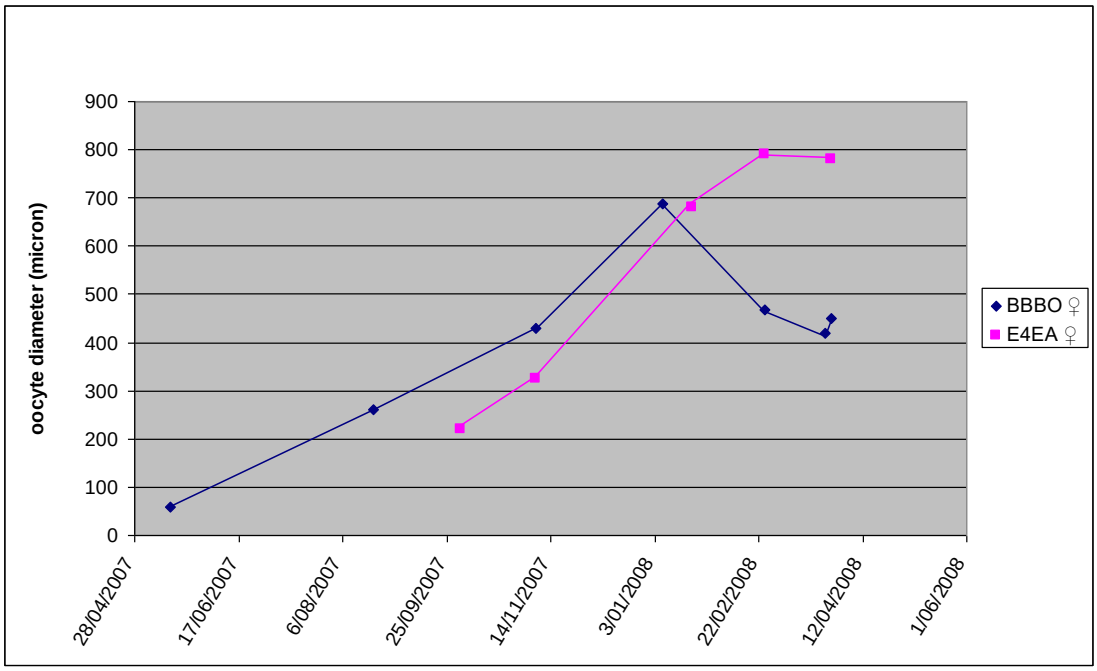

Figure 6 – Mean (± se, n = 10) cobia oocyte development 2007/08

Gonad development in 2008/09

These wild-caught female cobia again showed variable development in captivity through the 2008/09 spawning season (Figure 7).Three female fish showed steady development of oocytes from November through until March, with oocyte diameters of these fish reaching 500 μm or above, when they were judged suitable for induction. Two of these three females (BBBO and E4EA) also provided the more encouraging results in the earlier season.

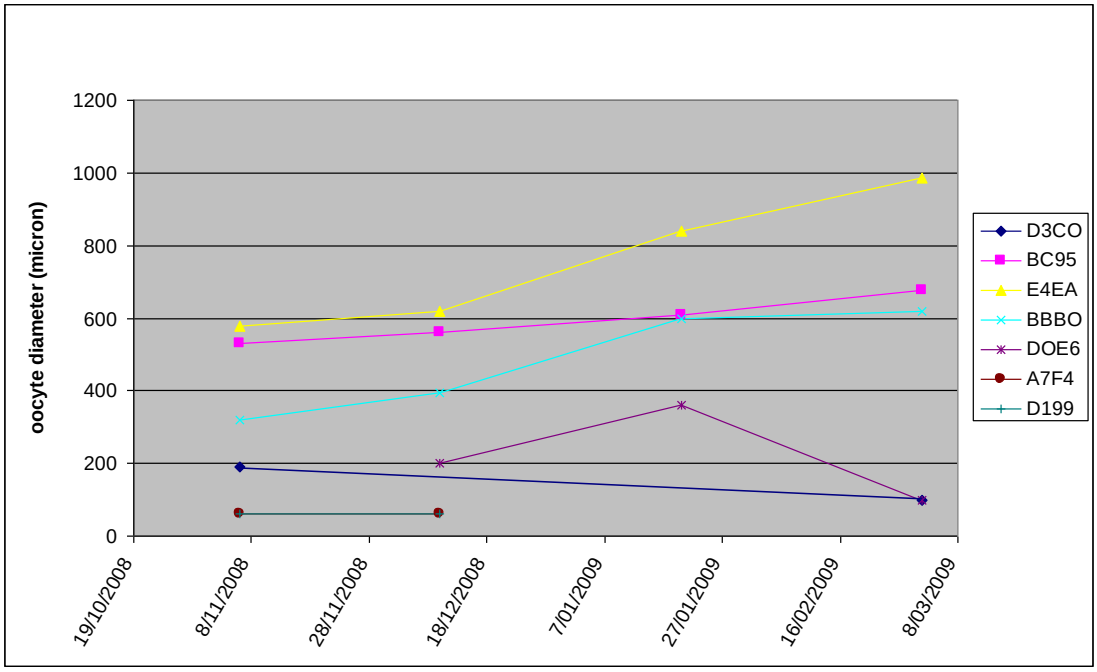

Figure 7 – Mean (± se, n = 10) cobia oocyte development 2008/09

The remaining four fish showed limited gonadal development evidenced by oocyte diameters of less than 400 μm. Female body size appeared to be a factor in these gonad development indices, where only fish above 15 kg developed oocytes larger than 400 μm.

Sperm development in 2008/09

Sperm activity also showed distinct seasonal patterns. Motility increased steadily from November 2008 until March 2009, and duration showed a rapid increase from November 2008 to January 2009 and then a plateau through until March 2009 (Figure 8).

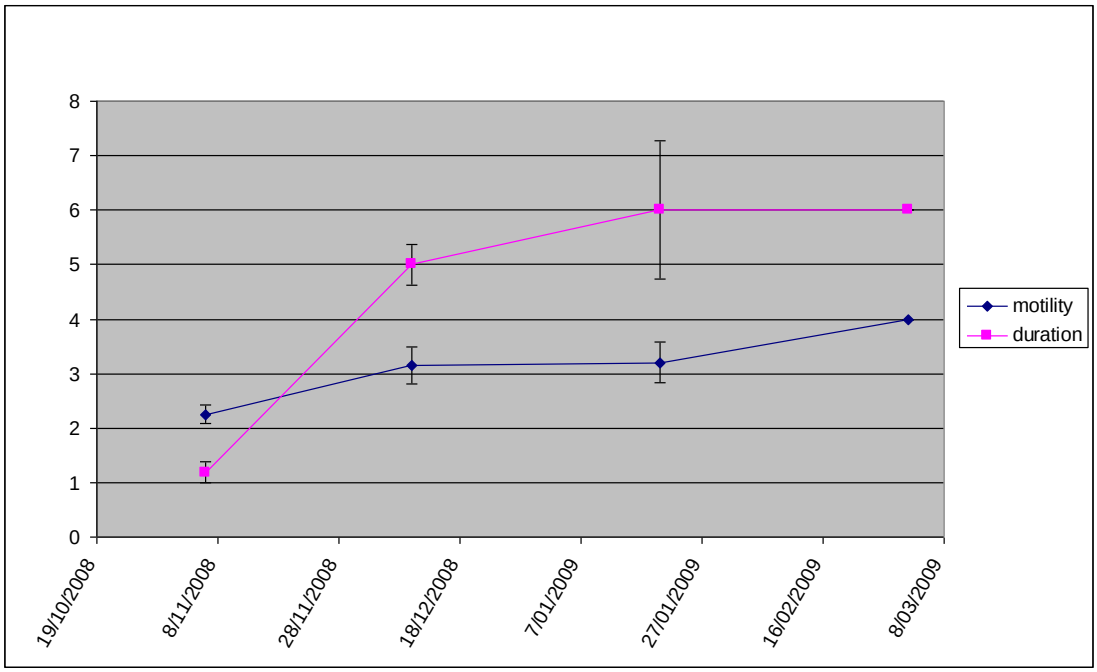

Figure 8 – Mean (± se, n = 3–5) sperm motility scores (0–5) and durations (min) in 2008/09

Hormone induction and spawning

Induction 1 in 2008

The first cobia female induced with injected hormones in the program failed to spawn over the following fortnight. There were no clear courtship or chasing behaviours observed during this time. Observations through the viewing window of the broodstock tank also suggested that there were no increases in the size of the induced female's abdomen due to the hydration of oocytes.

Induction 2 in 2008

The introduction of the two newly-induced broodstock fish into the spawning tank from the first induction attempt caused an immediate behavioural response from the resident (previously induced) male. It displayed a prominent pairing behaviour with the newly-induced female, and aggressive motions towards the newly-introduced male.

On Wednesday 23 January 2008 viable eggs were harvested from this second induction. A total of 4.2 million eggs with an estimated fertilisation rate of 76% were collected from the tank overflow. It was not possible to identify which females and males had participated in the spawning event, but assessments of oocyte diameters after this spawning suggests that either female could have participated.

The developing embryos were all at approximately one third epiboly at 6.45 am on 23 January 2008, which suggested that a single spawning event had occurred. Egg diameter averaged 1.23 mm and hatching occurred at 1 am on 24 January 2008. The hatching rate of fertilised eggs (after clearance of unfertilised sinking eggs) was >95%.

Induction 3 in 2008

In preliminary assessments of oocyte diameters undertaken before the third induction in 2008, the previously induced broodstock female BBBO appeared to have regressed. This was evidenced by a number of misshapen oocytes and a marked decrease in oocyte diameters (see Figure 6), as well as a significant quantity of free oil within the cannulated gonad sample. This female was therefore not induced and was moved to another broodstock holding tank (Tank 1 – see Appendix 2). The only other available female with suitable oocyte development for induction at that time was E4A4, which was the same female involved in the previous successful spawning four weeks earlier.

Two mornings after this third hormone induction, a total of 4 million eggs was collected with a fertilisation rate of 81%. Egg diameters averaged 1.3 mm. From the developmental stage of embryos at collection, the spawning time was estimated to be between 3 and 4 am. The hatching rate of fertilised eggs was >95%.

Induction 4 in 2008

The cobia broodstock used in the fourth induction in 2008 did not spawn. There was no pre-spawning behaviour observed and no noticeable increase in the abdominal size of females post induction to suggest hydration of the oocytes.

Inductions in 2009

Despite six induced spawning attempts in 2009 (see Appendix 2), hormone inductions were only successful once. The female that spawned successfully was the same fish (E4EA) that likely spawned on both occasions in the previous 2007/08 season. It had oocytes of about 840 μm when injected. A total of 5.9 million eggs with a fertilisation rate of 81.5% was collected from the spawning tank overflow on 23 January 2009. The spawning time was estimated to be around 4 am. Average oocyte diameter was 1.22 mm, and the hatching rate of fertilised eggs was >95%.

Discussion

In other parts of the world, cobia are reported to reach two spawning peaks: the first during spring and the second late in the summer or autumn (Arnold *et al*., 2002; Liao *et al*., 2004). Observations of sperm and oocyte development in this study suggests that the cobia at BIRC reached a developmental peak in mid- to late-summer. This difference may be a function of the environmental parameters of a subtropical region in which BIRC is located, compared to the developmental profile observed in tropical environs.

During the first season (2007/08) sperm activity increased to reach motility intensity scores of 3–4, and motility durations of 5–6 minutes from December to March (when monitoring ceased). Oocyte diameters of the two females studied also increased from April to peak between December and March (Figures 7 and 8). Two successful spawning events were achieved in 2008, one in January and the other in February. The second successfully demonstrated the production of viable oocytes from the possible repeat spawning of a female within one month, a single paired natural mating, and spawning outside of the peaks in the lunar cycle. These early results guided a mid-summer focus the following season.

During the second summer season at BIRC (in 2009), sperm activity appeared sufficient to facilitate fertilisation in all induction attempts (see Appendix 2). Males subjected to hormone therapy had sperm motility intensity scores ranging 3–5, and sperm motility duration from 4–10 minutes when injected. For females, oocyte diameters ranged from <150 μm to 985 μm with five induced females having oocytes larger than 500 μm when injected (Appendix 2).

Unsuccessful inductions in 2009 were typified by a lack of courting behaviour and no apparent physical changes to the females during the latency period. Whilst there appeared to be a relationship between fish size and ovarian development, there was no clear correlation between oocyte diameter and spawning success. Successful spawning events during the two seasons where restricted to a single night, with no further egg releases occurring until after further hormone therapy was administered.

There was no evidence of natural (un-induced) spawning. While it is generally recognised that natural spawning produces higher quality spawning outputs, hormone inductions successfully produced 4–6 million eggs with fertilisation rates above 75%, and yielded excellent hatch rates. These inductions also allowed better planning for culture activities that closely followed spawning events. While several hormone inductions in the study did not stimulate egg releases, those inductions that were successful produced large numbers of high quality eggs, with fertilisation rates comparable to that reported elsewhere (Arnold *et al*., 2002; Holt *et al*., 2007b).

It is unclear why many inductions for seemingly mature females in the study failed to generate the release of eggs. It is generally accepted that oocytes of marine teleosts are ready for hormone induction when they attain a diameter of 500 *µ*m or larger (Partridge *et al*., 2002; Weirich *et al*., 2007; Benetti *et al*., 2007). In many cases in the study, oocyte diameters where found to be 600 *µ*m or above, yet hormone induction failed to induce ovulation and spawning. This may be an artefact of working with wild fish which are reportedly more difficult to spawn than domestic stock, but other factors may also have played a part in this finding. While cannulated oocyte samples are considered to provide a reasonable guide to ovary maturity, they do only sample a small portion of the ovary, and may not fully document ovary condition. Since cobia are a partial spawner it was assumed that the largest oocytes in the cannulated sample would be those which matured following induction. However, a better assessment in the future may be to assess the entire cannulated sample to more accurately assess the maturity of the ovary.

The hormone induction method used may also need to be refined to provide more reliable spawning results. Further investigations of hormone dose rates, the timing of application and injection media may provide useful improvements. Manipulating sex ratios may also provide benefits by stimulating early gonad development and encouraging pre-spawning behaviours that may lead to final maturation and egg releases.

In other parts of the world cobia egg supplies are a limiting factor for industry expansion (Schwarz and Kaiser, 2008). Improvements in the reliability and quality of cobia spawning has been achieved by introducing domesticated broodstock (Holt *et al*., 2007a; Weirich *et al*., 2007; Benetti *et al*., 2008). The cobia juveniles produced in these trials were expect to reach maturity in coming seasons, and it was anticipated that these fish would be more amenable to hormone induction or natural spawning through environmental manipulations. Importantly though, longer-term planning and irregular additions of wild stock will be needed to ensure sufficient genetic diversity is maintained in breeding populations. This means that the improved productivity of wild-caught broodstock will remain a priority for cobia research into the future.

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Appendix 2

Broodstock inductions 2009

19 January 2009 – Tank 1

Tag no.	Weight	Sex	Gonad	Hormone dose
B39C	10.4	♂	Motility – 4–5 Duration – 10	300 µg = 29 µg/kg @ 6.25 am
BC95	27.0	♀	610 µm	1400 µg = 52 µg/kg @ 6.15 am
B890	12.2	♂	Poor sample	Nil

- No spawn on 21 January 2009
- No change in physical appearance, abdomen not distended
- No apparent change in behaviour – appear hungry

20 January 2009 – Tank 3

Tag no.	Weight	Sex	Gonad	Hormone dose
E689	12.7	♂	Motility – 4 Duration – 5	300 µg = 23.6 µg/kg @ 6.30 am
9F9C	10.1	♂	Motility – 2 Duration – 4	Nil Poor condition – physical damage/bite marks
DOE6	14.0	♀	360 µm	Nil
BBBO	31.6	♀	600 µm	1700 µg = 53 µg/kg @ 6.20 am

- Some pairing evident, E689 swimming directly under BBB0, no sign of chasing or biting at 8.00 am on 21 January 2009
- No spawn on 22 January 2009
- No change in physical appearance, abdomen not distended

21 January 2009 – Tank 2

Tag no.	Weight	Sex	Gonad	Hormone dose
A95D	15.1	♂	Motility – 3 Duration – 4	400 µg = 26.5 µg/kg @ 6.15 am Minor bite marks
E4EA	29.4	♀	840 µm	1600 µg = 54.4 µg/kg @ 6.10 am
D61B	19.2	♂	Motility – 4 Duration – 8	Nil

- Pairing evident
- Spawn of 5.9 million eggs collected on 23 January 2009
- 81.5% fertilised

2 March 2009 – Tank 1

Tag no.	Weight	Sex	Gonad	Hormone dose
B39C	11.5	♂	Motility – 4–5 Duration – 6	300 µg = 26 µg/kg
BC95	26.8	♀	675 µm	1400 µg = 52 µg/kg
B890	–	♂	Not sampled	Nil

- No spawn on 4 March 2009
- No change in behaviour or appearance

02 March 2009 – Tank 2

Tag no.	Weight	Sex	Gonad	Hormone dose
A95D	15.4	♂	Motility – 4 Duration – 6	400 µg = 25.8 µg/kg
E4EA	28.5	♀	985 µm	1600 µg = 56.1 µg/kg
D61B	–	♂	Not sampled	Nil

- No spawn on 4 March 2009
- No change in behaviour or appearance

2 March 2009 – Tank 3

Tag no.	Weight	Sex	Gonad	Hormone dose
E689	13.7	♂	Motility – 4 Duration – 6	300 µg = 23.6 µg/kg
9F9C	10.1	♂	Not examined	Nil
DOE6	15.0	♀	Primordial <150 µm	700 µg = 46 µg/kg
D3CO	13.6	♀	Primordial <150 µm	700 µg = 51.5 µg/kg

- No spawn
- No change in behaviour or appearance

3. Live food culture management at the Bribie Island Research Centre

Sizhong Wang, Trevor Borchert and Paul J. Palmer

Abstract

To better understand the conditions prevailing in microalgae cultures that are routinely used in low-water exchange green-water larvicultures at the Bribie Island Research Centre (BIRC), six cultures of *Isochrysis galbana* (T-iso) and eight cultures of *Nannochloropsis oculata* were studied for up to 11 days with daily measurements of cell counts, Secchi depths and total ammonia levels. These parameters were selected based on their direct effects on larvicultures and as practical guides for hatchery operators. As expected, there were strong correlations shown between all parameters that were monitored. Cell densities surpassed 20 x 10^6 cells mL^{-1} in *N. oculata* cultures and 2 x 10^6 cells mL^{-1} for T-iso cultures. For cultures between 7 and 10 days old, which are routinely used in larvicultures or are used to feed rotifers and artemia, Secchi depths were <16 cm and <30 cm, ammonia levels were <4 mg L^{-1} and <0.1 mg L^{-1}, and cell densities were >14 x 10^6 and >1.2 x 10^6 mL^{-1} for *N. oculata* and T-iso, respectively. The different culture media used for each of these microalgal species and the methods used to scale them up from stock cultures are described here, along with the standardised culture methods used for rotifers and artemia at BIRC.

Introduction

Intensive live food production is well recognised as a crucial aspect of fish, crustacean and molluscan larval production in hatcheries around the world. This is particularly important for low water exchange approaches where the contents and qualities of larvicultures are strongly influenced by the contents and qualities of the live feeds that are used. Of particular importance is the microbial flora, which can be a function of several factors including the site, the facilities and the way the cultures are managed (Verdonck *et al*., 1994). Work undertaken in this area at BIRC has suggested that assemblages of microorganisms that are beneficial for many marine fish larvae can be generated by a simple set of principles. These include keeping source cultures in a state of physiological youth, and providing adequate aeration and mixing to preventing organic materials accumulating on the bottoms of tanks where anaerobic bacteria may proliferate (Palmer *et al*., 2007).

In the hatchery program for cobia (*Rachycentron canadum*) at BIRC between 2007 and 2009, the methods of live food production applied were similar to those that had been in use there for many years in the successful rearing of several other marine fish larvae. Even though the green microalgae *N. oculata* had predominately been used as the base of green-water larvicultures during this time, relatively little work had focussed on its particular qualities at the stage when it was routinely used (i.e. as a 7- to 10-day-old culture). Several factors were unknown including levels of ammonia which could potentially cause problems for marine fish larvae (particularly for oceanic species like cobia), and the expected cell densities of various algal cultures at different culture ages. Furthermore, rather than specific cell density, Secchi depth had been the general guide to readiness-for-use in larvicultures and in live feed production programs, because this is quicker to evaluate and does not require microscopic observations. In the case of *N. oculata*, cultures with Secchi depths of 10–20 cm had previously been considered suitable for these uses (Palmer *et al*., 2007), but clarification was sought regarding the actual cell densities and ammonia levels that prevailed at these stages under normal conditions. Similarly, this information for other algal species like T-iso, which may be used to provide nutritional supplements to the standard green-water base, was also considered potentially useful.

To address these questions, and provide readily available information regarding live food production methodologies, several cultures of *N. oculata* and T-iso were studied with a view to their addition to low water exchange green-water larvicultures. In recognition of the important role of starter cultures in generating high quality mass cultures, and other live feed cultures in generating a healthy mix of microorganisms for larvicultures, methods used at BIRC in support of this work with cobia are also documented in general terms in this section. This includes the management of algal stock and multiplier cultures, and the management of rotifer (large strain *Brachionus plicatilis*) and brine shrimp (*Artemia* spp. hatching and 1–3 day on-growing) production systems.

Materials and methods

Laboratory-scale microalgal cultures and media

All microalgae stock cultures held at BIRC were originally purchased from the CSIRO Microalgae Supply Service at Hobart in Tasmania. Stock cultures (with volumes of 75 mL) are maintained without aeration in small (250 mL) sealed (sterilised cotton swabs covered with foil) Erlenmeyer flasks positioned on an automatic shaking table (Figure 9, left). The shaking table is designed to swirl the flasks for 5 min every 6 h, and is powered by a 12 volt pulley-driven system.

Working cultures (2 L and 4 L flasks and 10 L carboys) (Figure 9, right) are maintained in an air-conditioned room (21–25 °C) in front of a bank of fluorescent lights (daylight tubes on a 12 h light:12 h dark regime). Continuous aeration (filtered to 1 μm) is provided to all working cultures. CO_2 is injected into the main aeration line (only during daylight hours) at a rate which maintains the pH of all algal cultures at between 8 and 9.

Figure 9 – Shaking table with stock cultures (left) and working cultures showing top fittings (right)

The nutrient medium used for stock and working cultures is based on Guillard's F formula (Guillard, 1983). The nutrient medium is made up in concentrated form (Table 3) and added to sea-water at the rate of 1 mL L^{-1} prior to sterilisation (autoclaving).

The trace metals and vitamins used in the nutrient medium concentrate are firstly dissolved in distilled water to make concentrated solutions of each compound (Tables 4 and 5), which are then added to the nutrient medium concentrate.

Before using new flasks and carboys these are generally filled with dilute HCl (5%) and left to stand for at least 24 hours to remove residues remaining from their manufacture and/or storage conditions. Used containers are washed with detergent using a bottle brush, rinsed twice with fresh water and once with dilute

HCl (5%). They are then rinsed twice with fresh water and turned upside down on a drying rack where they remain until needed for the next culture.

Table 3 – Procedures for making 5 L of the nutrient medium concentrate (1000x)

1. Add 2 L of distilled water to a >5 L measuring container
2. Add 750 g of sodium nitrate ($NaNO_3$)
3. Add 10 mL of each of the 5 trace metal stock solutions (see Table 3.1)
4. Add 10 mL of vitamin B_{12} stock solution (see Table 3.2)
5. Add 100 mL of vitamin H stock solution (see Table 3.2)
6. Add 1 g of vitamin B_1 (thiamine hydrochloride) (see Table 3.2)
7. Add 1 L of previously autoclaved iron solution containing 45 g of ferric citrate ($FeC_6H_5O_7.3H_2O$) and 45 g of citric acid ($C_6H_8O_7.H_2O$)
8. Add 50 g of sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$)
9. Top up to 5 L using distilled water
10. When all chemicals have dissolved and well mixed, distribute the media between five 1 L storage bottles and store in the fridge for up to 6 months.

Table 4 – Trace metals used for nutrient medium concentrate

Mineral	Chemical	Formula	Stock solution
Cu	Copper sulphate	$CuSO_4.5H_2O$	10 g L^{-1}
Zn	Zinc sulphate	$ZnSO_4.7H_2O$	22 g L^{-1}
Co	Cobaltous chloride	$CoCl_2.6H_2O$	10 g L^{-1}
Mn	Manganese chloride	$MnCL_2.4H_2O$	180 g L^{-1}
Mo	Sodium molybdate	$Na_2MoO_4.2H_2O$	6 g L^{-1}

Table 5 – Vitamins used for nutrient medium concentrate

Vitamin	Chemical	Stock solution
Vitamin B_{12}	Cyanocobalamin	1 g L^{-1} (0.2 g 200 mL^{-1})
Vitamin H	Biotin	0.1 g L^{-1} (50 mg 500 mL^{-1})
Vitamin B_1	Thiamine hydrochloride	Added to concentrate as a powder

Both stock and working cultures use 1 μm-filtered seawater diluted with tap water to lower the salinity to around 30 ppt to 32 ppt. After the nutrient medium concentrate is added volumetrically to the unsterilised seawater in each culture vesicle, stock cultures are capped with a Steristopper® and foil cap while working cultures are capped with their respective rubber bung fittings (Figure 10). All culture vesicles are then autoclaved at 121 °C for 15 min. (Note: it is important not to re-autoclave seawater after adding the nutrient medium concentrate since this second treatment can cause excessive degradation of essential vitamins).

The inoculation of stock cultures is carried out in a laminar flow cabinet using aseptic techniques to help maintain auxenic conditions (Figure 10, left). This involves using a flame from a Bunsen burner to sterilise the tops of flasks prior to opening for transfers. The inoculation of working cultures is preformed using a sterilised (100 ppm chlorine bath) siphon tube that is connected to the airline downpipe after spraying with

ethanol (70%) (to again reduce the chances of contamination) (Figure 10, right). After use the transfer tubing is rinsed with tap water and replaced back in a chlorine bath.

Figure 10 – Stock culture transfer (left) and working culture transfer (right)

Volumes used to inoculate new cultures are very low for stock cultures (0.05–0.1 mL per 75 mL culture) but reasonably high for working cultures (10% of vesicle volume). This is because their aims are quite different: stock cultures are used to maintain a pure source, whereas working cultures are involved with its rapid multiplication. Accurate records of the culture plan and any particular problems experienced are kept to allow backtracking of procedures in the advent of contamination. Cultures are also clearly labelled with the species name, the inoculation date and the source of the inoculants. Microscopic inspections are carried out on a monthly basis or when gross observations suggest that a problem is developing (e.g. poor cell density, cloudy water, unusual colour or odour, excessive foam or scrum-line on flasks).

Stock cultures are generally kept on the opposite side of the room to working cultures, to distance them from the strong lights needed by the working cultures. At relatively low light levels, the stock cultures will grow slowly, minimising the frequency of transfers and associated risks of contamination. Under normal conditions, the stock cultures are transferred every 6 to 8 weeks, and working cultures are transferred weekly. As a general rule, old cultures are kept as a back-up until new cultures are proven to have successfully established.

Outdoor intermediate and mass microalgal cultures

A principle of strong inoculants in outdoor cultures helps to ensure that adequate cell densities are reached in a reasonable time frame and that relatively pure cultures are maintained throughout the production system. To ensure a strong seed is maintained at each transfer, scaling the algal cultures up from laboratory-based working cultures involves an intermediate stage prior to the larger 5000 L mass cultures. Translucent white 200 L plastic tubs with lids are used at BIRC for this intermediate stage for both algal species discussed herein (Figure 11, left). Mass cultures are routinely grown in 5000 L flat-bottomed fibreglass tanks (Figure 11, right).

Figure 11 – Intermediate 200 L algal cultures (left) and mass cultures in 5000 L tanks (right)

The procedures for cleaning, filling, sterilising and seeding tub- and tank-based cultures are the same. Tubs and tanks are scrubbed clean after each culture, rinsed with freshwater and allowed to dry in the sun before refilling. They are then filled with 1 μm-filtered (filter bag) seawater which is then chlorinated (10 ppm active chlorine) using 100 mL of liquid sodium hypochlorite per 1000 L of seawater. For effective sterilisation chlorinated seawater is allowed to stand overnight without aeration. Liquid chlorine that is commonly used for swimming pools (11–12 ppm active chlorine) and from a reputable dealer is preferred.

The next day the tubs or tanks are heavily aerated and the residual chlorine in the seawater is neutralised with sodium thiosulphate anhydrous (add 0.5 g per 10 mL chlorine used in the tanks), or sodium thiosulphate pentahydrate at a rate of 8 g per 1000 L seawater.

After checking (using chlorine test tablets) and confirming that the seawater has been fully de-chlorinated, fertilisers (Table 6) and inoculants are added. Intermediate tub cultures of both algal species are normally seeded with one 10 L working culture from the laboratory, and 5000 L mass cultures are generally seeded with one (T-iso) or two (*N. oculata*) intermediate tub cultures.

Table 6 – Fertilisers used for intermediate and mass cultures

N. oculata		**T-iso**
Ammonium sulphate	107 g 1000 L^{-1}	Aquasol™ soluble fertiliser (commercial fertiliser for home gardening and agriculture) 35 g 1000 L^{-1}
Superphosphate	10 g 1000 L^{-1}	
Urea	7 g 1000 L^{-1}	

Mass cultures of *N. oculata* (7–10 days old) are also commonly used to inoculate other mass cultures where necessary. In this case the seed water represents about 20% of the total volume of new cultures, and is passed through a 1 μm filter bag as it enters the new culture to help remove contaminants.

Mass-to-mass sub-cultures of T-iso have proven less reliable and so this is not generally practiced for this algal species. This may be related to its sensitivity to light, and this is addressed by shading newly seeded mass cultures with 70% shadecloth for 1–3 days after inoculation (particularly on fine days where cultures are receiving strong direct sunlight).

Mass *N. oculata* cultures that are to be used for fish larvicultures have only half of the normal amount of fertiliser added (e.g. 310 g of the bulk fertiliser mix instead of 620 g in a 5000 L tank). This is done to ensure that the levels of ammonia in the mature cultures are low so that it does not adversely impact the fish larvae. Rotifers on the other hand are very tolerant of high ammonia levels so full-strength fertilisers are used for *N. oculata* cultures destined for rotifer feeding. These are also passed through a 1 *µ*m filter bag as it is pumped into the rotifer culture tank to reduce levels of ciliated protozoans and other particulate contaminants.

Mass microalgal culture investigations

During March to May 2008 six cultures of T-iso and eight cultures of *N. oculata* were quantitatively monitored for up to 11 days. Cell counts, Secchi depths and total ammonia levels (Palintest) were recorded daily. Consistent with algal cultures destined for use in fish larvicultures, half strength fertilisers were used in the *N. oculata* cultures that were studied, while for T-iso the full complement of Aquasol™ was used during the trial.

Rotifer production

Flat bottomed 4000 L fibreglass tanks are used for rotifer production at BIRC. Procedures employed are tailored to algal production schedules but all are generally maintained on a weekly basis. This means that the same duties are needed on the same days each week, allowing for easier management of hatchery protocols. Cultures are initially prepared and seeded on a Friday. This involves pumping 3500 L of mature *N. oculata* culture (through a 1 *µ*m bag) into a previously cleaned and air-dried rotifer culture tank. This is seeded with about 40 rotifers mL^{-1} (approx. 140 x 10^6 rotifers per tank).

Rotifers used for the seed are previously harvested from a recently matured (usually 7-days-old) culture using a 52 *µ*m nylon mesh bag, and washed while still in the nylon mesh bag with the algal culture they will be seeded into. Three days later (usually the next Monday) the tank's water begins to clear as the algae is consumed by rotifers. At this point, the culture tank is topped up to full capacity with mature *N. oculata* culture.

Each day after this top-up the rotifer culture is fed with 100 g yeast after it is blended with about 1 L of tap water. Moist caked yeast is regularly purchased fresh from a local bakery and stored in a sealed container in the fridge for this use.

On the seventh day after seeding, fresh seeds are taken and the remaining rotifers are used for fish cultures. A density of 100–120 rotifers mL^{-1} is regularly achieved with this approach. No other enrichment products, pure oxygen, or algal concentrates are used in the process. Notably, if rotifers from a clear culture are being used for particularly "hungry" fish larvicultures, a short (1 h) period of boosting in mature algal culture is applied before the rotifers are added to the larviculture.

Brine shrimp hatching and on-growing

Brine shrimp hatching is routinely conducted in 1000 L conical bottom tanks where up to 600 g of dried cysts (250 000 cysts per gram) can be hatched at a time in one tank. The dried cysts are hydrated in 10 L freshwater with 10 mL chlorine for 10 min before transferring into the hatching tank containing preheated (27 °C) seawater. One 30 watt fluorescent light is placed over the hatching tank overnight to assist in the hatching process. A 1 kWw heater with thermal control is normally necessary to maintain the temperature while strong aeration is also provided via two 4 mm open-ended airlines.

Brine shrimp nauplii are ready for harvest after about 24 h, or as soon as most nauplii in the hatching tank have cleared their egg shells. These are harvested and washed with clean seawater in a 250 μm nylon mesh bag prior to feeding or on-growing.

In order to increase the biomass and quality of brine shrimp nauplii for more advanced fish larvae, the newly hatched nauplii are on-grown for 1 to 3 days before being fed. Conical 500 L tanks are used for this. These are normally initially filled (day 1) with 300 L of either *N. oculata* and/or T-iso, into which the nauplii are stocked at densities up to 130 mL^{-1}. The next day (day 2) a further 150 L of either *N. oculata* and/or T-iso is added to these tanks, and the nauplii are harvested, washed and used before the end of the third day. On-growing cultures are also preheated to 27 °C before introducing brine shrimp nauplii, and this temperature is maintained with 300 w thermal controlled glass heaters while strong aeration is again provided.

Results

Mass microalgal culture investigations

Selected pairs of water quality parameters considered to be useful in mass microalgal culture management were plotted and the regressions were calculated with the values of the respective correlation coefficient provided in each Figure.

Figure 12 provides the Secchi depths of *N. oculata* and T-iso mass cultures through an 11-day production schedule. Cultures of *N. oculata* had smaller Secchi depths throughout the range of ages studied, from seeding through to a maturity. Both algal species appeared to grow in a linear fashion in terms of cell densities, although *N. oculata* cultures had far greater cell densities (more than 10 times greater – Figure 13).

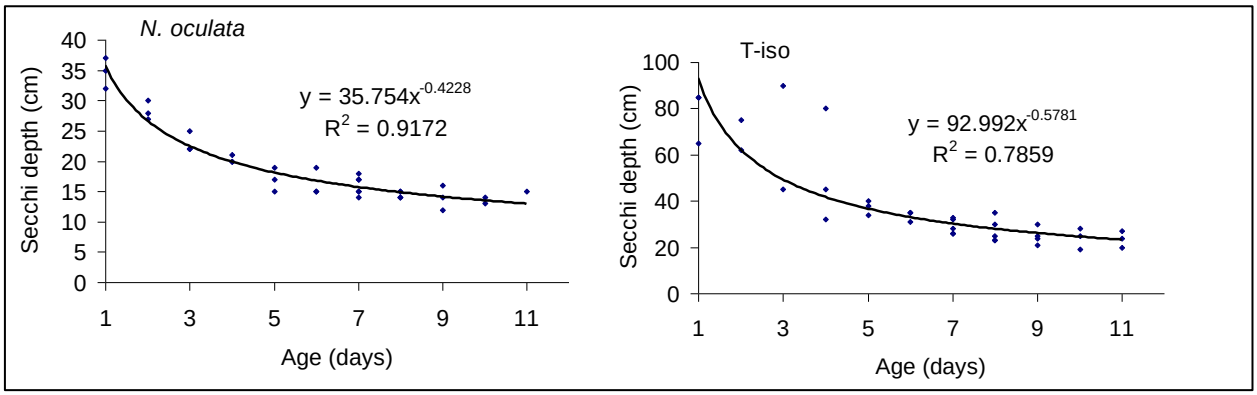

Figure 12 – Secchi depths of* N. oculata *and T-iso mass cultures at different ages

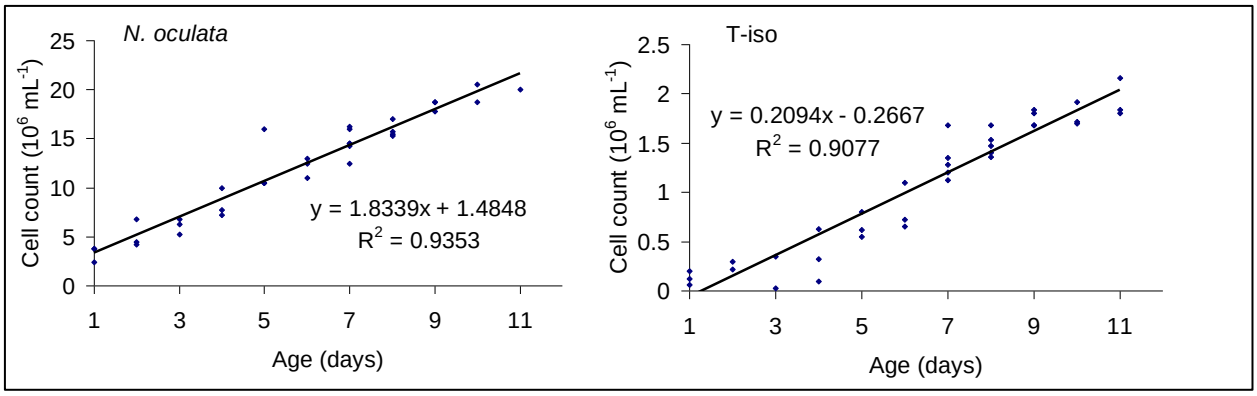

Figure 13 – Cell counts of* N. oculata *and T-iso mass cultures at different ages

Nannochloropsis oculata cultures had levels of ammonia that were many times greater than those of T-iso (Figure 14), despite the use of the half-strength fertilisation regime. There was a very strong correlation between Secchi depth and cell density for both algal species studied (Figure 15). Total ammonia levels also corresponded well with Secchi depths (Figure 16), and cell counts (Figure 17).

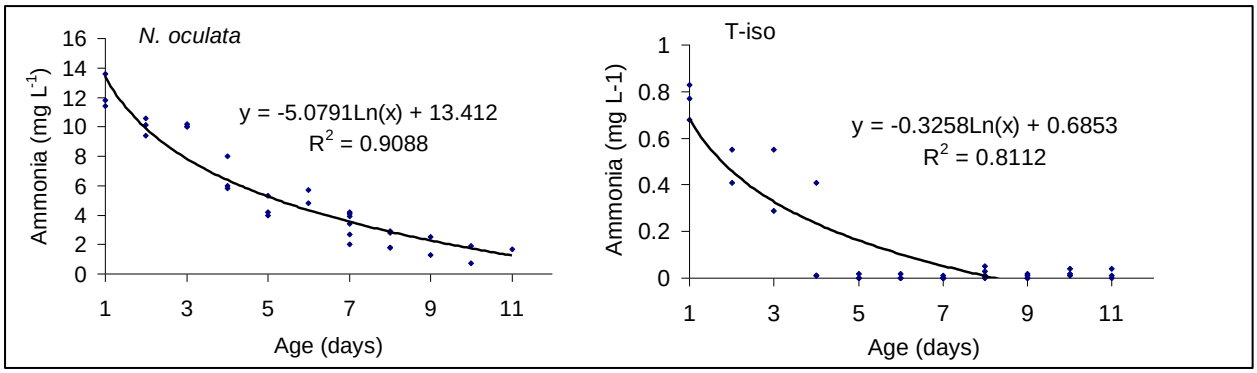

Figure 14 – Total ammonia levels in* N. oculata *and T-iso mass cultures at different ages

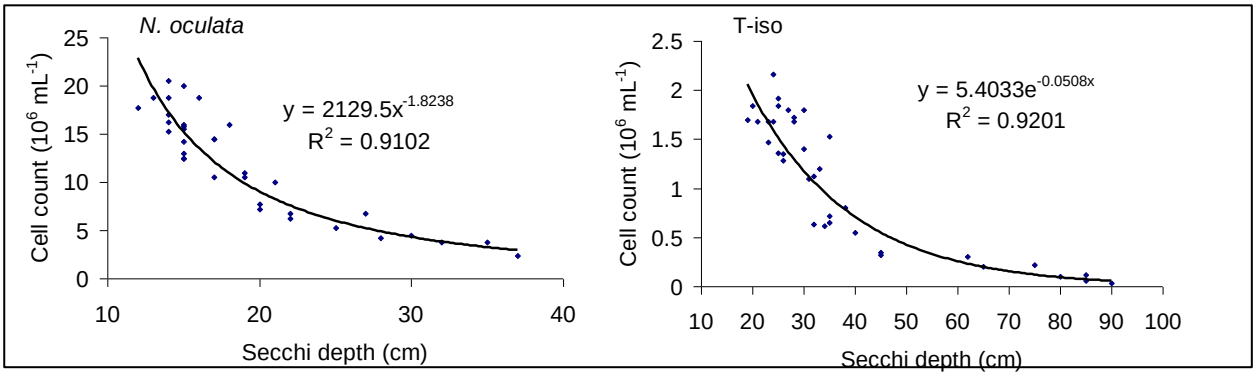

Figure 15 – Secchi depths and corresponding cell counts for* N. oculata *and T-iso mass cultures

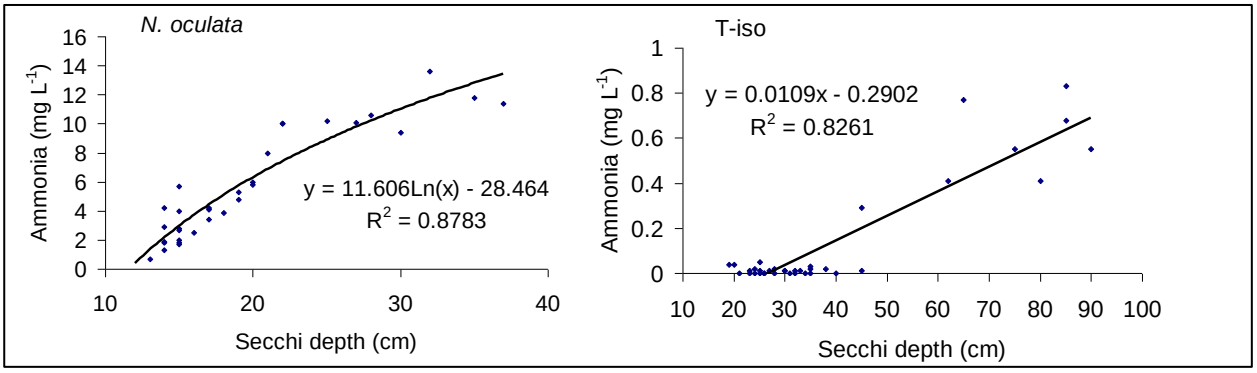

Figure 16 – Secchi depths and corresponding total ammonia levels in* N. oculata *and T-iso mass cultures

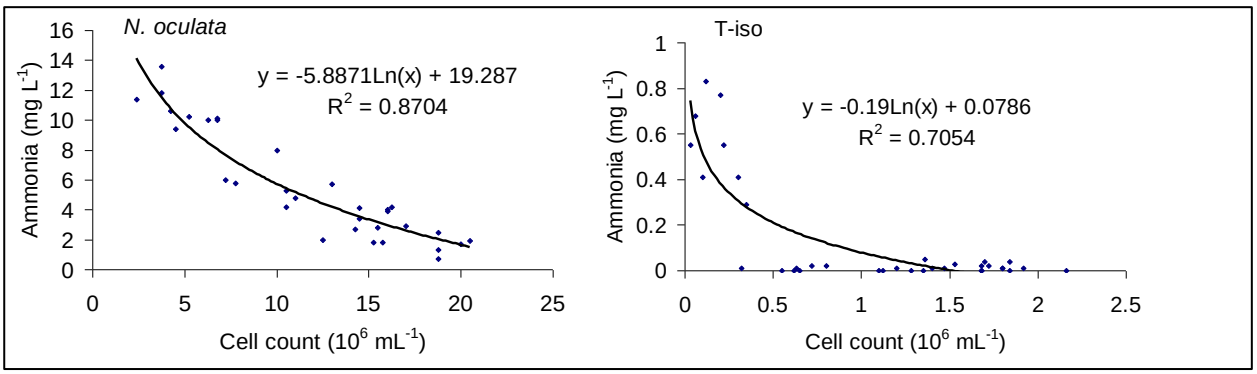

Figure 17 – Cell counts and corresponding total ammonia levels in* N. oculata *and T-iso mass cultures

Discussion

At BIRC, low-water-exchange green-water larvicultures have been successfully used for rearing many estuarine fishes. Pure cultures of *N. oculata* have routinely been used to provide the green-water environment, and where it was considered necessary, water stable microencapsulated nutritional boosters have been added directly to the larvicultures to improve the nutritional profile, especially the docosahexaenoic acid (DHA) content. However, these nutritional boosters are no longer available in their microencapsulated form, and there presently does not seem to be comparable products available that are suitable for this style of use. In anticipation that cobia larvae could require a more complete nutrition than *N. oculata* could provide on its own, efforts were undertaken to provide additional DHA levels in the larvicultures by also adding a complimentary algal species known to be rich in this particular fatty acid (Brown and Jeffrey, 1992; Martinez-Fernandez *et al.*, 2006). As the selected algal species for this purpose, T-iso was scaled up to mass cultures using similar facilities adjacent to the *N. oculata* production system, and was similarly studied in terms of its physical and chemical properties that are relevant for its use in a controlled green-water hatchery.

In general, cultures of both species of algae performed well during the research. Densities of *N. oculata* consistently reached over 20 million cells mL^{-1} in 11 days, when it was still in an exponential growth phase. T-iso was more temperamental before the shading protocol was applied, but then reliably reached densities of 1–1.5 million cells mL^{-1} within 7 to 10 days, when it also was still in exponential phase. These results indicate that the methods and management techniques used to grow these species of microalgae in mass cultures should have been ideal for the cobia larvicultures they were supporting.

The total ammonia results were of particular interest, since there were concerns that levels in the larvicultures were at times excessive. Levels in *N. oculata* cultures were 4 mg L^{-1} after 7 days, which is the age that is normally used for feeding to rotifers, and is the youngest age culture that has been recommended for use in larvicultures. Unlike fish larvae, rotifers are known to tolerate relatively high ammonia levels (Daintith, 1996), but these are washed after harvesting and before feeding the fish larvae, so their concentrates should not transfer excessive amounts of ammonia to the fish larviculture tanks. However, mass microalgae cultures do represent this risk to larvicultures if they are prematurely added before ammonia levels have declined to tolerable levels. For example, the amount of microalgae initially added into the green-water larval rearing tank is about one third of the total culture volume, resulting in ammonia levels that are about one third of that in the microalgal culture that is used.

Although there are no reports regarding the ammonia tolerance of cobia larvae, in other larval fish species it is advisable for ammonia levels to remain below 2 mg L^{-1} and ideally for complete safety below 1 mg L^{-1}. Therefore, to minimise the chance of high ammonia levels in the green-water culture tanks, the present results suggest that *N. oculata* cultures older than 10 days should be used. At that stage our results suggest that these cultures are still in exponential phase, and even after two weeks they appear to still be in very good condition. Another option could be to replace ammonia-based fertilisers with nitrate-based fertilisers, but it remains to be seen if similar cell densities and culture vigour can be achieved with this approach.

By comparison, the ammonia levels in Aquasol™-fertilised T-iso cultures were below 1 mg L^{-1} from the first day of culture, and this declined to below 0.1 mg L^{-1} after 5 days, representing lower related risks to larvicultures. However, the sometimes temperamental nature of T-iso makes it a less favourable conditioner of closed system green-water cultures. Compared with *N. oculata*, which very reliably subcultures from mass to mass cultures, T-iso often dropped out of the water column the day after seeding with mass cultures, which creates bottom fouling concerns in receiving larvicultures. Even though it was expected to provide

nutritional benefits, such as the provision of higher DHA levels, this lower reliability may diminish its benefits in routine hatchery operations.

There were other distinct differences between the two algal species studied in terms of their physical and chemical effects on water qualities. *N. oculata* cultures were shown to offer much greater levels of light shading, mainly because of the very high cell densities that are produced. The strong correlation between Secchi depth and cell density for both algal species studied also suggests that cell densities can easily be inferred through Secchi depth measurement. From a practical point of view, Secchi depth is far more easily measured, and the present data justifies its use on a routine basis to save time estimating numbers of cells. The relationship between Secchi depth and total ammonia was also somewhat better than cell density and ammonia, suggesting for both species this could also be a better proxy for direct ammonia measurement. Of course, different sites and physical culture conditions (e.g. tank design), along with seasonal effects on temperature, light availability and day-length can affect these relationships, so caution should be exercised when extrapolating in new environments.

Recommendations for further research

The high ammonia levels in *N. oculata* mass cultures are due to the chemicals used in the fertilisation media. To reduce this, new culture media could be evaluated, such as Aquasol™, which has been successfully used to grow T-iso in mass cultures. Generally speaking, *N. oculata* is easier to culture than T-iso, which suggests that this may be successful. Down-sides to this approach could be changed nutritional content or less favourable microflora which seems to be particularly good using current methods, and would need simultaneous investigation.

Although the rotifer culture methods that are presently employed at BIRC are very reliable, these cultures and the supporting *N. oculata* mass cultures, need to be maintained for 12 months of the year, when they are only necessarily used for a few months each year. New methods could be investigated which minimise the labour and costs of maintaining seeds from one season to another. This could involve scaling down to smaller-volume seed cultures that are maintained in the lab during the off-season, as is done for microalgae, or looking to store rotifer eggs or neonates via certain means (e.g. cryopreservation) which can be used to start new cultures each season. The reliability of these approaches and the resultant vigour of prevailing mass cultures would be aspects needing clarification.

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4. Rearing cobia larvae using controlled green-water cultures

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Abstract

Three mass larval rearing trials (two in 2008 and one in 2009) were conducted to assess the potential of controlled low-water-exchange green-water culture (GWC) techniques for early rearing of cobia (*Rachycentron canadum*) at large scale. In all three trials, 5000 L outdoor rearing tanks were prepared on the day before spawnings were due, and were seeded with cultured microalgae and rotifers the day before stocking day-of-hatch larvae. Stocking densities were 10 L^{-1} and 50 L^{-1} in the first trial, 50 L^{-1} in the second trial, and 30 L^{-1} in the third trial. Water temperatures during the culture period ranged from 24.2 °C to 31.0 °C, with high salinities (33–37 ppt) and pH levels of 7.9–8.3. Cultures were supplemented with rotifers for 9 days at 20 mL^{-1}, and co-feeding of brine shrimp was initiated on GWC-day 7. Water exchanges ranged from 20–50% day^{-1}, which coincided proportionally with increasing brine shrimp feeding levels from 2–5 mL^{-1} d^{-1}. Survival in 2008 was very high in all cultures for the first week, but waned over time to various lower levels (3.7–41.7%) at harvest (up to 23 days post-hatch). Results in the second year (2009) were similar, with high survival prevailing in all cultures through till day 9 when mortalities (approx. 30–40%) occurred in cultures using either *Isochrysis galbana* and/or *Nannochloropsis oculata* as phytoplankton bases (n = 3). Rotifer procreation in cultures which received both species of microalgae was generally lower than in cultures which received only *N. oculata*. Cobia survival (42.1–56.3%), growth (up to 8–9 mm long) and apparent condition was similar using either microalgae treatment. Following the successful attainment of an 8–9 mm fry length, two subsequent production strategies were undertaken: 1) an intensive tank rearing approach with further feeding using 2-day-ongrown brine shrimp prior to weaning; and 2) an alternative less intensive approach where fry were stocked at various densities into outdoor plankton-based nursery ponds prior to weaning.

Introduction

Cobia (*Rachycentron canadum*) is a migratory, pelagic fish species found in tropical, subtropical and temperate waters worldwide, with the exception of the eastern Pacific (Briggs, 1960; Shaffer and Nakamura, 1989). Spawning in these natural environments subjects their pelagic eggs and larvae to oceanic water qualities, which provides a general guide to larval culture conditions that are likely necessary for high health. For example, it can be assumed that oligotrophic waters with reasonably high salinities (35–36 ppt), stable warm temperatures (25–30 °C) and moderately alkaline pH levels approximating those of oceanic seawater (8.1–8.2) would be most suitable for its artificial propagation. Assuming that their larvae remain in the upper photic zone of these relatively deep waters, where primary production generates sufficient zooplankton as potential feeds, oxygen levels would also be close to saturation and light levels would vary from medium to low levels diurnally. In these natural habitats their larvae would seldom encounter significant levels of turbidity, dissolved organic matter or metabolic wastes like ammonia.

However, in intensive larval cultures there are often compromises which need to be made to enable practical production methods and provide adequate nutrition at much higher densities than occurs in the wild. In Taiwan, cobia are reared in intensive recirculating systems or more frequently in green-water ponds from hatching through to 30 g, with survival rates of 5–10% reported during the first 20 days of culture (Liao *et al.*, 2004). Once they reach 30 g (75 days post-hatch), they are transferred to larger outdoor ponds or inshore cages for further grow-out. The use of flow through and recirculating aquaculture systems to control environmental conditions is also common in many other countries during the larval and early juvenile stages

of cobia, with recent studies outlined by Benetti *et al.* (2007, 2008a, 2008b), Faulk and Holt (2005, 2006), Faulk *et al.* (2007), Hitzfelder (2006), Holt *et al.* (2007), Resley *et al.* (2006), and Webb Jr. *et al.* (2007).

A number of specialised larval rearing and live feed culture procedures have been developed for this species. These include protocols for egg disinfection (Tamaru *et al.*, 1999), weaning procedures for late larvae and early juveniles (Benetti, 2002), and live prey enrichment and green-water culture (Faulk and Holt, 2005). Furthermore, the fatty acid compositions of cobia eggs and larval feeding regimes have been examined (Faulk and Holt, 2003), along with the tolerance of larvae to different salinities (Faulk and Holt, 2006; Resley *et al.* 2006). While cobia have been shown to survive a broad range of temperatures and have been cultured between 16 and 32 °C (Liao *et al.*, 2004; Weirich *et al.*, 2004), Benetti *et al.* (2008a) found that at higher temperatures (>29 °C), the larvae grew faster, underwent metamorphosis earlier, and could feed on dry-diets quicker than larvae reared at lower temperatures.

There has also been considerable research into the growth and survival of larval and juvenile cobia in recirculating raceways and other aquaculture systems (Faulk *et al.*, 2007; Hitzfelder *et al.*, 2006). For example, Hitzfelder *et al.* (2006) found that initial densities of 5–10 larvae L^{-1} were optimal. This was of particular interest to the present study since we attempted to lift these production levels substantially with our previously standardised GWC approach (Palmer *et al.*, 2007). Other recent works with hatchery technologies for cobia include those by Benetti *et al.* (2007, 2008a, 2008b), Faulk *et al.* (2007), and Holt *et al.* (2007).

Benetti *et al.* (2008a) described the protocols of two larval rearing trials which incorporated the use of probiotics and prophylaxis, minimal microalgae use, and including commercially available ingredients for live feed enrichment. Survival rates ranging from 17.5% to 35% were achieved from egg to shipping-size fingerlings (1 g) with production of approximately 20 000 fingerlings per 12 000 L tank. Daily water exchange rates ranged from 100% at 3 dph to 500% from 17 dph onwards. Using indoor recirculating raceway systems, Faulk *et al.* (2007) found that cobia could be successfully cultured from hatching through to at least 35 g without negatively affecting growth or survival.

Due to our experience with green-water larviculture methodologies at BIRC, and the reported suitability of cobia to this style of culture, we applied our standardised GWC approach to cobia larvae on two separate occasions in 2008, and again in 2009. On each occasion our work was structured to provide fingerlings for use in experimental work and farm-based research, as well as to provide practically useful production information for industry. Our overall aims were to contribute to the development of hatchery technologies for cobia generally, and to assist industry in Queensland and Australia in developments with this promising species.

Materials and methods

Egg incubation and hatching

Fertilised eggs were obtained from 35 000 L broodstock tanks at BIRC on the mornings after successful spawning. Floating eggs were collected from the overflow of each tank after they had reached a reasonably high concentration in 200 L egg collectors with a mesh size of 500 μm. Concentrated eggs in the collectors were decanted into 20 L buckets and stocked directly into incubation tanks. This transfer generally took place six to eight hours post fertilisation. Incubation tanks consisted of 400 L “egg cup” shaped fibreglass tanks and 1000 L conical bottom tanks (Figure 18).

All tanks were supplied with UV sterilised 1 μm filtered seawater, and were maintained with a water temperature of 26–27 °C. Moderate aeration from an air ring around the base of a central pipe ensured that the developing embryos were mixed uniformly in each tank and constantly moving in a gentle circular pattern, with dissolved oxygen levels of 6 ppm or greater. A constant seawater (35 ppt) exchange (1–2 L min^{-1}) was applied during embryo incubation and after hatching. An external standpipe set the water level in the tank and the developing embryos and larvae were retained during water exchanges by internal screens with a mesh size of 300 μm.

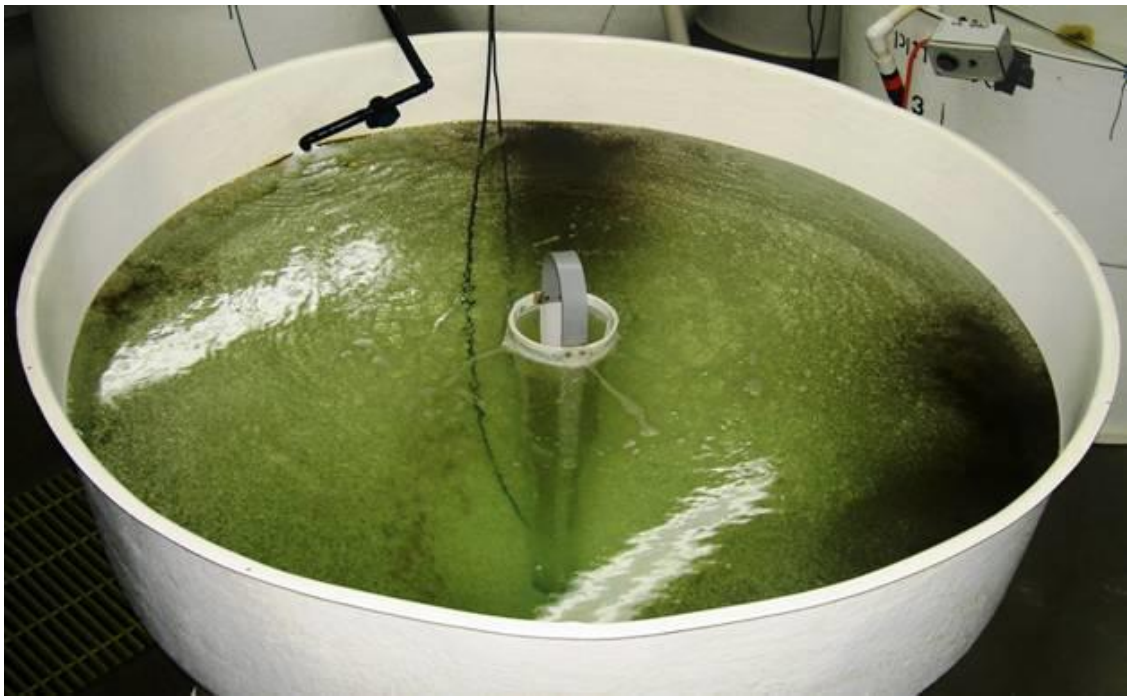

Figure 18 – Hatching tank containing several hundred thousand Day 2 cobia larvae in 2008

Shortly after stocking fertilised eggs collected from the spawning tanks, the aeration and water flow in the incubation tanks was suspended and a gentle circular motion to cause a vortex effect was created in the tank using a paddle. Following a settling period of approximately 5 min, non-viable eggs sank to the bottom of the tank and concentrated around the centre pipe, and these were easily removed with a small diameter (10 mm) siphon tube. Viable unhatched embryos were positively buoyant and therefore remained at or near the surface. This process was repeated within 1 h. A similar separation technique was also used following hatching to remove settable organic debris (e.g. non-viable eggs and discarded shells) to minimise bacterial loads. Regular samples were taken to monitor cell division and embryonic development. No chemical disinfection procedures were applied.

Egg densities in well-mixed hatching tanks were estimated volumetrically by taking several 10-mL samples and averaging the counts. Hatching densities used during the work generally ranged from 1000–2000 L^{-1}. Several hours after hatching, the fish larvae were slowly concentrated into about one quarter of the hatching tank's volume, larval counts (several 100 mL samples from a well-mixed known volume) were conducted, and the required numbers of larvae were volumetrically stocked into the GWCs. Similar methods of handling and distribution were applied to yolk sac larvae that were packaged in plastic bags for transport to other hatcheries. These were also decanted from the hatching tanks after gentle concentration, with care taken not to disrupt their fragile yolk sac membranes.

Green-water larval cultures

Larval rearing generally followed the protocols described by Palmer *et al.* (2007). GWC tanks were prepared and seeded with microalgae and rotifers prior to stocking, so that larvae were exposed to conditions approximating an exponential-phase plankton bloom. Figure 19 provides a view of the larval rearing area at BIRC, incorporating algae, rotifer and larval rearing tanks.

Figure 19 – Outdoor larval rearing area at BIRC with mass algal culture tanks in foreground

Larval rearing trials were conducted in up to six 5 000 L flat bottomed outdoor tanks with perpendicular sides. One layer of green 70% shade cloth was secured across the entire top of each larval tank so that shading and air ventilation were similar for all tanks. This combined with an upper layer of 70% shade cloth provided well shaded conditions inside the tanks. Tanks were each filled with 1200 L of filtered (1 μm) seawater which was sterilised with liquid hypochlorite (11–12% active chlorine) at a rate of 100 mL per 1000 L seawater. This was undertaken on the day that spawning was expected (GWC-day 0). After this, each tank was covered with shadecloth and allowed to sit overnight without aeration.

On the morning after spawning, the culture bases were dechlorinated with sodium thiosulphate pentahydrate at a rate of 8 g per 1000 L seawater (about 16 h after chlorination). Heating of GWCs to approximately 26 °C was achieved with a combination of 3 kW thermostat-controlled titanium immersion bar heaters and 300 w aquarium heaters. Aeration in each tank was set at moderate via nine evenly spaced air stones suspended just above the tank bottom.

Exponential-growth-phase microalgal cultures (600 L) (nominally *Nannochloropsis oculata* filtered through a 1 μm felt filter bag) and approx. 36 x 10^6 rotifers (large strain *Brachionus plicatilis*) were then (on GWC-day 1) added to each tank, thereby creating turbid conditions (tank bottoms obscured from view) and seeding the GWC tanks with about 20 rotifers mL^{-1}. Early the next day (GWC-day 2), the developing fish embryos began to hatch, and these were stocked into the GWCs later that day (between 2 and 4 pm). Before introducing larvae into the GWCs, they were acclimated for 5–10 min by slowly doubling the volume of larval concentrates with the water in each GWC.

Rotifer numbers in the GWCs were monitored twice daily (morning and afternoon) by assessing several volumetric (1 mL) samples. Rotifer additions were generally made up to day 9 if their densities fell below 20 mL^{-1}. Rates of aeration were low at stocking, gradually increasing to moderate a few days after stocking, and were high after 1 week. Initial uncertainty as to whether cobia larvae displayed any rudimentary swim bladder inflation meant that aeration was initially kept low[2], and that surface skimmers were deployed for the first few days of feeding in Trial #1. Since no sign of any primary swimbladder was detected in the first batch of cobia larvae, surface skimmers were not used in later operations, and slightly higher levels of aeration were applied after stocking.

Each of the cobia GWCs appeared to function in a similar way to those which have previously been applied to other species at BIRC. As the GWCs started to clear and as the tank bottom became visible (often on a daily basis), 100–300 L of an exponential phase microalgal culture was added. As in our previous work with other species (Palmer *et al.*, 2007) this algal addition was ideally undertaken in the morning before the natural light above the cultures became very strong, and before the pH of mass microalgal cultures rose significantly due to photosynthesis.

From GWC-day 7 and when brine shrimp were initially added to the GWCs, 300 L of previously sterilised and pre-heated seawater (1 *µ*m filtered) was also added after the microalgae to keep total ammonia levels below 2 mg L^{-1}. When the GWCs reached their maximum capacity (4800 L), water exchanges were carried out daily by previously discharging culture water through a screened (500 *µ*m) siphon hose (25 mm diameter) before the addition of microalgae and makeup seawater. Water exchanges ranged from 20% to 50% day^{-1}, which coincided proportionally with increasing brine shrimp feeding levels from 2–5 mL^{-1} d^{-1}.

The brine shrimp used in these studies were *Artemia franciscana* GSL Strain from INVE Pty Ltd. Their densities in cultures were monitored twice daily (morning and afternoon) with several volumetric (10 mL) samples. Newly-hatched brine shrimp were introduced into the daily GWC feeding regime on GWC-day 7 (on day 6 after larvae hatched[3]) at 1 mL^{-1}, when the cobia larvae were >5 mm long and appeared easily large enough to successfully ingest them. To ensure that all larvae had adequate time to fully adapt to this larger feed type, rotifer densities were maintained at between 10–20 mL^{-1} for a further 3 days.

On GWC-day 8, brine shrimp densities in the fish cultures were topped up to 2 mL^{-1} after they were on-grown in *N. oculata* for 24 h. For the next 2 days, single daily additions were made to maintain densities of 0.5–2 mL^{-1} using larger two-day-old brine shrimp that had been on-grown for 24 h in *I. galbana*. From GWC-day 11 onwards, applications of up to 4 mL^{-1} were required to ensure that they were not completely depleted, and towards the end of the GWC phase, two additions of brine shrimp were necessary each day to maintain densities above 0.5 mL^{-1}.

GWCs were harvested at various times after GWC-day 13 (12 days after hatch). At this stage the fry were longer than 8 mm and robust enough to handle concentration, number estimations and distribution. GWCs were harvested by turning the aeration off and allowing fry to rise to the surface (see Figure 20). From there they were decanted into a 500 L hemispherical holding tank with 1–2 L jugs. This estimation/holding tank (Figure 21) was filled with water from the respective GWC being harvested, gently aerated, and fitted with a 500 *µ*m central screen to permit volume adjustments. Fry were either cumulatively counted into the holding tanks, or were estimated volumetrically (several 100 mL or 550 mL samples) during a short period of heavy aeration and rapid additional mixing with a paddle. They were then either transferred to other GWC tanks,

[2] Excessive turbulence prevents larvae gaining access to air at the water surface and surface skimmers are generally used to clear any oily films that develop on the water surface – each of these factors can prevent or reduce swim bladder inflation in some species (Palmer *et al.*, 2007).
[3] Day 1 = day larvae hatched.

clear water tanks, or previously established nursery ponds which had a good population of natural zooplankton.

Figure 20 – Preparing a green-water culture for harvest – shade cloth covers have been removed and aeration is about to be turned off

Figure 21 – Fry in a holding tank after harvest from a green-water culture

Experimental design

In the first trial conducted in 2008 (starting on 24 January), day-of-hatch larvae were stocked into four GWCs at 2 pm (approx. 10 h post hatch). Two cultures (GWC 1 and GWC 2) were stocked at 10 L^{-1} (18 000 larvae per tank), and two cultures (GWC 3 and GWC 4) were stocked at the higher density of 50 L^{-1} (90 000 larvae per tank). A previously standardised approach to GWC management (as developed previously for other species) was applied up until GWC-day 19 when attempts were made to wean the larvae in GWCs using Biomar™ 0.3 mm starter.

In the second trial (starting on 28 February 2008), day-of-hatch larvae were stocked at 50 L^{-1} (90 000 larvae per tank) into two GWCs (at 2 pm; approx. 7 h post hatch). In both of these trials in 2008, *N. oculata* was exclusively used as the culture base up until GWC-day 6. From then a combination (50:50 *v/v*) of *N. oculata* and *I. galbana* was used on a daily basis to obscure the tank bottoms from view. This was undertaken as a precautionary measure to address the reported need for enriching the levels of essential fatty acids required by cobia in GWCs (Faulk and Holt, 2005; Ferreira *et al.,* 2008).

In the third trial conducted in 2009 (starting on 24 January 2009), a stocking density of 30 L^{-1} day 1 larvae was similarly used throughout the experiment, and cultures (n = 3) were maintained for the entire rearing period either with equal proportions (*v/v*) of these two microalgal species (Note: on a few occasions only *N. oculata* was added to all cultures). The statistical package Genstat was used to compare survival (ANOVA) and larval growth measurements (Repeated Measures ANOVA) for these different treatments in this third trial.

In all of these mass rearing trials, treatments were randomly allocated to tanks on the floor plan of the hatchery. For each culture, total lengths of 10 randomly selected fish were regularly measured to the nearest 0.5 mm under a dissecting microscope, when their general condition and stomach fullness was also assessed. Daily water temperatures, pH and dissolved oxygen levels were also assessed each morning with a YSI multi-probe meter, and total ammonia levels were assessed with a Palintest Photometer. Rotifer densities were monitored by averaging at least three counts in a 1 mL glass pipette, and brine shrimp densities were similarly monitored in 10 mL pipette samples (both assessed under a dissecting microscope). Estimates for survival were also made for each culture on a daily basis, using broad visual observations of the cultures by experienced technicians.

Results

Trial 1 in 2008

Table 7 summarises data collected in this first trial. Average temperatures (am) in the GWC tanks up until harvest were 25.7 °C; 25.4 °C; 25.8 °C and 25.7 °C for GWCs 1, 2, 3 and 4, respectively. The salinity of culture water at the time of stocking was 34.5 ppt and the pH was 7.9.

Good densities of larvae persisted in all GWC tanks for at least the first 14 days of larval life, but variable survival became evident after this point in some tanks. Larvae surviving in the tanks continued to actively feed on the brine shrimp that were being on-grown and enriched with *I. galbana*. Water exchanges were applied according to predetermined plans whereby on post-hatch days 9 and 10, tanks were topped up with 300 L of previously sterilised and pre-heated seawater. Water exchanges of 20% (GWC-days 11–13), 30% (GWC-day 14), 40% (GWC-days 15–16) and 50% (GWC-day 17 until harvest) were applied thereafter. The prevailing total ammonia (TAN) levels were only tested on GWC-day 14 (larval-day 13), and were 1.8 mg L^{-1}, 1.9 mg L^{-1}, 2.0 mg L^{-1} and 1.6 mg L^{-1} for GWCs 1–4, respectively.

Increasing mortalities with age prompted the harvest of cultures when the larvae were 16–23 days old. Ad hoc attempts to wean the larvae in GWCs using artificial diets polluted the tanks with uneaten feed and further complicated the results as well as each of the culture's management regimes. This prevented direct survival comparisons for tanks with different stocking densities, although our hatchery technicians estimated that percentage survival was relatively similar (80–90%) for all tanks for the first two weeks of operation. Survival estimates ranged from 41.1% (larval-day 16) to 6.2% (larval-day 21) (see Table 7 for a more detailed relative breakdown of results).

Table 7 – Summary of results from cobia rearing Trial 1 in 2008, including post-hatch larval age, the mean total length of 10 larvae, culture temperature, and estimated survival

GWC 1	**Stocked at 10 L^{-1} = 18 000 larvae**																						
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16							
Size (mm)	3.32		4.25	4.40		5.20	5.46	5.60	6.03	6.10		7.70	8.04										
T°C (am)	28.0	24.5	24.8	25.4	25.6	25.5	25.3	26.0	25.5	25.5	26.0	25.5	25.5	25.5	26.0								
Survival	high	high	high	high	high	high	high	high	high	high	high	high	high	high	med	41%							
GWC 2	**Stocked at 10 L^{-1} = 18 000 larvae**																						
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Size (mm)	3.32			4.20		4.95	5.44	5.60	6.15	6.30		7.50	8.21				9.62			13.16			
T°C (am)	27.0	24.2	24.5	25.0	25.0	25.8	25.3	28.8	25.0	25.5	26.0	25.5	24.4	25.2	26.0	25.8	25.5	25.5			25.0		
Survival	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	med	med	med	17.6%
GWC 3	**Stocked at 50 L^{-1} = 90 000 larvae**																						
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
Size (mm)	3.32	3.93	4.29	4.33		5.00	5.28	6.00	6.15	6.05	6.50	7.10	8.90				11.37			13.34			
T°C (am)	30.0	24.8	24.8	25.0	25.8	26.0	25.8	25.8	26.0	25.5	26.0	25.8	25.0	25.2	25.8	25.8	25.5	25.5			25.0		
Survival	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	med	med	med	low	6.2%		
GWC 4	**Stocked at 50 L^{-1} = 90 000 larvae**																						
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Size (mm)	3.32	3.93		4.40		4.70	5.11	5.75	6.03	5.60	6.25	7.4	8.1	8.27			10.97			13.54			
T°C (am)	31.0	24.5	25.0	25.2	25.3	26.0	25.5	26	25.5	25.5	26.0	26.0	25.0	25.4	26.0	25.8	25.6	25.2			24.4		
Survival	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	high	med	low	10.2%	

Trial 2 in 2008

Water quality parameters in the two GWCs at the time of stocking were 26.6 °C, 33.7 ppt, pH 8.16 and dissolved oxygen 7.2 mg L^{-1}. Water temperatures (measured at about 8 am each day up to harvest) averaged 26.2 °C for GWC 1 and 26.5 °C for GWC 2. On GWC-days 8 and 9, both GWC tanks were topped up with 200 L and 300 L, respectively, using previously sterilised and pre-heated seawater. Water exchanges of 20% (GWC-days 10 and 11) and 30% (GWC-days 12 and 13) were then applied.

High survivals (>80%) were apparent in both GWCs up until larval-day 9. At that stage although larvae were active there were no signs of brine shrimp in their stomachs. Ammonia levels were then tested and were found to be 2.75 mg L^{-1} and 3.15 mg L^{-1} in GWCs 1 and 2, respectively, which was considered excessive and immediately addressed with 50% water exchanges. Despite the dilution effect of increased water additions and exchanges (well above those planned in the culture management regime), high mortalities occurred in GWC 1 from larval-day 10, and in GWC 2 from larval-day 12. Ammonia levels were checked again in both GWCs 1 and 2 on larval-day 12 and were 3.2 mg L^{-1} and 2.3 mg L^{-1}, respectively. This result prompted closer study of ammonia levels, and sooner water exchanges in the rearing protocol in the third trial in 2009.

Both GWCs (in Trial 2 in 2008) were harvested on larval-day 14 and the remaining fry (total of approx. 7000) were transferred to one previously prepared 1600 m² nursery pond containing a good population of natural zooplankton (e.g. copepods). Survival for GWC 1 was estimated to be 3.72% (*n* = 3347) and for GWC 2 was 3.85% (*n* = 3662). At harvest fry averaged 9.27 mm for GWC 1 and 11.05 mm for GWC 2. Table 8 summarises other data collected during this second trial in 2008.

Table 8 – Summary of results from cobia rearing Trial 2 in 2008, including post-hatch larval age, the mean total length of 10 larvae, culture temperature, and estimated survival

GWC 1	**Stocked at 50 L^{-1} = 90 000 larvae**													
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Size (mm)	3.47	4.36	4.55	4.93	5.02	5.18	5.38	5.58	6.41	6.60		8.16		9.27
T°C (am)	25.8	26.7	25.2	25.0	24.8	27.4	27.2	27.5	26.7	27.0	26.5	25.0	27.0	25.0
Survival	high	high	high	high	high	high	high	high	high	med	low	low	low	3.72%
GWC 2	**Stocked at 50 L^{-1} = 90 000 larvae**													
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Size (mm)	3.47	4.4	4.65	4.7	5.16	5.51	5.80	5.79	6.67	7.10		9.31		11.05
T°C (am)	26.0	26.5	25.2	25.0	24.8	28.0	27.0	27.0	27.0	26.8	27.5	27.4	28.0	25.2
Survival	high	high	high	high	high	high	high	high	high	high	high	med	low	3.85%

Trial 3 in 2009

Considerations from the previous two trials gave rise to the standardised culture approach described in Figure 22. Water quality parameters in the six GWCs at the time of stocking were 26–26.5 °C, 36.5 ppt, pH 8.2 and dissolved oxygen 6.2 mg L^{-1}. Water temperatures during the trial (measured at about 8 am each day up to harvest) averaged 26.8 °C; 26.1 °C; 27.0 °C; 26.0 °C; 26.0 °C and 27.3 °C for GWCs 1–6, respectively. Table 9 summarises other data collected during this third trial in 2009.

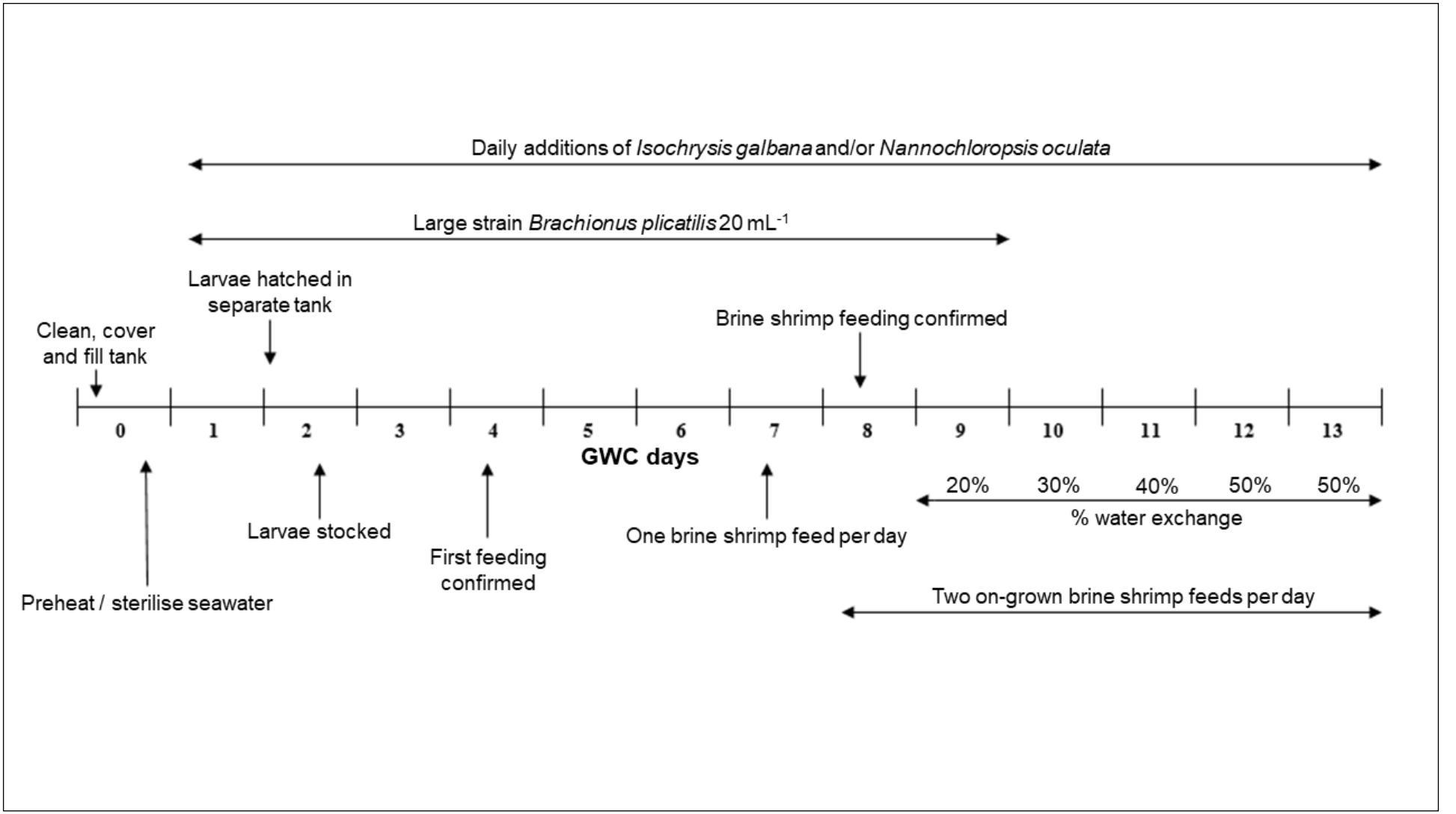

Figure 22 – Larval rearing regime for cobia in green-water cultures at BIRC during the third trial in 2009

Table 9 – Summary of results from cobia rearing Trial 3 in 2009, including post-hatch larval age, mean total length of 10 larvae, culture temperature, total ammonia (TAN) levels and estimated survival

GWC 1	**Stocked at 30 L^{-1} = 56 000 larvae : *N. oculata* + *I. galbana* (T-iso)**												
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13
Size (mm)	3.99	4.05	4.30	4.25	4.73	4.99	5.73	5.90	6.39	7.96	8.04		
T°C (am)	26.5	28	27	27.5	26	25	27	27	27	27	27		
TAN (mg L^{-1})	0.44		1.2		1.48	1.26	1.48	1.48	1.66	0.9	1.2		
Survival	high	high	high	high	high	high	high	high	med	med	med	46.1%	
GWC 2	**Stocked at 30 L^{-1} = 56 000 larvae : *N. oculata* only**												
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13
Size (mm)	3.99	3.78	4.09	4.74	5.17	5.12	5.56	6.27	7.14	7.13	8.51		
T°C (am)	26.5	27	27	26.5	26	26	25.5	25.5	26	25.5	25.5		
TAN (mg L^{-1})	0.51		1.1		1.54	1.48	1.92	1.54		1.25	1.00		
Survival	high	high	high	high	high	high	high	high	med	med	med	56.3%	
GWC 3	**Stocked at 30 L^{-1} = 56 000 larvae : *N. oculata* only**												
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13
Size (mm)	3.99	4.09	4.34	4.52	4.91	5.60	5.48	6.18	7.23	7.42	8.79		
T°C (am)	26.5	27.5	27	27.5	26	25	27	27.5	27.5	27.5	27.5		
TAN (mg L^{-1})	0.55		1.1		1.6	1.66	1.54	2.00		1.50	1.00		
Survival	high	high	high	high	high	high	high	high	med	med	med	50.8%	
GWC 4	**Stocked at 30 L^{-1} = 56 000 larvae : *N. oculata* + *I. galbana* (T-iso)**												
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13
Size (mm)	3.99	3.88	4.26	4.72	4.87	5.87	6.10	6.02	6.93	6.96	8.48		
T°C (am)	26	26	26.5	26	25	26	26	26	26	26	26		
TAN (mg L^{-1})	0.32		0.9		1.1	0.94	1.66	1.48		1.3	0.9		
Survival	high	high	high	high	high	high	high	high	med	med	med	54.3%	
GWC 5	**Stocked at 30 L^{-1} = 56 000 larvae : *N. oculata* + *I. galbana* (T-iso)**												
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13
Size (mm)	3.99	4.15	4.13	4.65	4.64	4.88	6.10	6.21	6.80	7.37	7.88		
T°C (am)	26	26.5	27	26.5	25	25	26	26	26	26	26		
TAN (mg L^{-1})	0.37		1.2		1.22	1.42	1.66	1.66		1.45	0.55		
Survival	high	high	high	high	high	high	high	high	med	med	med	42.1%	
GWC 6	**Stocked at 30 L^{-1} = 56 000 larvae : *N. oculata* only**												
Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13
Size (mm)	3.99	4.08	4.13	4.60	4.95	6.03	6.07	6.96	7.94	7.75	8.93		
T°C (am)	26	29	29	28	28	27	27	27	27	26.5	26.5		
TAN (mg L^{-1})	0.55		0.9		0.76	1.54	1.92	2.00	1.74	1.4	0.65		
Survival	high	high	high	high	high	high	high	high	med	med	med	55.4%	

On GWC-day 8, all six GWC tanks were topped up with 800 L of previously sterilised and pre-heated seawater. Water exchanges of 20% (GWC-day 9), 30% (GWC-day 10) and 40% (GWC-day 11) were applied. High survival prevailed in all cultures through to larval-day 9 when some mortalities (approx. 30–

40%) again became apparent in all cultures. This included those being supplemented with *I. galbana* and those with only *N. oculata* as the phytoplankton base (*n* = 3).

Rotifer procreation in cultures which received both species of microalgae was much lower than in cultures which received only *N. oculata* (Figure 23). This was demonstrated by the higher rotifer densities of cultures without T-iso in the 2–3 days after the initial seeding, and before significant predation by the larvae began. Interestingly though, as larval predation increased, rotifer populations in all three of the more productive cultures crashed to very low levels on GWC-days 4 or 5. Larval growth was similar (P>0.05) for tanks regardless of microalgal types, where fry were 8–9 mm long 11 days after hatching (Figure 24).

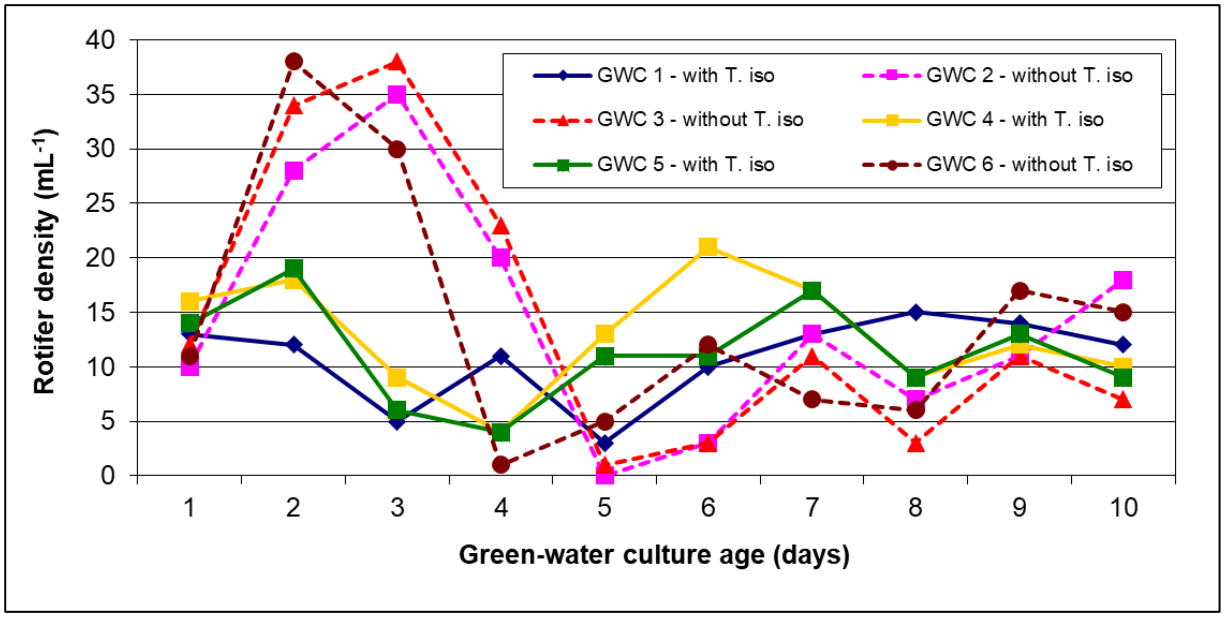

Figure 23 – Mean densities of rotifers (am) during Trial 3 in 2009 in green-water cobia larvicultures with and without T. iso supplementation

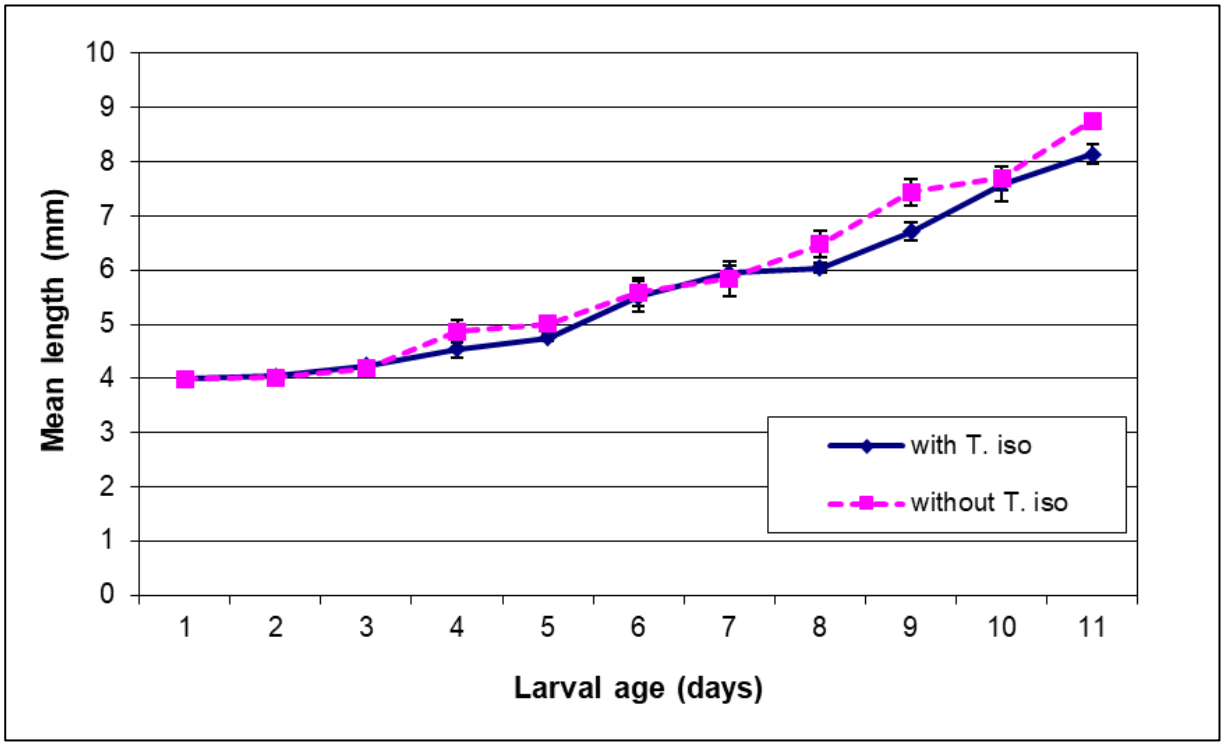

Figure 24 – Mean (± se) lengths of cobia grown during Trial 3 in 2009 in green-water larvicultures with and without T. iso supplementation (n = 3)

All six GWCs were harvested 12 days after the larvae hatched, yielding 25 800, 31 500, 28 450, 30 423, 23 591, and 31 000 fry from GWCs 1–6, respectively. This provided similar (P>0.05) mean (± se) 12-d survival estimates of 47.5 ± 3.59% for cultures with T-iso supplementation, compared with 54.2 ± 1.7% for those without T-iso supplementation (*n* = 3). The apparent health, vigour and condition of fry in the enumeration and holding tanks after harvest from the GWCs (see Figure 21) was also similar between treatments. These harvested fry (total number = 140 764) were transferred to three previously prepared 1600 m² nursery ponds at BIRC containing good populations of natural zooplankton (e.g. copepods). Fry from GWC tanks 1 and 2 were pooled together and transferred to Nursery pond 2, fry from GWC tanks 3 and 4 were transferred to Nursery pond 3, and fry from GWC tanks 5 and 6 were transferred to Nursery pond 4.

Discussion

In the intensive culture of marine fish larvae such as cobia, microalgae are often added to the water along with zooplankton (most commonly rotifers and brine shrimp). These methods are commonly referred to as "green-water culture" (GWC), and have formed the basis of controlled larviculture methods at BIRC for many years (reviewed by Palmer *et al.*, 2007). There is evidence for significant advantages from adding phytoplankton to larval fish rearing systems, with increased growth and survival demonstrated for a number of marine fish larvae including halibut (Naas *et al.*, 1992), turbot (Oie *et al.*, 1997), sea bream (Papandroulakis *et al.*, 2002), and many others. These beneficial effects are generally attributed to: 1) enhanced environmental conditions for feeding from increased turbidity, light scattering and attenuation, and visual contrast enhancement; 2) improved digestive functions; 3) enhanced nutritional value of prey; 4) antibacterial properties of the microalgae; and 5) improved water quality due to stripping of nitrogenous substances and increased oxygenation rates (Shaw *et al.*, 2006; Palmer *et al.*, 2007). Combinations of particular microalgae in larval cultures are generally considered beneficial to broaden the nutritional profile of live feeds. For example, Ferreira *et al*. (2008) tested the nutritional value of *I. galbana* for enriching rotifers, and found that it improved the nutritional value of the live feed used in larval fish cultures.

For cobia, Faulk and Holt (2005) have shown that the presence of live algae in recirculating rearing systems significantly improves larval survival. They showed that enriching rotifers and brine shrimp with live *I. galbana* or commercial preparations of dried algae such as Algamac™ (2000 and 3050) improved the growth and survival of cobia larvae. Faulk *et al.* (2007) also adopted a GWC technique for larval feeding of cobia in an indoor recirculating raceway system, and reported that cobia can be successfully cultured in this way from hatching through to at least 35 g without negatively affecting growth and survival. Gaumet *et al.* (2007) also concluded that the presence of live algae drastically improved cobia larval survival during their first week. On the other hand, one of the more recent studies by Benetti *et al.* (2008a) reported cobia larval rearing protocols which use probiotics, prophylaxis, artificial live-prey enrichment, and limited use of live microalgae. Those trials generated post-larvae (20–22 days post hatch) with survival rates estimated to be >50%, and demonstrated that the role of microalgae could potentially be substituted with commercially available ingredients in the future.

The present study has provided further evidence that cobia larvae can be successfully reared in GWCs, though in our case with low water exchange. Low water exchange conserves input ingredients and reduces heating and labour associated with screen management for cost effective production, and can also aid in biosecurity controls by reducing the potential for contaminants in exchange waters. But this approach needs to use high quality live feed cultures, and be measured so that water qualities are not compromised by metabolites such as ammonia which can be toxic under certain conditions. We did not detect any benefits from supplementing cultures with *I. galbana,* when *N. oculata* was the microalgal base. This suggests that nutritional benefits were not bestowed by the inclusion of this second complementary microalgal species, which is important considering the comparative ease that *N. oculata* can be cultured, and the much simplified approach of only culturing one microalgal species in hatchery operations. Since this work was also

conducted at much higher initial stocking densities (10–50 larvae L^{-1}) than those reported as optimal for this fish species elsewhere (5–10 larvae L^{-1}), the importance of adequate nutrition for fish health would have been high and the conditions challenging in our cultures.

Hitzfelder *et al.* (2006) studied the effects of initial stocking density on cobia larval survivorship, growth rates, and final density in a closed recirculating aquaculture system. That work revealed a general trend towards lower survivorship and growth at higher stocking densities (above 10 L^{-1}). Mortalities at higher densities were attributed to crowding stress which interfered with first feeding. Benetti *et al.* (2008a) later confirmed that the survival rates of fingerlings cultured in tanks that were initially stocked at lower densities (5 L^{-1}) were significantly higher. While we found no apparent reluctance of cobia to begin feeding at much higher densities (e.g. 30–50 L^{-1}) than in these studies in other countries, we did observe behavior several days later that indicated intense competition in some areas of the culture tanks. Our first trial also yielded lower overall survival at the higher density, although this was complicated by variable harvest ages.

In all three rearing trials conducted in the present study, the larvae were healthy and active on the day of stocking. In the first trial in 2008, the average length of newly-hatched larvae (3.32 mm) was slightly smaller than in the other trials, and that reported by Holt *et al.* (2007) (3.5–3.6 mm). On the day after hatching (larval-day 2), the larvae tended to become very mobile and formed dense schools at the water surface of the high density hatching tanks (see Figure 18 above). To address this tendency and the need for accurate stocking estimates, larval numbers were estimated and GWCs were stocked the day before (larval-day 1) when their distribution in the hatching tank could be spread more homogenously with additional aeration and physical mixing.

Excess larvae not stocked into GWCs were maintained in the hatching tanks without live feed for several days. During this time they appeared to remain healthy, drawing exclusively on their endogenous energy reserves. This agrees with Gaumet *et al.* (2007) who reported that cobia can be sustained by endogenous reserves for up to 12 days at 25 °C, and it further suggested that larval quality in our studies was high. Broodstock used in the present work (see Section 1) had shown no previous disease issues and had been prophylactically treated with formalin (150 ppm for 1 h), so unlike Tamaru *et al.* (1999) and Benetti *et al.* (2008a), fertilised eggs were not disinfected using formalin (100 ppm for 1 h). However, prior to stocking into GWCs the larvae were continuously flushed with UV sterilised seawater, which greatly reduced the potential for any disease transfer. Since our GWC methodology involves sterilising all other seawater that is used, larval cultures can be otherwise considered biosecure during normal operations.

On larval-day 3, the fish were active in the GWCs but were not aggregating greatly due to the evenly distributed aeration. By this stage their eyes had become pigmented, their mouths were open and their digestive systems had developed to a point where they appeared ready to feed. In our first trial, exogenous feeding (rotifers in their stomachs) was observed for the first time on their fourth day when they averaged 4.2–4.4 mm long, but in the second trial this occurred on their third day when they were 4.5–4.6 mm long. High numbers of larvae were clearly visible in all cultures during this period and by larval-day 4 they were starting to noticeably school at the surface and in particular areas near the edges of each tank. This was despite relatively high levels of turbulence applied in the cultures by the aeration. This highly energetic behavior seems to be a defining attribute which sets cobia apart from many other species at this stage. Holt *et al.* (2007) also noted that cobia larvae tend to stay near the surface for the first week, increasing their apparent crowding.

During larval-days 5 through to 7, the larvae were active and continued feeding well on rotifers, with continued good survival apparent in all tanks. It appeared at this stage that most larvae stocked had achieved their first exogenous feed, since all samples taken from cultures on GWC-day 5 showed clear signs of rotifers in their stomachs. Dense aggregations of larvae regularly formed at the water surface, and this was particularly prevalent in the more-highly stocked tanks of the first trial (GWCs 3 and 4). Larvae involved

in these aggregations even appeared to become stranded on the sides after jumping and sticking to the damp tank surface. Aeration was increased gradually on a daily basis to disperse the larger aggregations. Although a co-feeding regime of rotifers and brine shrimp was applied from GWC-day 7 (larval-day 6), fish samples taken showed that the majority of larvae did not start feeding on brine shrimp until larval-day 7 in the first trial, and until larval-day 8 in the second. Other reports on this aspect of cobia culture management include Benetti *et al.* (2008a) who described a similar overlapping of feeds at 6- and 9-days post hatch. That study found in two larval rearing trials that co-feeding lasted for a period of 2–3 days, and by 10-days post hatch the larvae had completely transitioned to brine shrimp.

Rotifer densities were less than optimal in our third trial, since they undesirably dropped to negligible levels on several occasion between GWC-days 3 and 8 (see Figure 23). This was caused by the larvae's intense feeding, and can generally be expected to create nutritional deficits and later mortalities in most fish species. It is therefore possible this may have led to some mortalities from larval-day 9, yet those cultures still produced a high proportion of healthy cobia fry which survived and grew well when stocked into the nursery ponds. Although the maintenance of adequate rotifer densities is a well-known critical aspect of larviculture that was at the forefront of our management arrangements, the prevailing high water temperatures at the time appeared to increase feeding rates of the cobia larvae to unprecedented levels. Under similar conditions of water temperatures over 28 °C, cobia larvae may be better cultured at lower densities and in larger tanks where greater buffers of available food can be maintained. For example, one 20 000 L parabolic tank that was stocked at 8–9 larvae L^{-1} in the present work, as a backup supply of cultured larvae (not reported in detail here), produced another 147 850 larvae, with outstanding results in terms of survival (approx. 85%) and growth up to larval-day 15 (9.61 mm), presumably through greater stability of feed availability and water qualities.

The apparent high-energy metabolism of cobia, relative to other species that we have previously studied, made us aware of the added potential for increased metabolic waste production and the associated build-up of total ammonia levels in low-water exchange cultures. Ammonia is known to be toxic to fish, especially when they are reared at high stocking densities (Ruyet *et al.*, 1997), and water quality can quickly deteriorate during larval rearing of fast growing species exhibiting high metabolic rates such as cobia. This coupled with the presumed oceanic existence of cobia larvae in the wild suggested that caution should be exercised in this regard. However, consistent with our previous findings for other species, we assumed that total ammonia would remain at non-toxic levels when adequate levels of water exchange were applied (see Palmer *et al.* 2007), so our schedule of typically-low water exchanges was applied. In the first trial, TAN appeared to be reaching our predetermined critical level of 2 mg L^{-1} on larval-day 13, and from this point the increasing daily water exchanges applied appeared to prevent further increases. Interestingly in this first trial, there were no orders-of-magnitude differences in TAN between tanks with markedly different fish densities. This implicated the algal cultures that were used, and in our second trial ammonia levels became excessive even earlier on larval-day 9, which stimulated a closer inspection of TAN levels in our algal culture protocols (Figure 14). Since Benetti *et al.* (2008a) had noted that relatively lower levels (e.g. those above 0.18 mg L^{-1}) in flow through tanks seemed likely to be detrimental to larval growth and survival of cobia, additional caution and greater levels of exchange were applied in our third trial. TAN was closely monitored for all tanks in the third trial (Table 9 above), where the upper levels of 2 mg L^{-1} did not appear to cause negative effects on the larvae.

The explorative additions of artificial weaning diets to the GWCs in the first trial after GWC-day 18 may have increased the levels of ammonia in tanks, but would also have increased and changed the nature of bacterial levels that were present. While this was part of our initial approach to try and wean the fish at an early stage and thus avoid the use of extensive nursery ponds, there are several difficulties faced by most larval fish species at this stage in intensive cultures. Although brine shrimp service the need for a larger live prey item

after rotifers in intensive fish cultures, they are not the natural prey item of most species for which they are applied, and thus have a range of nutritional deficiencies which operators seek to overcome with artificial boosting agents. Other possible causes for high mortalities at this stage include the increasing metabolic demands associated with growth during early developmental stages (Feeley *et al.*, 2007), the physiological stress caused by the switch from cutaneous to gill respiration (Benetti, 1992), and the problems associated with high density culture conditions, such as competition for feed and cannibalism (Faulk *et al.*, 2007).

Many of these difficulties are avoided with an approach of stocking larvae into nursery ponds soon after metamorphosis. This approach also takes into account the need to apply low-cost labour-saving options in countries like Australia. These economic considerations apply equally to larviculture, nursery and grow-out operations. The GWC methods applied in this study adhered to this approach by determining the simplest approach that can be applied to provide high quality seedstock of various stages. Although 5000 L tanks were used in this work, larger GWC tanks could easily be used to increase the process to larger industrial scales. The microalgae that was predominantly used in larvicultures (*N. oculata*) is easily mass cultured and appeared nutritionally competent to provide 13-day-old fry of a size and quality that are suitable for stocking into low maintenance plankton ponds. Such an approach uses the high quality natural feeds (e.g. copepods) that can easily be generated and which are most suitable for fish of that developmental stage.

Recommendations for further research

There are many areas which could be fruitfully explored in future cobia larviculture research. Some of these include the levels of tolerance for unionised ammonia by different stages of larvae, the appropriate live feed densities and optimal stages for rotifer and brine shrimp co-feeding and diet changes, and the effects that different temperatures have on feeding, metabolic rates, and the provision of adequate nutrition in commercial-scale production systems.

Refining protocols for green-water culture systems could potentially assist and sustain commercial operations by improving larval survival and increasing production capacities, leading to viable commercial cobia aquaculture in Queensland.

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5. Nursery pond management for cobia fingerling and juvenile production

P.J. Palmer, S. Wang, T. Borchert, L. Dutney and S. Nicholson

Abstract

Two seasons of nursery pond research at the Bribie Island Research Centre (BIRC) in 2008 and 2009 documented reliable and efficient fingerling production methods, and yielded several thousand cobia juveniles for industry trials. The relationship [wet weight = 2×10^{-7} x length$^{3.6514}$] best described cobia with lengths of up to 270 mm long and wet weights of up to 135 g grown in such ponds. The survival of fry (8–11 mm long) stocked into these ponds was thought to be higher in 2008 (60%) than in 2009 (20%) because of a lower stocking density and less in-pond cannibalism in the first year. The fish demonstrated rapid growth and were easily weaned onto artificial diets in the ponds. Prior to weaning the fish were shown to selectively feed on large copepods that were generated with a combined organic/inorganic pond-fertiliser regime.

Introduction

Nursery ponds are an integral part of fingerling production techniques for a wide range of fish species in Queensland and elsewhere in the world. Freshwater fish fingerlings in Australia have long been produced in earthen ponds from their first feeding stages. Species such as silver perch (*Bidyanus bidyanus*) and golden perch (*Macquaria ambigua*) are routinely stocked into fertilised ponds soon after the ponds are filled to take advantage of the early succession of zooplankton which occurs (Rowland, 2009). This approach is also undertaken in other countries for freshwater species such as striped bass (*Morone saxatilis*) (Geiger, 1983a). Zooplankton populations in previously prepared ponds have been shown to follow repeatable trends in population levels and successional species, which allow desirable points in this succession to be predicted for larval stocking and the availability of suitable feed organisms and water qualities (Geiger, 1983b).

This approach has also been shown successful for several marine fish in brackish-water ponds. In the early 1990s, techniques developed in Texas USA (McCarty *et al.*, 1986; Rutledge, 1988) for the red drum (*Sciaenops ocellatus*) were successfully applied to barramundi (*Lates calcarifer*) in north Queensland (Rutledge and Rimmer, 1991). Compared with more intensive tank-based larval rearing protocols, the pond-based methods offered faster growth and lower levels of labour. Despite some production-related difficulties (e.g. ectogenic meromixis: Rimmer, 1993) many commercial barramundi producers adopted this approach in the 1990's over intensive hatchery production.

The reliability of pond-based fingerling production has also been shown to be enhanced for several freshwater and marine species by providing some initial laboratory-based feeding. Arumugam (1986) showed this for golden perch, and the work by Barlow *et al.* (1993) showed how dramatic changes in feeding strategies and behaviour in species like barramundi can affect growth and survival in ponds. Research with barramundi and several other marine fish species at BIRC has suggested that growing larvae through to metamorphosis in hatchery-based cultures prior to stocking into nursery ponds is a particularly reliable fingerling production strategy (Palmer *et al.*, 2007). This was the preferred approach for fingerling production efforts in the Maroochy River stocking program conducted from 1995, where several hundred thousand sand whiting (*Sillago ciliata*) and dusky flathead (*Platycephalus fuscus*) fingerlings were required on demand (Palmer *et al.*, 2000). Pond fertilisation strategies developed during that work (McGuren and Palmer, 1997) have provided a guide to ongoing work in this area, and to this research with cobia.

To investigate the potential for use of brackish-water nursery ponds in cobia fingerling production, cobia fry were stocked into previously prepared ponds at BIRC in 2008 and 2009. The pond fertilisation and management techniques that were used (and which are documented herein) were very successful at

producing high quality fingerlings and juveniles. The pond zooplankton populations which prevailed, and the coinciding gut contents of the growing cobia were studied in 2009, to provide a better understanding of this species in such systems and to help optimise future seed production efforts.

Materials and methods

Facilities at the Bribie Island Research Centre

The larger in-ground ponds at BIRC were used for these cobia nursery experiments. Each of these ponds has an area of approx. 0.16 ha and volume of 3.2 mega litres (40 m x 40 m x 2 m deep). They are lined with a high density polyethylene (HDPE) plastic liner and have screened monk drains for water discharge. Supply seawater was pumped from an offshore (450 m) intake on the Bribie Island surf beach, and this was filtered with 300 μm sock-filters as it entered each pond. All ponds were protected by overhead bird netting.

Pond preparation and management

One pond in 2008 (Pond 2) and three ponds in 2009 (Ponds 2, 3 and 4) were used for this work. Pond filling commenced the day after it was confirmed that cobia embryos from a recent spawn had successfully hatched. In 2008 this was on 28 February, and in 2009 it was on 25 January. Pond preparation and management was similar to that reported by McGuren and Palmer (1997) for other species. Briefly, 2 bags of dolomite (24 kg bag^{-1}) were spread evenly on the bottom of each pond before filling. This application rate (300 kg ha^{-1}) has been found to help minimise pH shifts when the initial algal blooms develop. Each pond was then filled to about 80% of its capacity over one or two days. This ensured that there was room for dilution of the algal bloom without flushing zooplankton from the system (viz. by topping the pond up without overtopping). One paddle wheel (1 hp) was operated continuously in each pond to provide circulation and mixing. Organic and inorganic fertilisers were applied according to the details below:

Nursery pond fertilisation regime

Initial dose (ideally during pond fill)

Organic fertiliser:	total of 20 kg pond^{-1} (125 kg ha^{-1}), consisting of 50% (10 kg) course material such as lucerne chaff (62.5 kg ha^{-1}) and 50% (10 kg) fine material such as rice pollard (62.5 kg ha^{-1}).
Inorganic fertiliser:	total of 12 kg pond^{-1} (75 kg ha^{-1}), consisting of 9.5% (1.14 kg) mono-ammonium phosphate (7 kg ha^{-1}), 47.5% (5.7 kg) urea (36 kg ha^{-1}) and 43% (5.16 kg) potassium nitrate (32 kg ha^{-1}).

Follow-up dose (twice weekly where necessary)

Organic fertiliser:	total of 4 kg pond^{-1} (25 kg ha^{-1}), consisting of 50% (2 kg) lucerne chaff and 50% (2 kg) rice pollard.

The aim of this pond fertilisation regime was to achieve and maintain a Secchi depth water transparency of about 50 cm. Phytoplankton densities of this nature are generally known to encourage a high abundance of naturally occurring copepods, but higher phytoplankton densities can cause increasing pH and biological oxygen demands, which represent a risk to larval and juvenile fish. Accordingly, follow-up fertiliser additions were suspended when Secchi depth readings were less than 50 cm, and the addition of seawater was implemented if Secchi readings reached 30 cm. When Secchi depths were greater than 50 cm (typical of lower algal densities), the abovementioned bi-weekly follow-up fertiliser additions were implemented. When algal blooms subsided leading to clear pond waters (bottom visible), artificial dye (ocean blue) was used at

0.5 times the manufacturers application rate guidelines, to minimise macro-algae growth in the pond. Temperature, dissolved oxygen (DO), salinity, pH and Secchi depths were assessed twice daily (early morning and mid-afternoon) for each pond.

Fry stocking procedures

In 2008, 14-day-posthatch fry were stocked on 12 March, and in 2009 12-day-posthatch fry were stocked on 4 February. To minimise the stress during the transfer to the nursery ponds, harvest procedures involved scooping fry from the water surface of the 5000 L larval rearing tanks. These were concentrated in 500 L conical tanks containing 300 L of larval culture water. Volumetric samples (100 mL) were taken from these concentrates during vigorous mixing to estimate the numbers of fry harvested and stocked. Fry were transported over a short distance (100 m) to the nursery ponds in half-full 20 L buckets. These buckets were slowly filled to the brim with pond water over 5–10 minutes to slowly acclimatise the fry to the water qualities in each particular pond. The fry were then gently released into the open water of each respective nursery pond.

A relatively low stocking rate was used in 2008 (4.4 m^{-2}) compared with that used in 2009 (34–36 m^{-2}). In 2008, the fry were an average of 9–11 mm long when 7 000 were stocked into the nursery pond. In 2009, the fry were 8–9 mm long when 57 000, 58 000, and 54 000 were stocked into ponds 2, 3 and 4, respectively.

Feeding artificial diets

In 2008, due to the relatively low stocking density of fry and a reasonably good copepod density in the pond, no artificial diets were added to the pond for the first week after stocking fry. After one week, broadcast feeding twice per day (morning and afternoon) was initiated around the entire periphery of the pond, and two auto-belt feeders were deployed on opposite sides of the pond to continuously add feed during daylight hours. Ridley's "barra dust" was used in equal proportions for both feeding strategies with a total of 2 kg added per day. The daily feeding amount was gradually increased about one week later, based on an increase in observed feeding responses. Soon after this the sequential range of Ridley native fish crumbles were offered to apparent satiation twice daily.

In 2009, the fish stocking densities were much higher than in 2008, so artificial feed was offered at an earlier stage and at higher amounts. Twice daily broadcast feeding (total of 1 kg barra dust per day) started on the day after stocking fry, and the next day four auto feeders were also deployed offering an additional 2 kg of dust per day to each pond. On the fifth day post stocking, a build-up of uneaten feed was becoming apparent under the feeders, so auto-feeder amounts were halved for the next few days. From 10-days post stocking, a total of 3 kg was fed daily to each pond with an equal split between broadcast and belt feeding. As in the previous year, crumble diets made up increasing percentages of the feed offered as soon as feeding responses were observed. This began with a 50% dust + 50% crumble No.1 mix from 6-days post stocking, whereby the dust inclusion ceased completely 11 days later (29 days after hatch).

Zooplankton monitoring

During 2008, the abundance of the zooplankton was monitored qualitatively by irregularly visually observing samples taken using a 63 μm oblique-tow-net. In 2009, water samples were taken twice weekly from each pond to quantitatively assess the abundance of zooplankton. This was undertaken over a 5-week period starting 4 days after the ponds were filled. The sampling procedure involved pumping a known volume (100 L) of pond water from the mid-water column of each pond and collecting particles filtered from this sample with a 63 μm nylon screen. The pumping apparatus was a submersible flexible impeller pump which was lowered on a boom to the preferred water depth (after Farquhar and Geiger, 1984). This pump was controlled with a shore-based switch to ensure the 100 L collection tub did not overflow. The water sample was taken about 15 m downstream and within the mixed flow of the pond's paddle wheel. This ensured that

the water column was well mixed to avoid the stratification effects that are typically caused by the diurnal vertical migration of motile plankton. Particles filtered from the water sample were collected in a small jar (100 mL) and re-suspended in a known volume (1 L) of water for volumetric counts. Freshwater was used in this re-suspension to slow the motility of organisms for enumeration procedures. Enumeration involved taking three 1 mL subsamples from the well-mixed re-suspension and counting: 1) the number of copepods (nauplii + copepodites + adults); and 2) the number of other zooplankton organisms which may have been food for the cobia fry. These “other zooplankton” were identified whenever possible. Additionally, to assess the relative grazing pressures on different sized copepods in each pond, 20 to 50 randomly selected copepods in each pond sample were measured in total length.

Cobia gut content analyses

In association with pond plankton studies in 2009, 10 fish from each pond were sampled each week to assess their gut contents and compare the sizes of copepods inside their stomachs with those present in the ponds. This was undertaken for three weeks when it was revealed that the sampled fish had only eaten artificial diets and thus were fully weaned. The fish sampled were euthanised with an overdose of Aqui-S™, measured (total body length to the nearest 0.1 mm) and weighed (to the nearest 0.01 g). Five randomly selected fish from each euthanised sample were dissected to obtain the entire gut contents for analysis. Twenty to 50 intact copepods from each gut content were separated and measured (total length) as with the pond plankton samples. Mean copepod sizes in fish’s stomachs and in corresponding ponds were compared using repeated measures ANOVA.

Results

Survival and growth of cobia fry

Production in 2008

In 2008 the nursery pond was drain-harvested on the 13 May, 62 days after stocking (at 4.4 m^{-2}) with fry. Harvest estimates were calculated based on the cumulative tare weights of buckets containing 100–150 fish. A total of 47 kg of fish was harvested. Four sample buckets containing a total of 5546 g of fish yielded 492 fish, giving an average fish weight of 11.272 g. This provided an estimate of 4186 fish harvested, giving a survival rate of 59.8%.

Production in 2009

Progressive harvests of nursery ponds were undertaken in 2009 (see Table 10). This involved scooping juveniles from the upper water column with hand nets after they were attracted to the sides of the ponds with feed additions. When the effectiveness of this procedure eventually waned the pond was drain-harvested. On the whole, survival was considerably reduced by cannibalism during the protracted culture period, which was necessitated by delays in supply dates for industry growth trials. However it was then our preference to supply juveniles for the industry trials as soon as practically possible after harvest from the nursery ponds, to ensure that fish condition was not adversely affected by a protracted tank-holding period.

Table 10 – Nursery pond production of cobia at BIRC in 2009

Pond	2	3	4
Stocking density	35.6 fish m^{-2}	36.3 fish m^{-2}	33.8 fish m^{-2}
Harvest dates	26 March; 28, 29, 30 April; 1 May	19, 20, 26, 27 March	17, 18 March
Time in pond	50–86 days	43–51 days	41–42 days
Overall survival	18.5%	12.9%	20.1%

The overall survival was also lower in Pond 3 due to a mortality event that occurred when the pond was allowed to drain overnight in preparation for a drain harvest the following day. This was presumably related to low oxygen conditions, since no other clinical signs of disease could be identified. Adding those fish which died to the total production figure gave an adjusted survival for that pond of 19.5%, which was similar to that achieved in the other two ponds.

Figures 25 and 26 demonstrate the steady growth of cobia in these nursery ponds in terms of total body lengths and wet weights, respectively. During the first two weeks of growth in the nursery ponds the fish were not weighed, due to perceived random sampling difficulties and their very low wet weights. However from the third week in the ponds, they were easily captured with hand nets and they demonstrated an exponential increase in body weights. From the fifth week in the pond they maintained a mean wet weight increase of 10–20 g per week.

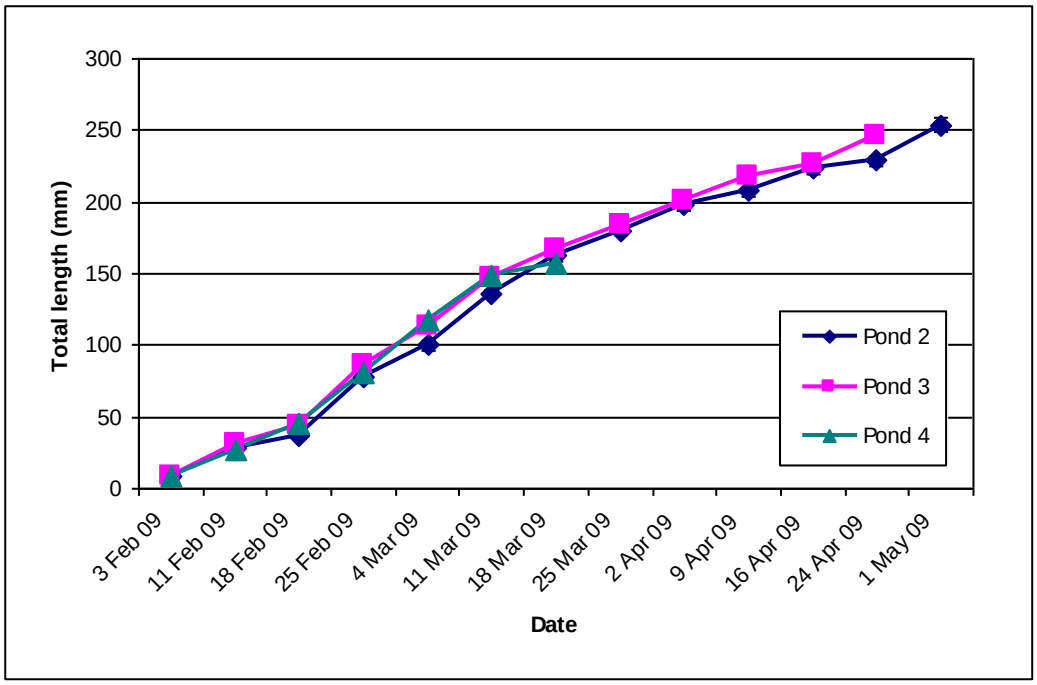

Figure 25 – Mean (± se) total lengths of cobia grown in different nursery ponds at BIRC in 2009

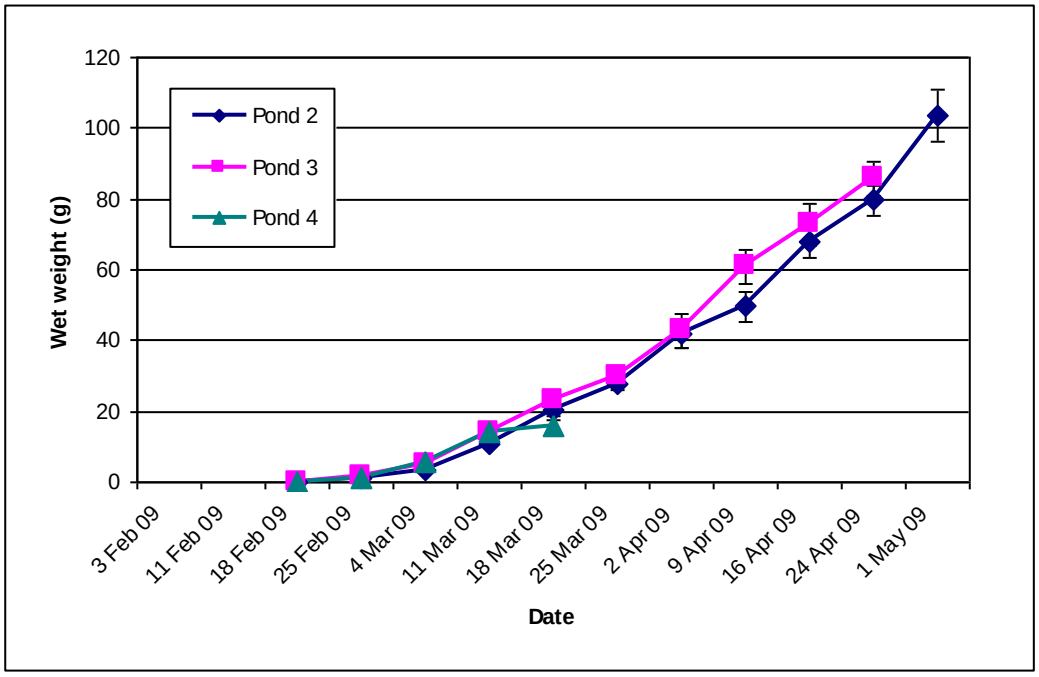

Figure 26 – Mean (± se) wet weights of cobia grown in different nursery ponds at BIRC in 2009

Figure 27 shows individual total body lengths and corresponding wet weights of cobia fingerlings grown in the three different nursery ponds at BIRC in 2009. Even though the duration of culture and size of fish varied for each pond, relatively similar length-weight relationships were apparent. The Pond 3 length-weight relationship (wet weight = 2 x 10^{-7} x length$^{3.6514}$) had the best fit for fish up to 270 mm long and with wet weights of up to 135 g.

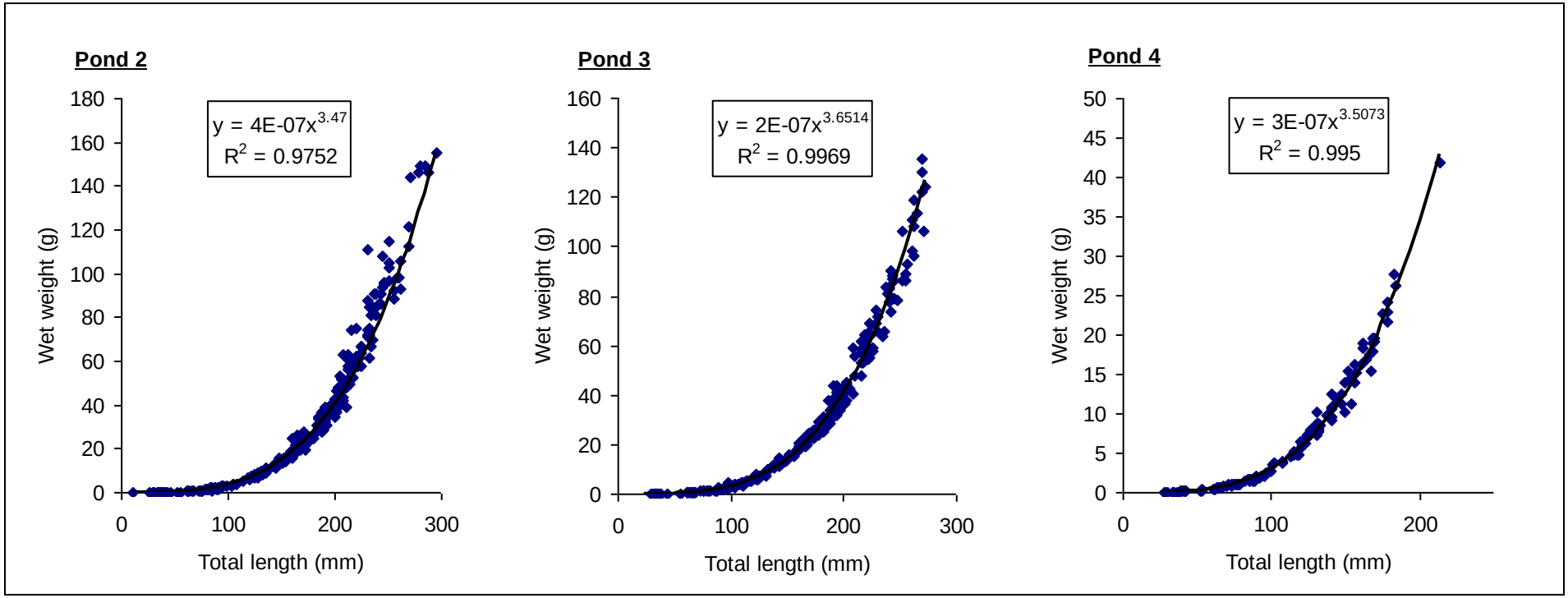

Figure 27 – Total body length verses wet weight relationships for cobia juveniles grown in three different nursery ponds at BIRC in 2009

Zooplankton in ponds

The majority of zooplankton that occurred in the nursery ponds were copepods (Figure 28). Those categorised as “other zooplankton” were mainly identified as molluscan larvae. In the first three weeks after filling the pond there were small numbers of bivalve larvae observed, and in the last two weeks monitored, densities of gastropod larvae increased significantly. These gastropod larvae were probably from the spawning of sea hares (*Stylocheilus striatus*). Although there was some variation in copepod densities between ponds, all ponds peaked in the second week after fill. Also, these peaks in copepod densities tended to coincide with the lowest Secchi depth readings. Pond 4 had the most prolonged phytoplankton and copepod blooms.

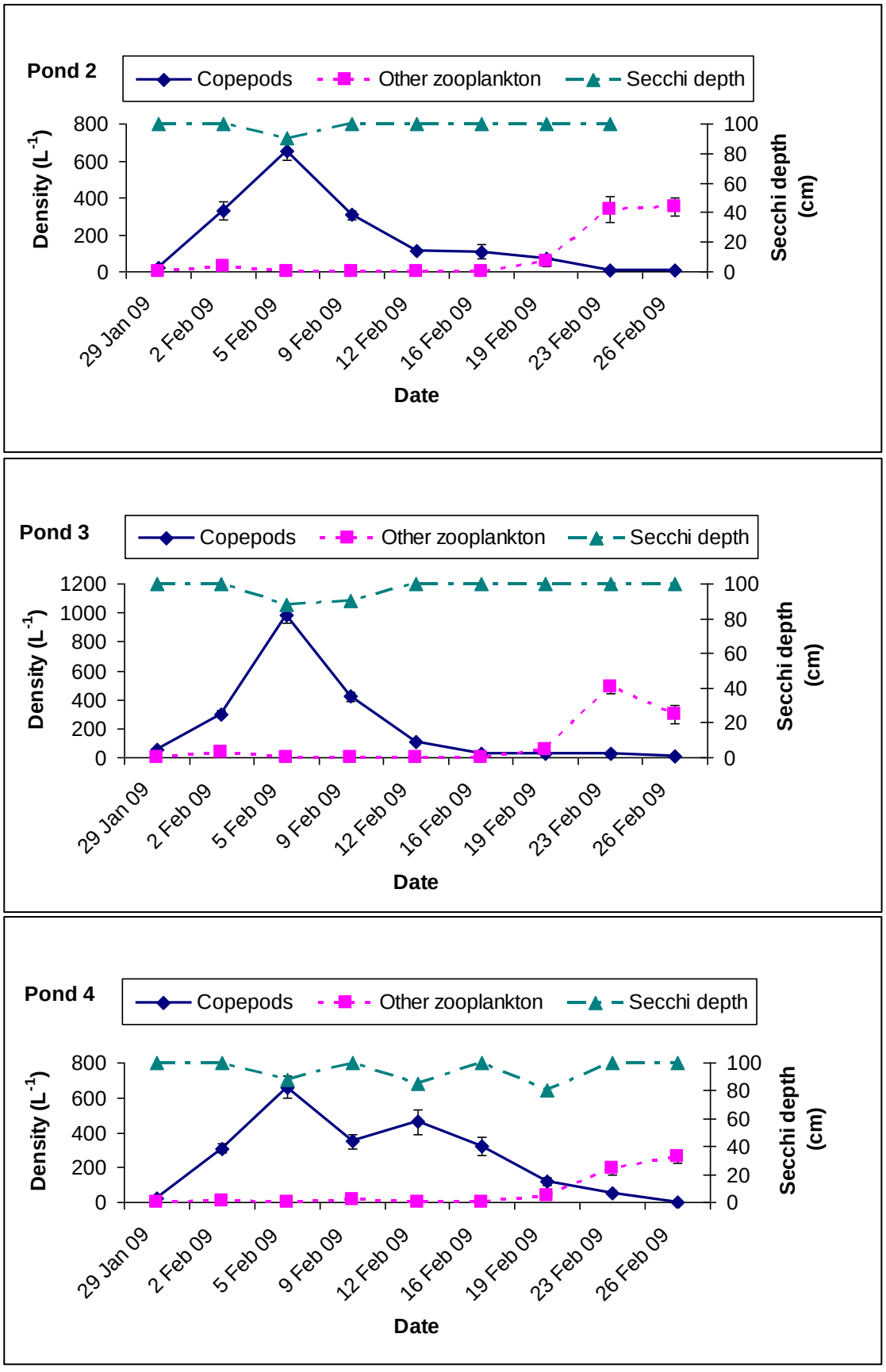

Figure 28 – Zooplankton densities and Secchi depths[4] in nursery ponds during the rearing of cobia at BIRC in 2009

[4] Note that Secchi depth readings had a maximum of 100 mm due to pond depths.

Cobia gut content analyses

Copepods were the only zooplankton type that could be identified in the excised gut contents of fish sampled from each pond during the first fortnight after stocking. Of particular interest was the distinct lack of artificial diet in any gut content analysis during their first week in the pond, despite the addition of liberal amounts of appropriately sized artificial feed during that time. After three weeks in the nursery ponds, very few copepods could be detected amongst the ingested artificial feed. The sizes of copepods in the stomachs of fish during this relatively short documented period of zooplankton feeding were larger ($P<0.05$) than those in the corresponding ponds (Table 11).

Table 11 – Comparisons of copepod sizes in the stomachs of cobia juveniles and their respective nursery ponds at BIRC in 2009

Sample date	Days after pond filled	Fish age (days after hatch)	Days after stocking pond with fry	Mean (± se) length (mm) of fish			Mean (± se) length (μm) of copepods in gut samples			Mean (± se) length (μm) of copepods in ponds		
				Pond 2	Pond 3	Pond 4	Pond 2	Pond 3	Pond 4	Pond 2	Pond 3	Pond 4
12 Feb 2009	28	19	8	30 (± 1.9)	33 (± 1.4)	33 (± 2.4)	654 (± 16.8)	663 (± 16.0)	680 (± 15.9)	566 (± 26.3)	571 (± 22.9)	385 (± 29.7)
18 Feb 2009	34	25	14	49 (± 5.7)	43 (± 3.7)	50 (± 5.2)	748 (± 35.1)	Artificial diet only	727 (± 18.8)	570 (± 36.1)	417 (± 35.4)	724 (± 31.7)

Figure 29 provides a picture of the excised stomach contents of one of the fish sampled on the 12 February 2009. The compacted mass of copepods could easily be teased apart to reveal undigested material in the foregut. Further down the digestive tract the material was less distinct making accurate enumeration impossible.

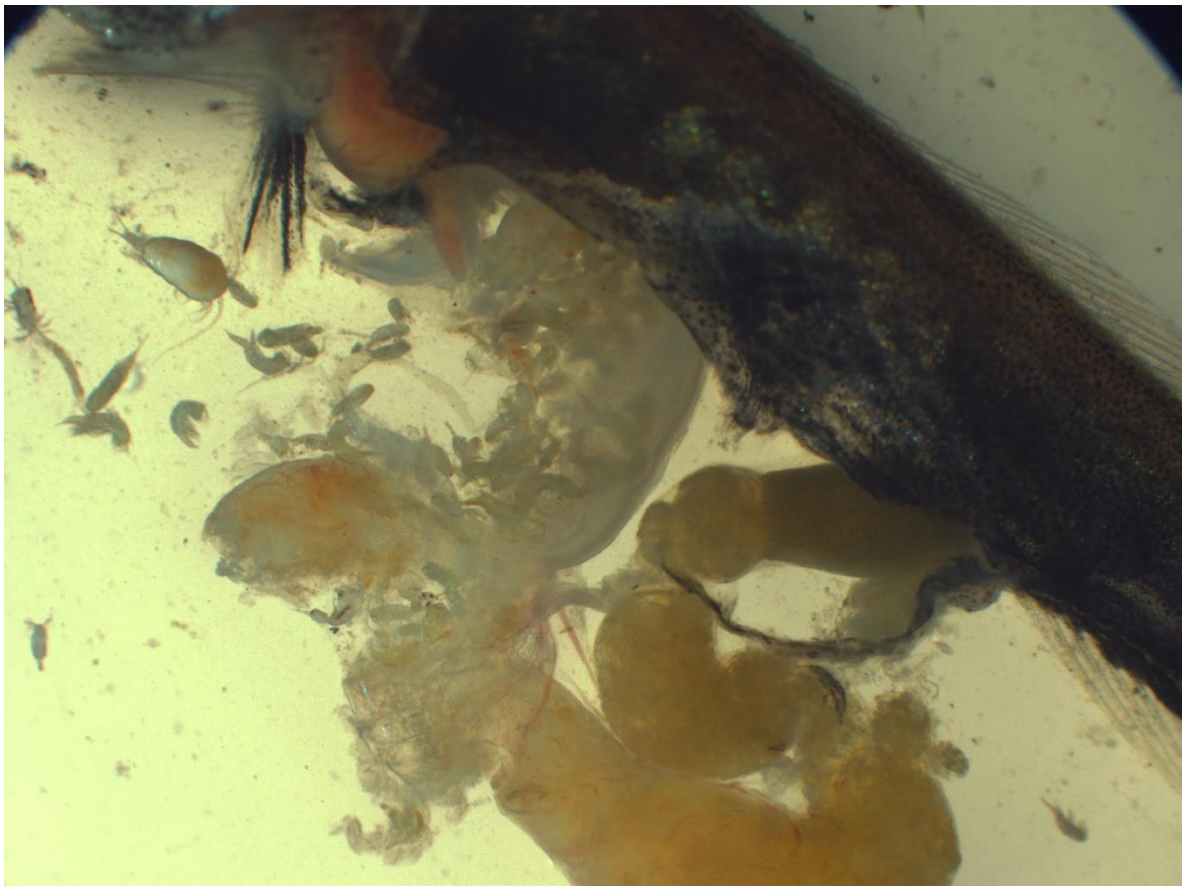

Figure 29 – Excised stomach contents of a cobia juvenile after growing for one week in a nursery pond at BIRC

Discussion

The stocking of cobia fry into nursery ponds at BIRC in 2008 and 2009 was timed to coincide with the maximum densities of copepods in the ponds. All three ponds monitored in 2009 reached maximum recorded densities in excess of 650 copepods L^{-1} soon (1 day) after stocking. Although these zooplankton blooms were relatively short lived, they provided the necessary nutrition to allow reasonably high survival and growth. Survival was higher in 2008 (60%) than in 2009 (20%). This was probably due to two factors including the lower stocking density used in 2008 (4.4 m^{-2} versus 34–36 m^{-2} in 2009), and because the fish were harvested at a much earlier stage in 2008, before the cannibalism which was evident in 2009 had taken its toll. Nevertheless, the levels of survival achieved in 2009, when considered against the larger and more advanced juveniles produced (e.g. 11 g versus 100 g), and the very low levels of labour required to produce them to this larger size, provide scope for industrial considerations regarding commercial production strategies.

This overall production strategy, whereby larvae are intensively grown to a size of 8–10 mm long and then stocked into prepared plankton ponds, provides a reliable and cost-effective fingerling production method for cobia. In several fish species so far studied at BIRC (see Palmer *et al.* 2007), live brine shrimp-based diets start to become problematic when the fry reach a size of about 8 mm. At this stage, on-growing and boosting live brine shrimp is necessary along with early weaning onto specialised artificial diets. If these are not nutritionally suitable this can risk the health and vigour of larvae and cause significant subsequent losses. To date, a controlled hatchery phase followed by an

outdoor nursery pond phase has provided better more reliable results, and this is especially important when subsequent programs of research and development rely on fingerling production successes.

As fish larvae approach metamorphosis, they enter a period of rapid growth and high nutritional demands. Any delays in feeding on demand, or less than optimal availabilities or qualities of feeds, can quickly lead to nutritional deficiencies which invariably have adverse and irretrievable effects. Natural processes involving a bloom of zooplankton productivity can address this high nutritional demand without an in depth knowledge of the species' larval and juvenile requirements. This may be particularly important for cobia which is by far the most vigorous species we have so far dealt with in the hatchery environment. Several other factors are also thought to contribute to this successful nursery-pond approach including the reduction of fry densities so that competition is minimised, maintaining more stable water qualities, and providing more favourable environmental conditions which reduces stressors and increases visual cues for vigorous feeding and growth.

The average sizes of copepods in the stomachs of cobia were larger than the average sizes in their corresponding ponds, suggesting that selective feeding on the larger copepods was occurring. This gut content work may have also highlighted the most appropriate size for weaning and the densities of copepods that are necessary for sufficient natural feeding in this species. No cobia were found to have artificial feed in their stomachs until two weeks post-stocking. This was despite liberal additions of fine particle fish-meal based feeds to the ponds on a regular and continuous basis. The smallest cobia found to be feeding on the artificial diet had an average length of 43 mm (Pond 3–25 days after hatch). At that stage (18 February), slightly larger juveniles (49–50 mm average lengths) in the two other ponds that still had reasonable densities of copepods (approx. 100 L^{-1}), did not show any signs of having ingested artificial feeds. It is therefore likely that the lower copepod densities (in the order of 25 L^{-1}) that occurred in Pond 3 at that time resulted in an increased propensity for those cobia to wean. Much higher copepod densities were achieved in ponds in these cobia trials, than in similar ponds in previous work with other species at BIRC (see McGuren and Palmer, 1997; Palmer *et al.*, 2000). Also, cobia stocking densities were much lower due to their expected higher demand for food compared with some of smaller inshore species previously studied (e.g. sand whiting and dusky flathead).

In general, when stocking plankton ponds with fish larvae or fry, it is very useful to have data on the successional time periods and types of zooplankton resources that are likely to be available. In most situations this can only be derived by specifically studying the pond system in question, because many different physical and biological factors can affect pond ecosystem dynamics. Geiger (1983a, b) established the effects of zooplankton composition and density on striped bass survival and growth, and proposed a pond management strategy which used combinations of organic and inorganic fertilisers and zooplankton inoculants. That work suggested that efforts should seek to maximise crustacean zooplankton feeds for 2 weeks before and 2 weeks after fish stocking. Subsequent work (Geiger *et al.*, 1985) also found that adult copepods were the most predominant prey item for striped bass.

Following similar work by Rutledge and Rimmer (1991) with barramundi in saltwater rearing ponds, extensive pond culture has been widely used for a variety of species in Queensland. Unlike that work in North Queensland, which advocated the stocking of first feed larvae, research at BIRC has focussed on stocking more advanced larvae (fry) to avoid environmental problems caused by such factors as heavy rainfall, which may cause poor swim-bladder inflation or create unsuitable salinity and temperature gradients in the water column (ectogenic meromixis) (Rimmer, 1993). The competency of larvae also improves with age such that after several days they are generally much

more able to forage for natural prey items and find refuges from potential predators. For example, Arumugam (1986) demonstrated that a 3-day laboratory-based feeding period was sufficient to increase the survival of golden perch stocked into nursery ponds.

The behaviour of cobia juveniles in the nursery ponds at BIRC were a particular feature of this species' production. They were always easily seen in the water column allowing regular survival confirmations, although an educated eye was necessary to judge the scale of the population. Soon after stocking they tended to float with the current generated by paddle wheels, without apparent purpose or need to reposition themselves at depth. This was apparently their typical zooplanktivorous feeding mode. After one week they began congregating in the downstream vicinity of each auto-feeder station, although as previously indicated, it was not until another week later that juveniles could be found with artificial feed in their stomachs. Their change in behaviour from a roving zooplanktivore to a more predatory existence (waiting avidly for addition of pellet diets) seemed to begin when the fish were about 40 mm long. This is much larger than other higher order predators like barramundi which undergo this behavioural change at lengths of 16–18 mm (Barlow *et al.*, 1993).

Another behavioural feature of cobia fingerlings was their vigorous feeding at sizes greater than 150 mm. This availed a large proportion of the fish in each pond to be harvested with hand nets after repeatedly attracting the fish to the sides of the pond with additions of artificial feed. Although this method of harvest was laborious, it allowed for easy distribution of stock and to conveniently spread the load of harvest and handling over several days, which helped reduce the risks of excessive oxygen demand in fish transporters and short-term holding tanks.

Recommendations for further research

One of the most important factors identified in this work was the very rapid growth that this species demonstrates throughout its life cycle. In practical terms this presents logistic hurdles that need to be addressed well ahead of time. Harvest times on each occasion were later than preferred which created difficulties in the size of transportation vessels that were necessary to manage the rapidly increasing biological load of larger fish. It is therefore recommended that future operations of this nature pay particular attention to the growth rates demonstrated in this work and seek to harvest and move stock to grow-out facilities at appropriate stages.

The oxygen demand of this species also seems to climb significantly soon after harvest, and at different times when they may be stressed. This caused several losses of recently harvested stock in the program, and could be the subject of future investigations with focus on the transportation and movement of stock via various means.

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6. Health and growth of cobia in tanks and ponds

Luke Dutney, Trevor Borchert, Steve Nicholson

Abstract

This study examined the feasibility of producing cobia in prawn production ponds in Queensland and the effect of geographic location on production efficiency. Commercial grow-out trials were conducted in ponds on four commercial prawn farms that were located in two distinct geographic zones (north and south).

Cobia grew to 4–5 kg in north Queensland (specific growth rate (SGR) = 1.40–1.42) compared to 2–3 kg in south Queensland (SGR = 1.21–1.27) in the first year of growth. Production yields ranged from 1.8 to 14.5 tonnes per hectare per year, with higher yields obtained in north Queensland. Food conversion ratios ranged from 1.8 to 2.3:1. Faster growth rates obtained in the northern zone were attributed to water temperatures remaining consistently 5 °C higher than the southern zone through winter. This allowed for maximised growth in the northern zone for the most part of the year. Low water temperature in southern Queensland was implicated in a reduced growth rates and a moderate mortality event at the Bribie Island Research Centre (BIRC).

The results of the commercial trial demonstrate the technical feasibility of cobia production in commercial prawn ponds in Queensland. Production of cobia in prawn ponds is well suited to a tropical environment; whereas there is a need for temperature control in subtropical regions during the winter period.

Introduction

In 2007 the Department of Employment, Economic Development and Innovation—now the Department of Agriculture and Fisheries (DAF)—began building a program designed to assist industry to investigate the opportunity for cobia production by Queensland aquaculture producers, primarily as a diversification option for established operators. Gold Coast Marine Aquaculture (GCMA), a highly successful prawn farming operation recognised the desirable biological traits and market appeal to propose cobia as a potential diversification option. Their intention was to trial the production of cobia in prawn grow-out ponds.

Following the successful development of spawning and larval rearing protocols at BIRC, GCMA were supplied with larvae and juvenile cobia to facilitate commercial evaluation of grow-out techniques and market appraisal for the species. Indications of success with initial trials at GCMA led to the development of a larger collaborative project with other industry members in Queensland.

The aim of the second stage of the program was to:

- Assist farmers to conduct preliminary investigations on farms under commercial conditions.
- Benchmark, in broad terms, the performance of cobia under a range of on-farm operating conditions.

The wider collaborative effort set a platform to examine cobia production under a variety of conditions, using differing infrastructure and production techniques, which were distributed over distinct geographic regions. The project aimed to implement an industry-wide program of research to stimulate information sharing and cooperation, and an expansion of research and development with

this species in the future. In making these arrangements, DAF supplied weaned fingerlings to aquaculture operators under individual research contracts.

Global production of cobia has focused primarily on inshore cage culture (Liao *et al.*, 2004; Benetti *et al.*, 2007; Schwarz and Svennevig, 2009); whereas prawn production ponds, being relatively shallow (<2 m) earthen ponds with a relatively large surface area (typically 1–2 ha), present a novel system for producing cobia. This report details the combined findings from on-farm trials conducted to examine the feasibility of producing cobia in commercial prawn production ponds.

Materials and methods

Industry participants

A brief description of the industry participants and the production methods employed:

- Gold Coast Marine Aquaculture – a prawn farm located in South East Queensland. Temperature controlled, lined raceway nursery facilities were used to overwinter juveniles. Grow-out was conducted in one 1 ha prawn grow-out pond. 4400 fish were stocked.
- Pacific Reef Fisheries – a prawn farm located in North Queensland. Grow-out was conducted in two 1 ha ponds. 4000 fish were stocked into each pond.
- Coral Sea Farm – a prawn farm located in North Queensland. Grow-out was conducted in two 0.5 ha ponds. 2400 fish were stocked into each pond.
- Creel Seafood – a prawn farm located in South East Queensland. Grow-out was conducted in one 1 ha pond. 1000 fish were stocked.
- BIRC – fish were held on site at BIRC to allow observation of growth and behaviour under trial conditions. 1100 and 500 fish were held in two 16 000 L outdoor concrete raceways supplied with flow through seawater.

The grow-out ponds were prepared in a similar manner to that used for prawn production. Aeration was supplied using paddle wheels varying from 2–20 hp/ha. Aerators were coordinated to produce circular current within the pond to ensure water mixing and movement and facilitate waste accumulation in the centre of the pond (Figure 30 and 31).

Figure 30 – Feeding cobia stocked in a prawn production pond located in southern Queensland

Figure 31 – Sampling cobia from a prawn production pond in northern Queensland

Live transportation

Juvenile cobia were transported to the respective facilities using a commercial fish transport truck fitted with six 2000 L tanks, each supplied with pure oxygen through ceramic diffusers. Stocking densities varied depending on the size of the fish and the duration of transit. Dissolved oxygen was maintained above 10 ppm and sodium bicarbonate was used to maintain pH above 7.

Upon arrival at the stocking location the fish were acclimatised to pond conditions by flushing the transport tanks with pond water. Acclimation times varied from 1 to 4 hours depending on the variation between the water quality parameters of the transport tanks and the grow-out ponds.

Production statistics

Periodic weight measurements were recorded depending on management practices for each operation. Harvesting began when fish reached an average weight of 2 kg at which time harvest data was used to provide growth measurement.

Specific growth rate (SGR) was calculated as follows (after Priestly *et al.*, 2006):

$$\text{SGR (\%/day)} = \frac{\ln (W_f) - \ln (W_i)}{\text{time (days)}} \times 100$$

Where W_f and W_i were the final and initial wet weights of the fish, respectively.

Husbandry practices varied between farms; however the general practice was to feed to apparent satiety twice daily with floating pellets. Feed conversion ratio (FCR) was calculated as biomass increase divided by the total amount of food added. It did not account for any fish mortality.

Zoning

For the purpose of demonstrating the differences in geographic location farms were grouped in to northern and southern zones. Figure 32 shows the approximate placement in each of the zones.

Figure 32 – Locations of northern and southern trial farms

Figure 33 shows a sample of the cobia produced at one farm.

Figure 33 – Sampling cobia during grow-out

Results

Live transportation

Fish were transported to all facilities in good health with negligible (<1%) or no mortality. Fish were observed feeding actively within 24 hours post stocking. Conditions in the live transportation tanks are provided in Table 12.

Table 12 – Cobia live transporter conditions during the course of the study

Fish size (g)	Density (kg/m³)	Duration (hours)
22	15	15
22	6.25	20
85	17.5	2
110	23.5	18.5
120	33.3	2.5

Production statistics

Figure 34 shows the average weight of cobia produced on two farms in each of the zones during the first year post stocking. Higher growth rates were obtained on farms located in northern Queensland with average body weight reaching 4–6 kg compared with 2–3 kg on southern farms. The tendency for the growth data to plateau towards the end of the growing period is a function of selectively harvesting the larger fish rather than an indication of reduced growth rates.

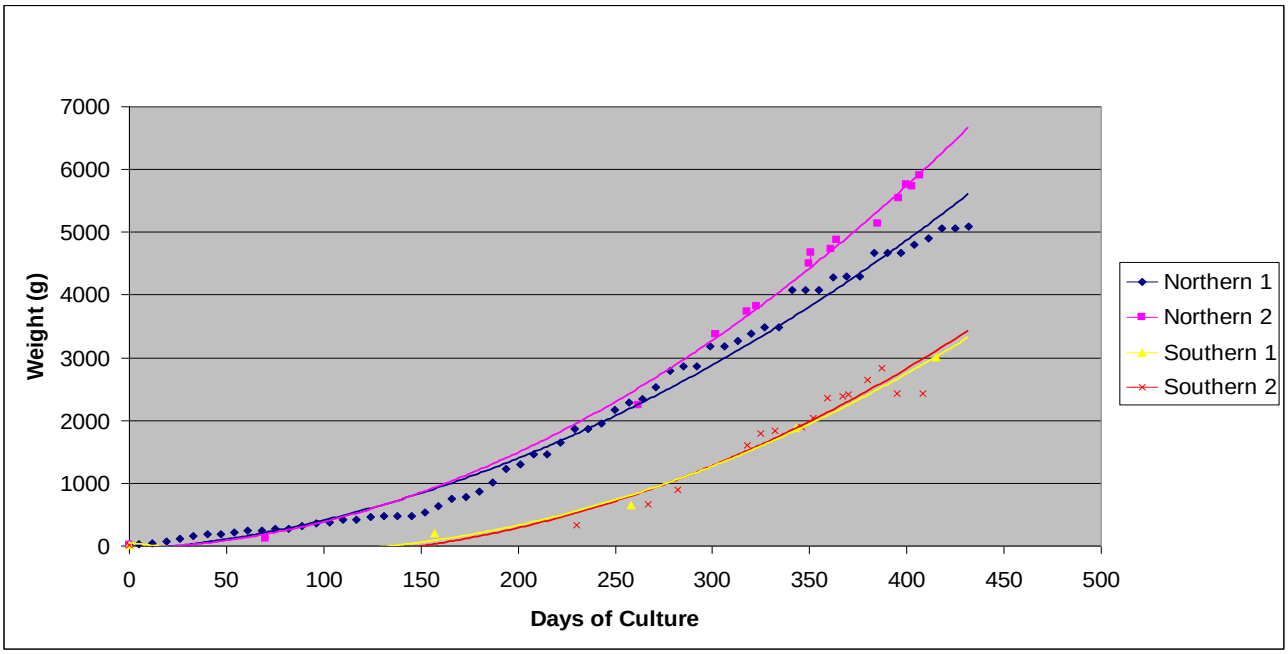

Figure 34 – Growth rate of cobia during the production period. (Second-order polynomial trend lines provided. Growth equation and r² values provided in Table 13).

Specific growth rates, measured as per cent increase in body weight per day, and production yield, measured as tonnes per hectare per year (t/ha/yr), were higher in the northern zone. Survival was excellent in the northern zone and variable in the southern zone (Table 13).

Table 13 – Production statistics for cobia produced in northern and southern prawn ponds

	Production yield (t/ha/yr)	FCR	Survival (%)	SGR (%/day)	Growth curve and regression (r²)
Northern 1	14.5	2.1	89	1.42	y = 0.0251x2 + 2.2877x - 75.507 R2 = 0.9881
Northern 2	15.2	2.5	82	1.40	y = 0.0341x2 + 0.8192x - 44.516 R2 = 0.9958
Southern 1	1.4	2.3	65	1.21	y = 0.0268x2 - 3.9698x + 46.862 R2 = 0.9936
Southern 2	9.8		90	1.27	y = 0.0282x2 - 4.2586x + 12.502 R2 = 0.9622

The length-weight relationship obtained from cobia grown on a north Queensland farm is displayed in Figure 35.

Temperature comparison

The pond temperatures in the northern zone were continually 5 °C warmer through winter and spring. During the peak of summer the temperatures of each zone were comparable (Figure 36).

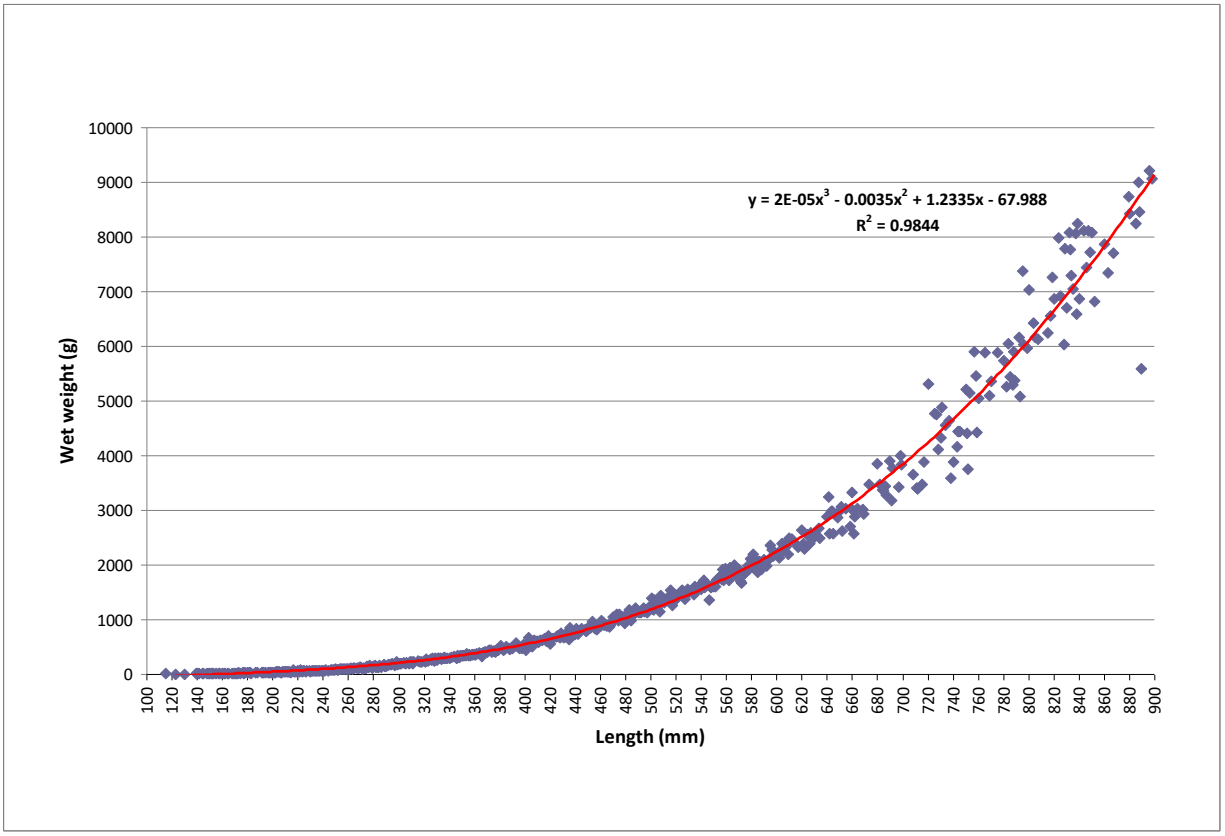

Figure 35 – Length-weight relationship for cobia grown on a northern farm. (Third-order polynomial trend line and growth equation – Data supplied by Pacific Reef Fisheries).

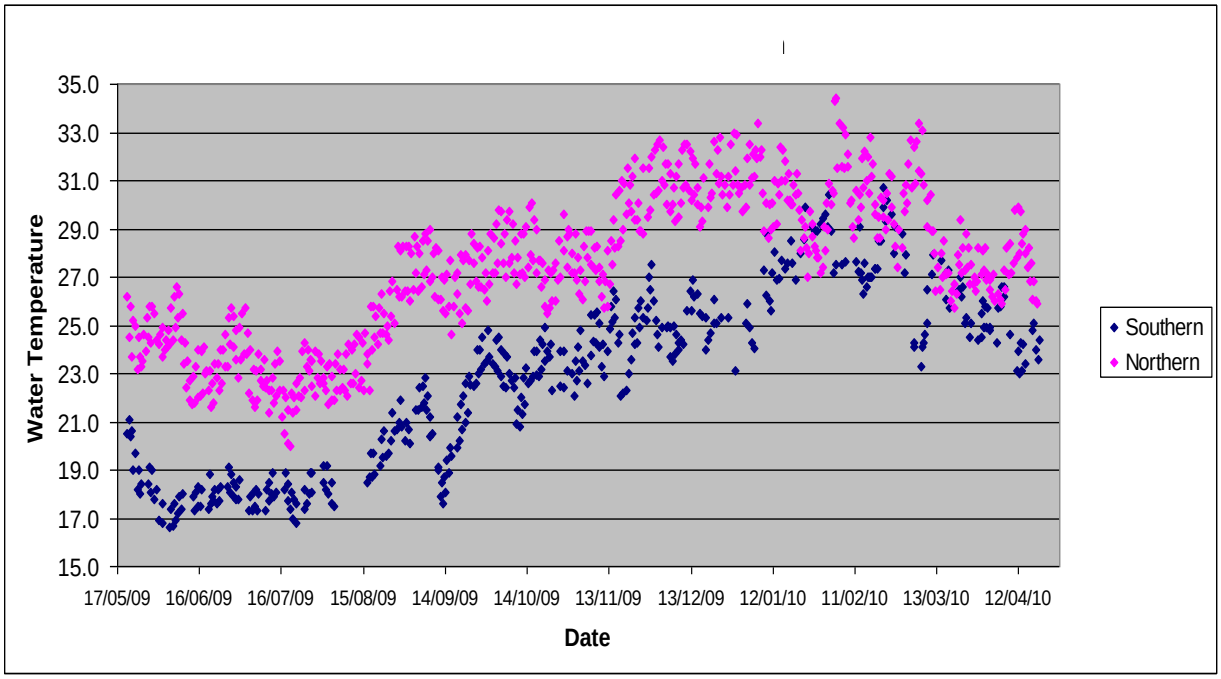

Figure 36 – Comparison of pond water temperatures from one southern and one northern farm

Salinity

The salinity of pond water on northern farms varied considerably according to the wet and dry seasons (Figure 37). Salinity recordings were above and below that of oceanic conditions in which cobia naturally inhabit, reaching 37 ppt in the dry season and falling to 8 ppt during the wet season. Reduced salinity did not appear to have an effect on production statistics.

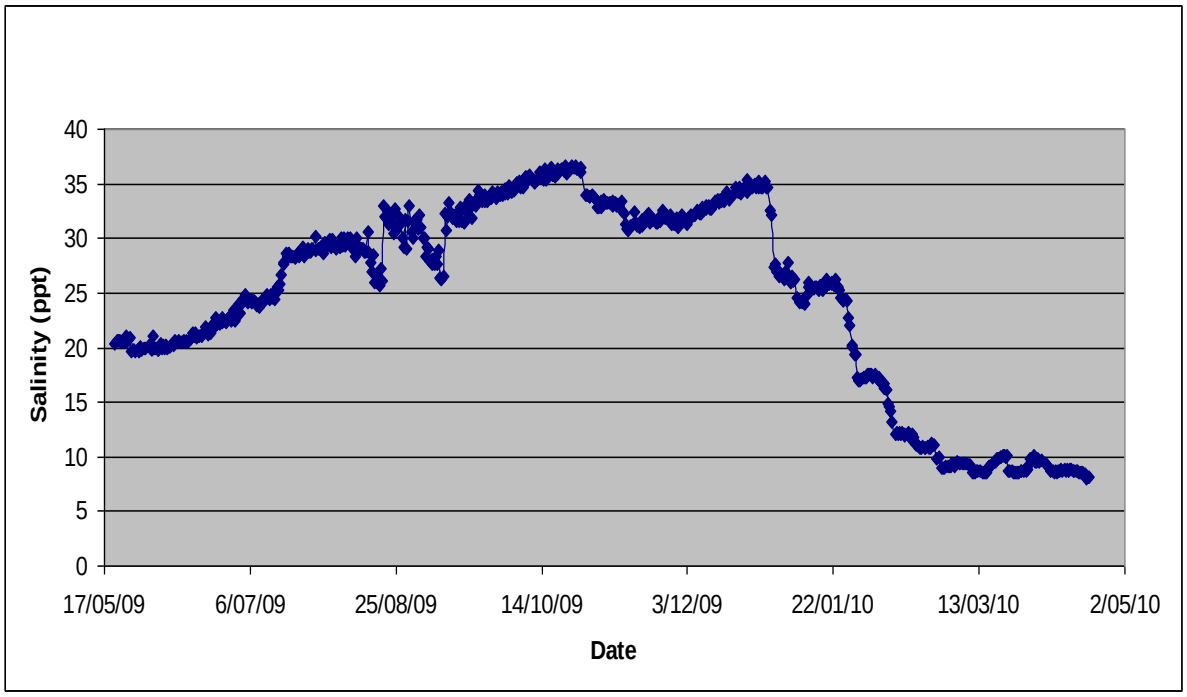

Figure 37 – Daily salinity measurements taken from a north Queensland farm

Health

No significant disease or parasite outbreaks were reported on the farms; however fish held at BIRC during winter suffered heavy mortality as a result of bacterial infections (see Appendix 3). *Photobacterium damselae* and *Vibrio harveyi* infections were identified as the causative agent for kidney disease and resulted in heavy mortality (see Figure 38). The likely cause of this infection was the extended exposure to sub-optimal temperatures creating a chronic stressor which compromised the immune system of the fish.

Figure 38 – Cobia infected by* Photobacterium damselae *(pasteurellosis) – note the green coloured liver and massively swollen kidney

For example, prior to this condition presenting the water temperature in the outdoor production system was close to 16 °C for several days: temperatures were below 18 °C for around 30 days and below 20 °C for 80 days during winter (Figure 39). Feeding response was reduced as water temperature approached 20 °C and stopped completely below 18 °C. Mortalities started to appear after four weeks exposure to sub-20 °C water (Figure 40): Raceway 2, which was stocked with 500 cobia suffered 35% mortality, and Raceway 6, which was stocked with 1100 cobia suffered 5.5% mortality.

To address this, beginning on 1 September 2009, both raceways were placed on two 10-day antibiotic treatments using Amoxycillin-clavulanic acid (Amoxyclav™), according to the conditions shown in Appendix 3. The efficacy of this antibiotic treatment is likely to have been assisted by increased water temperatures coinciding with the later stages of the treatment period.

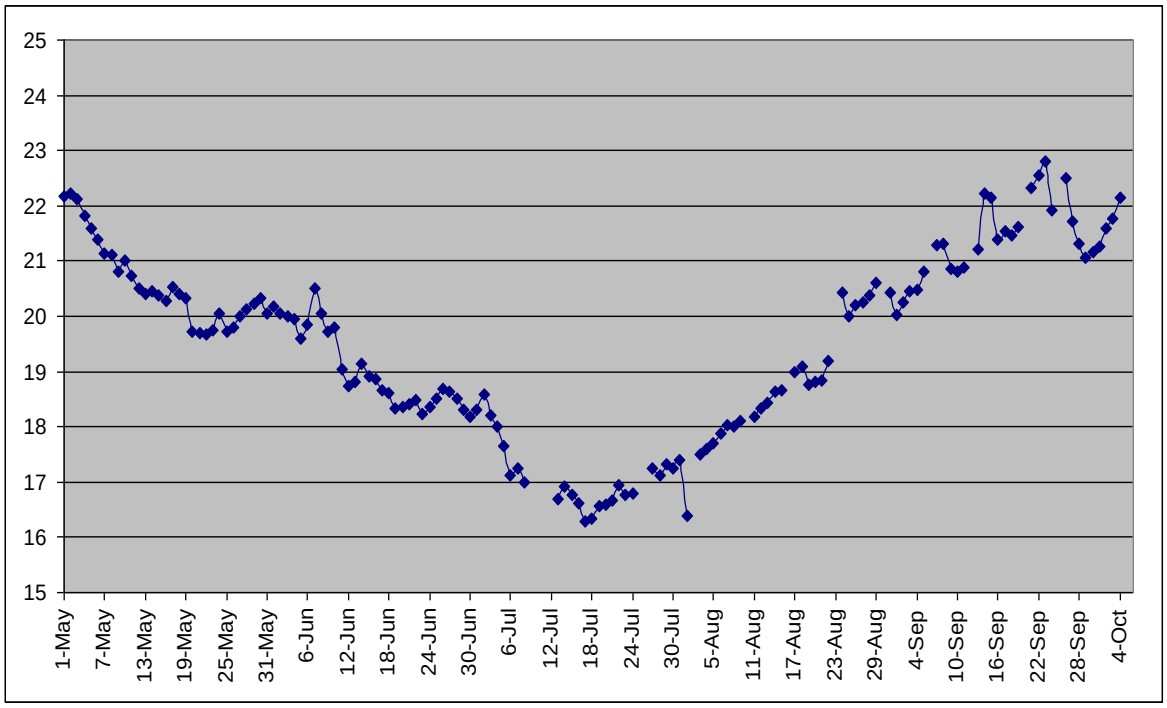

Figure 39 – BIRC raceway water temperatures (°C) during winter 2009

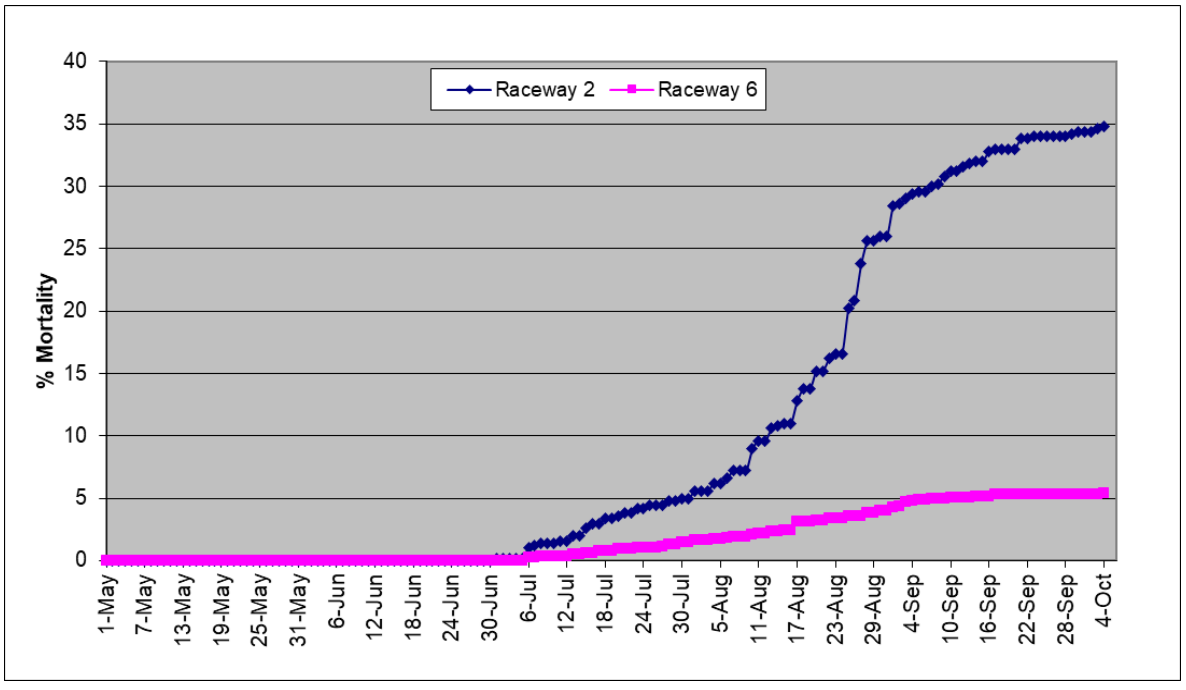

Figure 40 – Cumulative per cent mortality of cobia in each of the raceways during the winter period

Post-harvest research

Australia's wild catch of cobia is typically less than 30 t/year (Fry and Griffiths, 2010). A small commercial catch offers little competition to cultured product in the marketplace: alongside this comes a lack of product recognition. A new product does however offer a wide scope for product and market development without preconception or preceding reputation. Production output from the farm-based trials (Figure 41) was expected to be in the vicinity of 60–70 tonnes of whole fish.

Fish were harvested for market at sizes from 2 kg through to 12 kg. The majority of fish were sold whole chilled, and feedback on product quality was excellent. Suitable prices were obtained from wholesalers and through direct marketing of product. Farms generally took a steady approach to sales, with a view to minimising oversupply and subsequent price fluctuations.

Further investigation is required to determine the most suitable size to market cobia. This will be determined by the incorporation of both market demand and production efficiency.

Figure 41 – Cobia (2–3 kg) ready for market

Discussion

The project successfully demonstrated the technical feasibility of producing cobia in prawn grow-out ponds. The growth rates and FCR obtained on prawn farms located in northern Queensland were similar to those of cobia grown in off shore sea cages. Benetti *et al.* (2007; 2010) reported growth rates of 4–6 kg in 12 months, an FCR of 2:1 and survival of 75% or higher when cobia were grown in low-density offshore sea cages. Although our FCR data is comparable to global standards, there is a need to improve feeding efficiency to improve the economic efficiency of cobia production. Feeding efficiency may be improved through the development of more specific diets that are aligned with seasonal variations in metabolic rate. For example, a winter diet could focus on maintaining optimal health, and contain attractants that may improve feeding response when water temperatures are suboptimal. Alternatively, a summer diet could be focused on maximising production and conversion efficiency.

Reduced growth rates in the southern zone are most likely due to low water temperatures during winter relative to those of the north. This is supported by Sun *et al.* (2006) who reported a 44% increase in cobia growth with a 4 °C temperature differential. Liao *et al.* (2004; 2007) reported low appetites and suppressed growth from sea cage operations in subtropical Taiwan when water temperatures fell to 18 °C or below. *Photobacterium* spp. and *Vibrio* spp. infections, similar to those observed at BIRC, have also caused significant mortalities in sea cages (Liao *et al.*, 2004; 2007). The results of the current study suggest that while cobia appear to handle short-term exposure to low temperature, long-term exposure suppresses appetite resulting in a decrease in body mass and suppressed immunity. The need to reduce the health risk and maximise production to maintain commercial viability, suggests that producing cobia in a subtropical climate will require overwintering facilities in the form of heated nursery tanks or temperature controlled, raceway type ponds. This requires increased capital investment and higher running costs and would require a thorough economic evaluation to examine the cost-benefit of providing this infrastructure.

The production of cobia in low salinity has also been reported by Atwood *et al.* (2004). Their study found cobia survival was unaffected until salinities dropped below 8 ppt, suggesting that while cobia produced in the current study appeared to be unaffected by low salinities, any further reduction may have had an impact. Other studies of the low-salinity tolerance of cobia support the likelihood that 5–8 ppt is approaching their lower tolerance levels. Resley *et al.* (2006) found that while cobia grew equally well at 5 ppt compared with 15 and 35 ppt, survival was reduced at 5 ppt, most likely due to immune suppression. This presents a risk to cobia production in ponds, especially in the tropics where salinity can drop to these levels or less during the wet season.

This study demonstrates that cobia production is technically feasible in prawn ponds in Queensland; however it does not incorporate an economic analysis of cobia production. Such analyses are sensitive to site specific input costs and market prices obtained. Economic analysis should be conducted for a specific operation that considers the management and production requirements of the particular site. This study does however provide some of the data required to populate production modelling that is used to develop economic analyses.

Summary

- Cobia production is technically feasible in prawn ponds. This has been demonstrated by rapid growth rates and good survival.
- Cobia are more suited to a tropical climate. To be produced outside this region temperature control or overwintering facilities are required.
- Economic analyses and modelling are yet to be conducted to determine the profitability of cobia production.

Recommendations for further research

- There is a need to improve feeding efficiency. This could be done by refining feeding practices, developing diets that are more species specific and perhaps incorporating seasonally adjusted or winter diets.
- While sufficient product is now available to test markets there will be a requirement to further develop outlets as production increases to a commercial scale.

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Appendix 3

Cobia pathology report in 2009

8. SEP. 2009 9:48 BIO SCIENCES LAB NO. 616 P. 1/3

BIOSECURITY SCIENCES LABORATORY

Department of Primary Industries & Fisheries Animal Research Institute 665 Fairfield Road YEERONGPILLY QLD 4105

SUBMITTER :

L Dutney
Bribie Is Aquaculture Res Centre
144 North Street
WOORIM QLD 4507

Your submitter code is :

DUTLWOO

Please quote this code on future accessions

Pathology Report

Enquiries - Ph: (07)33629471 Fax: (07)33629440

Date Sent : 27/08/09
Date Received: 28/08/09
Reason for Submission: diagnostic

Accession No: 09-124564
Species : fish
Pathologist : R. Chong

Property No: QHCB5000
Owner : B I R C
D E E D I
144 North Street
WOORIM Qld 4507

DIAGNOSIS

Animal ID: cobia

Diagnosis : No diagnosis

Comment :

History

Moribund cobia with swollen abdomen experiencing mortalities of 50 fish over 6 weeks at a rate of approximately 3-4 fish per day in 2 raceways located in a pond. Approximately 1600 fish of size 150g at risk. More mortalities in the 1 raceway with 500 fish compared to the raceway with 1100 fish. Necropsy by BIRC staff : fluid retention in peritonuem, extremely swollen kidney, and green liver. White nodules noted in the kidney cut surface. Antibiotic therapy with amoxycillin-clavulanic acid prescribed on basis of gross pathology consistent with bacterial infection.

Diagnosis

Results provided on samples as received. Responsibility for sampling and delivery rests with submitter.

Distribution *** fax : (07)34083535 ***

SUBMITTER
Principal Manager, Fisheries Policy,
Fisheries Division, Floor 2 PIB

FILE

../..

Department of Primary Industries and Fisheries Queensland
Biosecurity Veterinary Laboratories: 13389

This laboratory is accredited by the National Association of Testing Authorities, Australia. The tests reported herein have been performed in accordance with its terms of accreditation. This document shall not be reproduced except in full.

Accession No: 09-.24564

1. Systemic and infectious disease with significant damage to the kidney and spleen.
2. Granuloma and necrotizing forming pathology consistent with bacterial infection.

Await results of bacteriology.

BACTERIOLOGY

Cultured for: aerobes.

Animal	Specimen	Test Result
1	spleen	*V.harveyi* (1)
2	spleen	*Photobacterium damselae* (2)
3	spleen	*V.harveyi* (3)
4	spleen	*Photobacterium damselae*

(1) amox-clav acid - s , ampicillin - r , cotrimoxazole - s
enrofloxacin - s , oxolinic acid - s , streptomycin - i
tetracycline - s , trimethoprim - s .

(2) amox-clav acid - s , ampicillin - r , cotrimoxazole - s
enrofloxacin - s , oxolinic acid - s , streptomycin - i
tetracycline - s , trimethoprim - s .

(3) amox-clav acid - s , ampicillin - r , cotrimoxazole - s
enrofloxacin - s , oxolinic acid - s , streptomycin - i
tetracycline - s , trimethoprim - s .

HISTOLOGICAL EXAMINATION

Cobia 1 : Spleen - congested with red blood cells, increased reticular activity with lymphocytic cells. Kidney - 1 large granuloma with 2-3 other small granulomas. Dilated glomeruli and tubular lumen with plugging by degenerate cellular material. Gills - curled lamellae and cellular tags (artifactual ?). No abnormalities observed in stomach, intestine, pyloric cecae, heart sections.

Cobia 2 : Spleen - reactive reticular network with congestive pockets with red blood cells. Liver - multiple microgranulomas and fatty vacuoles. Kidney - necrotizing tubercles x 7. Gills - curled lamellae. No abnormalities observed in heart, stomach, pyloric cecae sections.

Cobia 3 : Peritoneum - severe localised necrotizing peritonitis surrounding an artery, next to the intestine. Spleen - reactive and

Accession No: 09-.24564

congestive pockets with red blood cells. Kidney - necrosis of glomeruli, tubular cell debris plugging and formation of tubercle in section. No abnormality noted in heart.

Cobia 4 : Liver - multiple microgranulomas. Stomach - many lymphocytes in the connective tissue of the muscular layer. Head kidney - multiple tubercles. Kidney - very dilated tubules with extensive protein blebs and some red blood cells in the lumen. This indicates glomerular damage and loss of protein/blood. No abnormalities observed in heart, gills, pyloric caeca.

Gram stain of kidney/spleen and liver sections to be completed.

--> Final Page <--

for Manager, Regional Veterinary Laboratory
08/09/09

Amoxycillin-clavulanic acid treatment

Conditions of Medication Use

Medication Name : Amoxycillin-clavulanic acid (Amoxyclav 200 mg or 250 mg or 500 mg/tablet)

Dosage : 40 – 80 mg/kg fish in food once daily for 10 – 30 days depending on response. Not for use in water bath medication.

Application : Crush tablets, mix calculated daily dose to include in diet. A gelatin binder may be required to minimise loss of medication from the fish food.

Estimated biomass / water volume to treat : Measure weight of fish and calculate biomass in tanks to be treated.

Amount of active constituent of medication required: Sufficient active ingredient for fish biomass treatment in 10 - 30 days . After 10 days of medication, review response. If mortalities resolve ie. less than 3-4 fish per week, discontinue medication. If mortalities reduce but continue at greater than 3-4 fish per week, continue medication. A 10 day treatment requires 240 kg x 40 mg x 10 days = 96,000 mg. Review the biomass after 10 days and recalculate the drug dosage accordingly. Total prescribed active drug amount = 96,000 mg x 3 = 288,000 mg or 288g of Amoxycillin-clavulanic acid (equivalent to 576 tablets of amoxyclav 500, or 1152 tablets of amoxyclav 250 or 1440 tablets of amoxyclav 200). No repeats on this prescription.

Minimum With-Holding Period : The with-holding period is 500 degree days (20 days at 25C water temperature) from the last day of treatment. That is no fish is to be used for human consumption until after the full with-holding period without medication has been completed.

General Precautions : Calculate and cross check amounts of medications to use accurately. Always consider performing a test batch with the product to minimise the risks of adverse reactions in fish before applying the product to all affected fish/shellfish stocks. Fish/shellfish with advanced disease may die despite treatment. Withdraw treatment promptly in the event of an adverse reaction. Prepare adequate drug-free, clean and aerated water to assist the recovery of fish/shellfish following any immersion treatment and for those that may display an adverse reaction. Do not use any expired product.