Aquaculture 319 (2011) 41–52

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Recurrent outbreaks of viral nervous necrosis in intensively cultured barramundi (*Lates calcarifer*) due to horizontal transmission of betanodavirus and recommendations for disease control

Paul Hick [a], Glenn Schipp [b], Jerome Bosmans [c], John Humphrey [b], Richard Whittington [a,*]

[a] Farm Animal and Veterinary Public Health, Faculty of Veterinary Science, University of Sydney, 425 Werombi Road Camden, NSW 2570, Australia
[b] Berrimah Veterinary Laboratories, Department of Resources, Northern Territory Government Makagon Road, Berrimah, NT 0828, Australia
[c] Darwin Aquaculture Centre, Northern Territory Government, Channel Island, Darwin, NT 0828, Australia

ARTICLE INFO

Article history:
Received 31 May 2011
Accepted 27 June 2011
Available online 2 July 2011

Keywords:
Barramundi
Betanodavirus
Cross-sectional survey
Viral nervous necrosis
Lates calcarifer
Transmission

ABSTRACT

Betanodavirus is a significant constraint to marine finfish aquaculture worldwide and the development of control strategies will depend on accurate data about mechanisms of infection. A repeated cross-sectional survey for betanodavirus infection using a real-time reverse transcriptase-polymerase chain reaction assay and virus isolation was conducted in barramundi (*Lates calcarifer*) at a hatchery in Australia to determine whether transmission occurred vertically from the broodstock or horizontally from the environment. Six consecutive production batches were studied in 2007–2008. Subclinical infection was detected in Batch 2007-1 with onset of infection after 26 d of age, reaching a true prevalence >90.9% (lower 95% probability limit) by 40 d. Two days later an outbreak of VNN with 100% mortality occurred in 12 d old larvae in Batch 2007-2, due to infection with an identical betanodavirus, which was not detected in this batch 7 d previously. A point source epidemic initiated by horizontal transmission from the environment (probably seawater) to Batch 1 then Batch 2 was confirmed. Betanodavirus was not endemic in the hatchery and was probably not vertically transmitted because: (i) infection was not detected in the eggs or larvae of any batch; (ii) infection was not detected in 4 out of 6 batches; (iii) infection was not identified in 20 broodstock; iv) freedom from infection of life history stages was determined with a very high degree of confidence; v) similar data were derived from archival samples from a batch in 2005. The data suggested that outbreaks were initiated because of vulnerabilities in the water supply and spread because biosecurity measures failed. The age of fish appeared to be a major risk factor for susceptibility to disease.

1. Introduction

Viral nervous necrosis (VNN) disease of fish is caused by infection with viruses in the genus *Betanodavirus*, family *Nodaviridae*, and may be a serious constraint on the culture of more than 30 species of fish globally (Munday et al., 2002). Approaches to VNN control are based on preventing transmission of infection particularly by the vertical route from the broodstock. For example, successful control of VNN was reported in striped jack (*Pseudocaranx dentex*) by exclusion of betanodavirus infected broodstock which were identified using a reverse transcriptase-polymerase chain reaction (RT-PCR) assay (Mushiake et al., 1994). In European sea bass (*Dicentrarchus labrax*), the disease was controlled by exclusion of broodstock in which betanodavirus specific antibodies were detected in blood samples (Breuil et al., 2000). Disinfection of fertilised eggs by washing with ozone in seawater has also been recommended to control vertical transmission of betanodavirus. Experimental infection of Atlantic halibut (*Hippoglossus hippoglossus*) eggs was prevented by washing the eggs in seawater with 0.3 $mg.L^{-1}$ ozone for 30 s (Grotmol and Totland, 2000), and the survival rate of striped jack larvae produced by infected broodstock was increased when the fertilised eggs were exposed to a total residual oxidant (TRO) concentration of 0.5 $\mu g.L^{-1}$ for 1 min (Arimoto et al., 1996). However, there are conflicting data. In Queensland Australia, exclusion of barramundi broodstock based on the results of a nested RT-PCR assay performed on blood samples did not reduce the incidence of VNN (Anderson and Oakey, 2008).

Horizontal transmission of betanodavirus has also been implicated in several outbreaks (Grotmol et al., 1999; LeBreton et al., 1997; Peducasse et al., 1999). The findings of one outbreak investigation were confirmed experimentally by the demonstration of disease in European sea bass cohabited with sea bream (*Sparus aurata*) that had subclinical betanodavirus infection (Castric et al., 2001). Other horizontal transmission study experiments were reviewed by Munday et al. (2002).

Viral nervous necrosis is clearly a complex disease in which the outcome of betanodavirus infection can be influenced by host factors

* Corresponding author. Tel.: +61 2 93511619; fax: +61 2 9351 1618.
E-mail address: richard.whittington@sydney.edu.au (R. Whittington).

0044-8486/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2011.06.036

such as age (Aranguren et al., 2002; Arimoto et al., 1993), and environmental factors such as water temperature (Skliris and Richards, 1999; Tanaka et al., 1998; Yuasa et al., 2007). An association between VNN outbreaks and husbandry-related stress is commonly reported. Stress factors observed include suboptimal feed, suboptimal water quality, crowding, transport and repeated spawning of broodstock (Athanassopoulou et al., 2003; Johansen et al., 2004; Mushiake et al., 1994; Tanaka et al., 1998).

In Australia, VNN has been reported in recent years from several marine fish species in aquaculture, but outbreaks in hatchery-reared barramundi (*Lates calcarifer*) with up to 100% mortality have been recognised in northern Australia since before 1990 (Glazebrook et al., 1990). More recently, production of barramundi in this region has been negatively impacted by intermittent epizootics of VNN, despite implementation of control measures based on current knowledge of the disease.

The aim of this study was to investigate the epidemiology of VNN in an intensive barramundi hatchery, in particular to determine whether the infection was acquired by vertical or horizontal transmission, because this would inform development of new disease control strategies. A detailed, prospective, cross-sectional survey was undertaken to test for betanodavirus infection in fish at different stages of the production cycle, and so to determine where and when an outbreak of VNN was initiated.

2. Materials and methods

2.1. Survey design

A prospective, cross-sectional survey was designed to detect betanodavirus during routine production of barramundi at the Darwin Aquaculture Centre (DAC) (Table 1; Fig. 1). The DAC is located on Channel Island in Darwin Harbour, Northern Territory. The hatchery produced 5–6 production batches each year, where a production batch was defined as the progeny selected for on-growing from two spawning tanks, each containing one female and two male broodstock. After mortality and selective culling of the smallest fish, approximately 1 million juvenile barramundi were sold to grow-out producers throughout Australia each year. The sampling frame was defined as individual production batches at multiple development stages. A minimum sample size of 150 randomly selected individuals was applied to the earliest life history stages to obtain sufficient power to detect infection at a prevalence of >1%, after which ≥30 individuals of later life history stages were randomly selected to detect a prevalence of >10% assuming use of tests with sensitivity and specificity of 100% (Table 1). These design prevalences were conservative for betanodavirus, which is highly infectious and spreads very rapidly. Broodstock, animals from the environment surrounding the DAC and wild fish populations in the region were sampled opportunistically due to logistical and economic reasons.

2.2. Sample selection

A computer programme generated the random numbers used to select samples (Epi Info 2000, Centres for Disease Control, Atlanta).

2.2.1. Eggs

Eggs were sampled at the time of ozone treatment when they were concentrated and mixed in a 10 L bucket. Pools of 50 mL of eggs were obtained using a dip net, each pool comprising 10 scoops taken from 1 of 4 randomly selected depths in the bucket. A separate pool of eggs was taken before and after ozone treatment for each spawning tank on both nights of spawning, resulting in 8 separate pools for each batch. A sub-sampling procedure was used to obtain the samples for

Table 1
Sample collection strategy for betanodavirus surveillance during barramundi production at the DAC.

Age (days)	Development stage	Total length (mm)	Location	Production event	Population size	Number of samples	Tissue type	Preparation	Sample tested (assay unit)
0	Eggs	0.8	Spawning tank	Egg collection	1.5×10^6	150	Pooled eggs	Concentrate by centrifugation, remove seawater and wash	Pool of >100 individuals
0				After ozone disinfection		150			Pool of >100 individuals
0–1		1.5	Hatching tank	Monitoring of hatching		150			Pool of >100 individuals
2	Larvae	2	Larval rearing system	Transfer to larval tanks	1.2×10^6	150	Pools of larvae	Concentrate by centrifugation,	Pool of >100 individuals
5		3				150		remove seawater and wash	Pool of 30–60 individuals
8		4				150			Pool of ~30 individuals
11		5				150			Pool of 10–30 individuals
13–20	Juvenile	6.5–8			1.1×10^6	150	Entire fish	Rinse in 70% ethanol	Pool of 3–5 individuals
21–30		9–22	Nursery tanks	Transfer to nursery	7×10^5	150	Head	Rinse in 70% ethanol + excise head	Individual fish 1 sample/fish
37	Juvenile	30		Grading	4×10^5[b]	150	Head		
44		45		Grading		150			
51		60		Grading		150	Brain and 2 eyes pooled	Aseptic dissection & pool tissues	
58		75		Grading		150			
65–80		100	Grow-out	Transport to farms	$1–3\times10^5$	150	Brain and 2 retinas pooled		
90–150	Grow-out (sub-adult)	120–300	Farms [a]	–	5×10^4/pond	Opportunistic	Brain, 2 pooled retinas	Aseptic dissection, pool retinas	2 samples/fish
540		600		Harvest			Brain, and 2 retinas		3 samples/fish
>540	Broodstock	>800	Broodstock tank	Spawning	35–45	Opportunistic	Many	Complete necropsy	Individual tissues

[a] All other samples were from DAC.
[b] The population size was reduced by selective culling of the smallest fish according to sales requirements.