Journal of Invertebrate Pathology 125 (2015) 81–86

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Short Communication

# *Zoothamnium duplicatum* infestation of cultured horseshoe crabs (*Limulus polyphemus*)

Andrew P. Shinn [a,b,*], Alexander P. Mühlhölzl [c], Christopher J. Coates [d,*], Christoforos Metochis [a], Mark A. Freeman [e]

[a] *Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK*
[b] *Fish Vet Group Asia Limited, 99/386 Chaengwattana Building, Chaengwattana Road, Kwaeng Toongsonghong, Khet Laksi, Bangkok 10210, Thailand*
[c] *Marine Biotech Limited, Stirling, Stirlingshire, UK*
[d] *Biological and Environmental Sciences, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK*
[e] *Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur 50603, Malaysia*

ARTICLE INFO

*Article history:*
Received 9 July 2014
Revised 26 November 2014
Accepted 2 December 2014
Available online 10 December 2014

*Keywords:*
American horseshoe crab
Epibiont
Treatment
Aquaculture
Parasite

ABSTRACT

An outbreak of the sessile peritrich *Zoothamnium duplicatum* in a pilot, commercial-scale *Limulus polyphemus* hatchery resulted in the loss of ~96% (40,000) second/third instar larvae over a 61 day period. peritrich growth was heavy, leading to mechanical obstruction of the gills and physical damage. The peritrichs were controlled without resultant loss of juvenile crabs by administering 10 ppm chlorine in freshwater for 1 h and the addition of aquarium grade sand; a medium into which the crabs could burrow and facilitate cleaning of the carapace. Peritrich identity was confirmed from a partial SSU rDNA contiguous sequence of 1343 bp (99.7% similarity to *Z. duplicatum*).



## 1. Introduction

The American horseshoe crab, *Limulus polyphemus*, is an ecologically and commercially valuable marine invertebrate that has been exploited by numerous industries for over a century. Horseshoe crabs have been used as an ingredient in fertiliser and livestock feeds, as bait for whelk (*Busycon* spp.) and eel (*Anguilla* spp.), and most notably, for the production of the highly sensitive endotoxin detection assay, Limulus Amebocyte Lysate (LAL) (reviewed by Walls et al., 2002). LAL is derived from a series of proteins stored within the cytoplasmic granules of *L. polyphemus* blood cells (amebocytes; Coates et al., 2012a). After blood (hemolymph) has been extracted from the animals they are returned to their capture point, and although the industry states that typical mortalities are between 5% and 15%, other studies suggest that these can be high as 30% (Hurton and Berkson, 2006; Leschen and Correia, 2010). Recent evidence indicates that extracting large volumes of blood may not result immediately in horseshoe crab death, but induces sub-lethal effects that diminish animal fitness (Anderson et al., 2013).

Given that horseshoe crabs appear relatively easy to house *ex situ* (Smith and Berkson, 2005; Coates et al., 2012b), there is commercial aquaculture interest in maintaining and rearing sustainable captive held populations of *L. polyphemus* (Carmichael and Brush, 2012; Kwan et al., 2014). There remains, however, a lack of knowledge regarding the aetiologies/pathologies of infectious/non-infectious diseases of captive and wild populations. Degenerative lesions on the shell (carapace) and soft tissues of horseshoe crabs (irrespective of age) appear to be the most frequently reported disorder, attributed to pathogenic algae, fungi and Gram-negative bacteria (reviewed by Nolan and Smith, 2009).

The objectives of this study were to characterise the aggressive epibiont infestation (*Zoothamnium duplicatum*) that covered the entire carapace of juvenile horseshoe crabs, and to develop a non-invasive treatment. The presented data offers a relatively rare insight into horseshoe crab-protozoan antibiosis. The abundance of *Z. duplicatum* most likely facilitated fouling within the hatchery, providing conditions favourable for the proliferation of other noxious microorganisms.

* Corresponding authors at: School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK (A.P. Shinn and C.J. Coates).
*E-mail addresses:* andy.shinn@fishvetgroup.com (A.P. Shinn), c.j.coates@stir.ac.uk (C.J. Coates).

http://dx.doi.org/10.1016/j.jip.2014.12.002
0022-2011/