Aquaculture 396–399 (2013) 59–65

Contents lists available at SciVerse ScienceDirect

Aquaculture

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

Hematodinium infections in cultured Chinese swimming crab, *Portunus trituberculatus*, in northern China

Caiwen Li [a,*], Shuqun Song [a], Yun Liu [a], Tiantian Chen [a,b]

[a] Key Lab of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[b] College of Environmental Science and Engineering, Ocean University of China, Qingdao 266061, China

ARTICLE INFO

Article history:
Received 28 January 2013
Received in revised form 18 February 2013
Accepted 18 February 2013
Available online 1 March 2013

Keywords:
Parasitic dinoflagellate
Crab
Aquaculture
Transmission
Life cycle
Phylogenetic analysis

ABSTRACT

Since 2004, the parasitic dinoflagellates *Hematodinium* emerged as causative agent of the "milky blood disease" in cultured *Portunus trituberculatus*, *Scylla serrata* and *Exopalaemon carinicauda* along the coastal areas of southern China; while, no *Hematodinium* infections had been reported in northern China. We sampled *P. trituberculatus* in polyculture ponds and adjacent coastal waters from two sites in Shandong Peninsula, the major culture region in northern China which contributes to one third of the national gross outcome of the crab species. *Hematodinium* infections were identified in 10% of *P. trituberculatus* randomly sampled from polyculture ponds, while no infections were observed in wild crabs collected from adjacent coastal waters. Filamentous trophonts and amoeboid trophonts were observed in diseased crabs. The *Hematodinium* sp. shares similar morphologic features and causes typical pathological changes in hepatopancreas, heart, and muscles of infected hosts as other *Hematodinium* spp. The laboratory trials indicated that this parasite was infectious and caused pathological alterations to crab hosts as those diseased crabs from culture ponds. Molecular analysis indicated that the isolates were closely related to those reported in southern China. Our findings indicate an alarming broad distribution of the infectious pathogen along the coastal areas of China. The polyculture pond system widely used in major culture regions in China may prompt transmission or spread of *Hematodinium* among susceptible hosts, which urges pressing investigation of its major routes of transmission in such culture system along with better understanding of its life cycle.

1. Introduction

Species of *Hematodinium* are parasitic dinoflagellates infecting marine crustaceans (Shields, 1994; Stentiford and Shields, 2005). *Hematodinium* have severe pathogenic effects on their hosts. Infected hosts show lethargy, discolored carapaces, and having milky hemolymph that does not clot, and die eventually due to malfunction of major tissues/organs. Unlike planktonic dinoflagellates, this parasitic dinoflagellate mainly resides and multiplies in the hemolymph or hemocoel of their crustacean hosts. Considering the negative ecological effects of the parasitic dinoflagellates, *Hematodinium* has also been noticed as harmful alga bloom species by its broad definition (Burkholder et al., 2006; Frischer et al., 2006).

Hematodinium was originally reported in the European crabs *Carcinus maenas* and *Liocarcinus depurator* (Chatton and Poisson, 1931). The genus of *Hematodinium* is currently placed in the family of *Syndiniaceae* which is composed of predominantly parasitic species. Only two species of *Hematodinium* have been described, the type species *Hematodinium perezi* (Chatton and Poisson, 1931) and a second species *Hematodinium australis* (Hudson and Shields, 1994). Outbreaks of *Hematodinium* have significantly impacted stocks of various commercially fished crustaceans worldwide, such as snow crabs *Chionoecetes opilio* (Meyers et al., 1990; Taylor and Khan, 1995), tanner crabs *Chionoecetes bairdi* (Meyers et al., 1987, 1990), blue crabs *Callinectes sapidus* (Messick, 1994; Messick and Shields, 2000; Newman and Johnson, 1975; Shields and Squyars, 2000) and Norway lobsters *Nephrops norvegicus* (Field et al., 1992). *Hematodinium* has been recently reported as the causative agent of the "milky blood disease" in the Chinese swimming crabs *Portunus trituberculatus* (Xu et al., 2007a), mud crabs *Scylla serrata* (Li et al., 2008; Xu et al., 2007b) and cultured ridgetail white prawn *Exopalaemon carinicauda* in southern China (Xu et al., 2010).

Shandong Peninsula is one of the major culture regions of Chinese swimming crab *P. trituberculatus*, where more than 30,000 t were harvested in 2009, contributing to one third of the gross outcome of industry in China (China Fishery Statistical Yearbook, 2010). In 2011, *P. trituberculatus* cultured in this region suffered unusual mortalities with no overt symptoms. Thus in 2012, we randomly collected crabs from two sites, where no overt mortality occurred at that time, and processed for pathogenic diagnosis as requested by local farmers. A parasitic dinoflagellate *Hematodinium* sp. was identified in diseased *Portunus* crabs, which shares similar morphological, pathological and ultrastructural characteristic features as the *Hematodinium* sp. reported in southern China; molecular identification and phylogenetic analysis

* Corresponding author. Tel./fax: +86 532 82898995.
E-mail address: cwli@qdio.ac.cn (C. Li).

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http://dx.doi.org/10.1016/j.aquaculture.2013.02.022

indicated that the isolates were closely related to those reported from southern China; the parasitic dinoflagellate was infectious and caused similar pathological alterations to crab hosts in laboratory trials.

2. Materials and methods

2.1. Chinese swimming crabs P. trituberculatus

During July–November 2012, 80 crabs were randomly collected from aquaculture ponds and their adjacent coastal waters along Shandong Peninsula (Fig. 1). Water temperature varied between 20 °C and 26 °C in those sites, and salinity ranged from 25 to 27. Crabs weighed from 108 to 220 g (142 ± 35), and body length ranged from 13 to 16 cm (14 ± 0.8). Crabs were maintained in aerated plastic containers and transported to the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China. Crabs were temporarily held in aerated glass aquaria until being further processed.

The status of *Hematodinium* infection was examined using the hemolymph smear assay described by Stentiford and Shields (2005). Briefly, the juncture between the basis and ischium of the 5th walking leg of each the crab was sterilized with 70% ethanol, then ~200 μl of crab hemolymph was withdrawn using a sterile syringe and a 27 ga. needle. An equal volume of neutral red (0.05% w/v) in 1 × phosphate buffered saline (PBS) was mixed with a few drops of hemolymph on a glass slide, and then examined with a microscope at 100× and 400× (Olympus BX53) to diagnose the presence of parasites by its distinctive uptake of the vital stain. Remaining hemolymph was load into 1.5 ml microcentrifuge tube preloaded with 800 μl ethanol (100%) and kept at −20 °C for DNA extraction.

2.2. Inoculation trials

The naïve crabs, prescreened to be negative using the hemolymph smear assay and PCR diagnosis (as described in Section 2.4), were housed together in the re-circulating 200-gal system (salinity = 26 ± 1, temperature = 18 ± 2 °C, equipped with preconditioned biological filters, UV light) for experimental treatments. Fifteen crabs were injected with 100 μl (approximately 1×10^5 parasites) hemolymph from the infected donor crabs. Inoculums were injected into crabs at the juncture (sterilized with 70% ethanol) between the basis and ischium of the 5th walking leg. All of the crabs were monitored daily for mortality. Dead or moribund crabs were assessed for *Hematodinium* infections using hemolymph smears as above, and tissues were dissected and processed for histology as described in Section 2.4. At the end of the experiment (11 days after inoculation), surviving crabs were killed and processed as above to determine the status of *Hematodinium* infections. Over the time course of the experiment, crabs were fed with pieced clam once a day; water quality was monitored and maintained within reasonable limits: ammonia (0–0.3 ppm), nitrite (0–0.5 ppm), and pH (7.4–8.0).

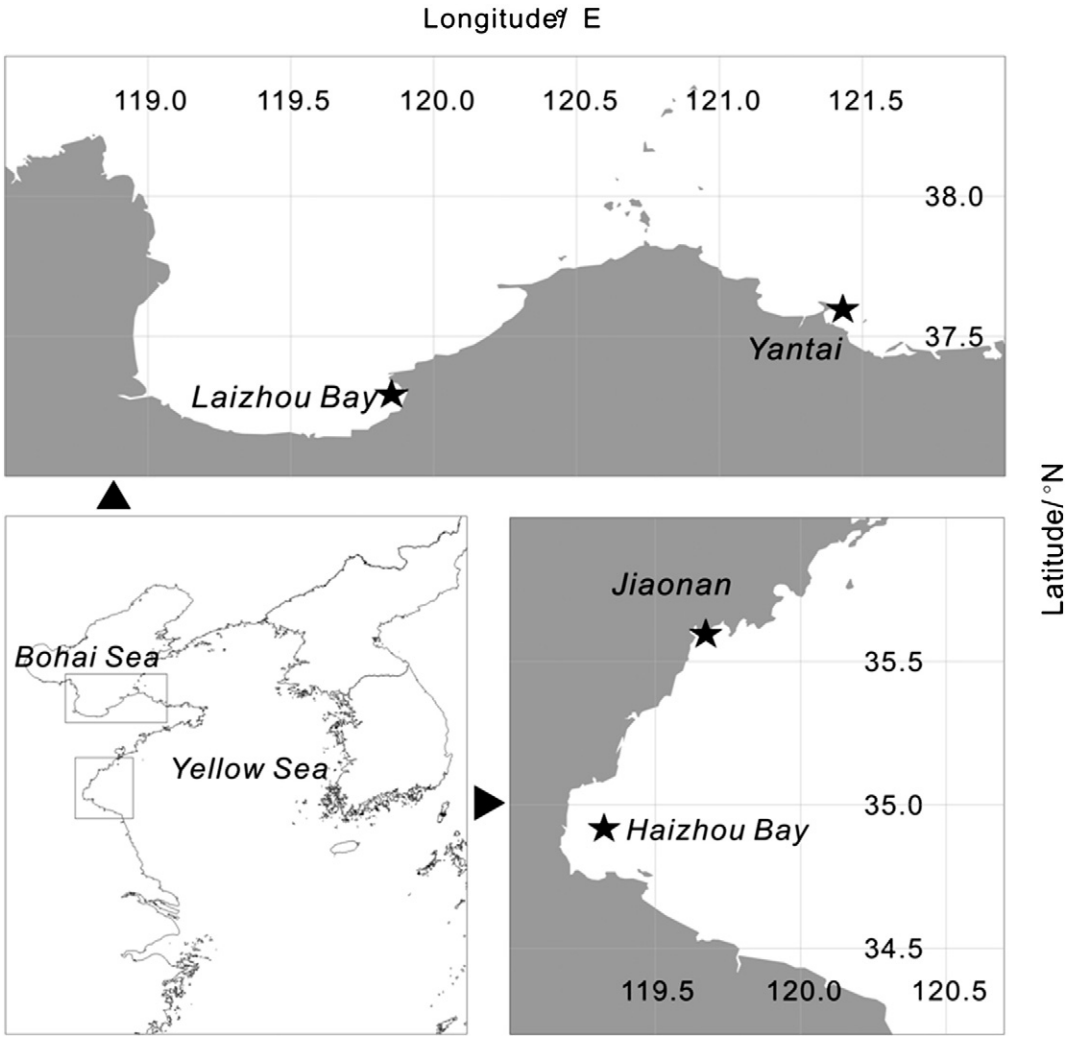

Fig. 1. Chinese swimming crab *P. trituberculatus* sampling locations along the coastal areas of Shandong Province, China. 1. Jiaonan; 2. Haizhou Bay; 3. Yantai; and 4. Laizhou Bay.