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ScienceDirect
Aquaculture 268 (2007) 274–287
Aquaculture
www.elsevier.com/locate/aqua-online

Microbiological aspects of phyllosoma rearing of the ornate rock lobster *Panulirus ornatus*

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Abstract

Rock lobsters of the Palinuridae are the most valuable wild fisheries sector in Australia and are currently target aquaculture species. Significant challenges exist however to produce commercial scale quantities of post-larvae due to an extended larval phase which acerbates a high rate of larval attrition caused by inadequate nutrition and a challenging microbial environment. Here we investigate a diverse and varied bacterial community in four compartments of the larval-rearing system: the water column, the biofilm, live feeds and the phyllosomas themselves. External fouling of phyllosoma by filamentous *Thiothrix* sp. was documented by scanning electron microscopy (SEM) and fluorescence *in situ* hybridisation (FISH). Internal proliferation of bacteria coinciding with mass mortality of phyllosoma was observed in histopathological analysis and identified as *Vibrio* sp. by specific labelling of sectioned hepatopancreas tissue using FISH. Of particular interest in relation to larval mortalities was a range of *Vibrio* species, isolated from the four rearing compartments, closely affiliated with *V. alginolyticus*, *V. parahaemolyticus*, and *V. harveyi*. The presence of bacterial quorum sensing signal molecules within the system was demonstrated in both biofilm and phyllosoma environments during a larval-rearing run. Interestingly, a large increase in quorum sensing signal molecules was detected in phyllosoma corresponding with mass mortality.

Keywords: Rock lobster; *Panulirus ornatus*; Microbiology; Scanning electron microscopy; Fluorescence *in situ* hybridisation

1. Introduction

Lobsters, of the suborder Pleocyemata, are one of the most valuable seafood commodities. Presently nearly all production of marine lobsters is based on harvesting adults from wild populations and is dominated by the clawed lobsters of superfamily Nephropidae, from the north Atlantic. The spiny or rock lobsters, of the family Palinuridae, make up approximately 33% of world harvest (FAO, 2004). Of the Palinuridae, 28% of total world harvest is represented by the temperate rock lobsters, primarily of the *Jasus* and *Palinurus* genera, with the remainder consisting of subtropical and tropical species. Global demand for some rock lobster species exceeds wild harvest supply and hence there is considerable interest in the development of a rock lobster aquaculture sector to expand production of selected species. To be sustainable, however, the life cycle must be closed to secure stable and consistent production of fry to supply grow-out operations.

A critical point in the closed life cycle production of aquaculture species is the hatchery phase. The only commercially viable crustacean aquaculture sectors are

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doi:10.1016/j.aquaculture.2007.04.067

those farm species that have a short larval phase (Lee and Wickins, 1992). Foremost of these successful sectors are penaeid prawns, which have a larval phase of only 15–20 days. In sharp contrast, the Palinuridae have a prolonged larval phase lasting anywhere between 6 to 22 months or more (Báez, 1983; Berry, 1974; Booth, 1989; Chittleborough and Thomas, 1969; Dexter, 1972; Johnson, 1956; Kittaka and Ikegami, 1988; Kittaka and Kimura, 1989; Kittaka et al., 1988; Kittaka, 1997; Lesser, 1978; Lewis, 1951; Michel, 1969; Prasad and Tampi, 1959; Prasad et al., 1975; Radhakrishan and Vijayakumaran, 1995; Saisho, 1966; Tong et al., 2000). The shortest larval phases known of the rock lobster *Panulirus* genera, based on wild plankton collection analysis, are those of *Panulirus elephas* and *P. ornatus*, estimated to be approximately 6 months (Prasad et al., 1975; Radhakrishan and Vijayakumaran, 1995).

The phyllosoma cycle is diecdysic with a short intermoult period followed by a longer pre-moult period, whereas an anecdysic cycle, predominantly an intermoult phase with a shorter pre-moult cycle, characterises the metamorphosis from phyllosoma to puerulus (Anger, 2001). Generally, larval development occurs through a series of 7–13 morphological stages. Within these, there are additional supernumerary moults or instars without morphological changes. Overall, it is generally thought that multi-instars within a particular larval stage are indicative of sub-optimal larval development and it is highly desirable to minimise the larval phase by having only 1 instar per developmental stage.

Efforts towards phyllosoma larval culture began in 1911 on *P. interruptus*, with further efforts on *Jasus lalandii*, *J. edwardsii*, *J. verreauxi*, *J. frontalis*, *Panulirus argus*, *P. elephas*, *P. homarus*, *P. inflatus*, *P. japonicus*, *P. longipes*, *P. penicillatus*, *P. polyphagus* and *P. stimpsoni* (Bardach et al., 1972; Booth and Phillips, 1994; Kittaka, 1997; Moe, 1991; Radhakrishan and Vijayakumaran, 1995; Tong et al., 2000; von Olst et al., 1980). In most studies phyllosoma mortality was so great that the larval cycle was not completed. Of those that were successful only a few pueruli were produced, again due to high attrition rates of phyllosoma (Booth, 1989; Kittaka, 1988; Kittaka and Ikegami, 1988; Kittaka and Kimura, 1989; Kittaka et al., 1997, 1988).

A major technological challenge for spiny lobster aquaculture includes maintaining high health status throughout the extended larval phase. As the major cause of phyllosoma mortality is believed to be due to bacteria, especially opportunistic pathogens, it is essential to obtain an understanding of the microbial community and dynamics within the larval-rearing system (Bourne et al., 2004). As part of this effort we have examined the larval-rearing environment as four microbiological compartments, 1) the water column, 2) the biofilm, 3) the live phyllosoma feed (*Artemia*) and 4) the phyllosomas themselves. The division of the system into compartments is useful to obtain an understanding of the microbial community as well as interactions and dynamics of the entire larval-rearing system. We have employed a polyphasic approach to study the microbial community, incorporating direct microscopic analysis, culture-based and molecular microbiological methods, as well as analyses of quorum sensing (QS) molecules in phyllosoma and biofilm samples. In this study, we provide an overview of our investigations into microbiological aspects of larval rearing of the ornate rock lobster *Panulirus ornatus*. The ultimate goal is to develop an effective strategy for microbial management to improve phyllosoma survival.

2. Materials and methods

2.1. Larval-rearing technology

An outline of the maintenance of wild-caught adult *P. ornatus* broodstock and larval-rearing technology is provided in Bourne et al. (2004). Broodstock were fed daily *ad libitum*, a combination of squid, mussels and supplemented occasionally with prawn pellets. All phyllosomas were fed on *Artemia* (Prime Brine Shrimp, A Grade). Cysts were decapsulated in 50 g batches per 50 L hatching tank at 28 °C. *Artemia* were boosted from a stock solution, 50 g L^{-1} of DC Super Selco. Ten to 21 h after *Artemia* hatched they were supplemented with 0.6 g L^{-1} of stock DC Super Selco and enriched for 17 to 24 h. Subsequently *Artemia* were treated with 25 mg L^{-1} formalin, 8 mg L^{-1} oxytetracycline, 9 mg L^{-1} erythromycin and 20 mg L^{-1} streptomycin before addition to larval-rearing tanks. Phyllosoma were fed twice daily at 0900 and 1600 h. *Artemia* were added to obtain a density of 1 to 4 mL^{-1}. If *Artemia* remained in the tank by the next feeding the density was topped up to reach the 4 mL^{-1} target.

2.2. Histopathological analysis

Phyllosoma larvae were fixed in Davidson's fixative (Hasson et al., 1997) for 24 h prior to processing. All samples were sectioned (5 μm) and stained (Mayer's hematoxylin and eosin) using routine histological procedures and examined using light microscopy (Bancroft and Stevens, 1990). Phyllosoma sections from early in the larval-rearing run (day 1 post-hatch) were directly compared with phyllosoma sampled at the end of a