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Infection of *Nematopsis* oocysts in different size classes of the farmed mussel *Perna viridis* in Thailand

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ABSTRACT

We report on the infection intensity of *Nematopsis* oocysts in different size classes of the green mussel *Perna viridis* farmed in Chonburi province, eastern upper Gulf of Thailand. Infection of the apicomplexan parasite was investigated from November 2003 to November 2004 in small (2.3±0.2 cm shell length, n=130), medium (5.6±0.3 cm, n=130) and large (8.6±0.4 cm, n=130) size classes of the green mussel. Heavy prevalence of infection was found in all size classes from November 2003 to June 2004, whereas low prevalence was observed from July to October 2004. From statistical analysis, prevalence of the parasite did not differ between the three mussel size classes. However, infection intensity calculated per unit weight of dried gill tissue was significantly lower in the smallest size class.

From light and transmission electron microscopy, the *Nematopsis* oocysts were ellipsoidal 12.6±0.3 μm wide and 17.4±0.9 μm long and 12.0±0.3 μm wide and 16.6±0.5 μm long, respectively. Oocyte wall thickness was between 0.5 and 0.7 μm. Oocysts were located within a parasitophorous vacuole (PV) 22–30 μm in diameter. The PVs were in turn engulfed within phagocyte sacs produced by the mussel with diameters ranging from 30–95 μm depending on the number of oocysts within. Microscopic examination suggested that this infective stage of the parasite causes severe damage to *P. viridis* gill tissue in conditions of heavy infection causing inflammation and disruption of gill epithelia.

1. Introduction

The Apicomplexan protozoan *Nematopsis* is a parasite commonly infecting molluscan intermediate hosts with decapod crustaceans as definitive host (Lee et al., 2000). The first species of *Nematopsis* was discovered in the mantle of the razor clam *Solen vagina* in France, while the earliest nominal species referred to the genus was *Nematopsis schneideri* Leger, 1903 in the gills of *Mytilus edulis*, also reported in France (Sprague, 1970). Subsequently, *N. schneideri* Leger, 1903 became the type species and the monotype.

Although the importance of protozoan parasites in farmed bivalves is recognized, the biology and life-history of these microorganisms in Thailand's tropical waters have not been extensively investigated. Many species of bivalves in the area are heavily infected by *Nematopsis* (Tuntiwaranuruk et al., 2004) including *P. viridis*, the primary cultivated bivalve species in Thailand in terms of landings. Annual losses in the green mussel industry in this area have primarily been attributed to poor environmental conditions. However, infections by *Nematopsis* might play a role in the weakening of these bivalves. *Nematopsis* sp. may be associated with mass mortalities of cockles and clams in Portugal (Azevedo and Cachola, 1992) due to their harmful effects on the host.

Our research reports upon the prevalence of *Nematopsis* in different size classes of the green mussel, *P. viridis* monitored over a 13 month period and determines the infection intensity of *Nematopsis* in relation to mussel size. An account is also given of the histological appearance of heavily infected gill tissue and ultrastructure of the infecting protozoa.

2. Materials and methods

Green mussel, *P. viridis* were collected from local farms in farming areas from Ang-Sila district in the Chonburi province coastal zone located at 13° 19.97' N, 100° 54.94' E in Thailand (Fig. 1). From November 2003 to November, 2004, 10 small, medium and large mussels were collected monthly corresponding to a 13 month culture

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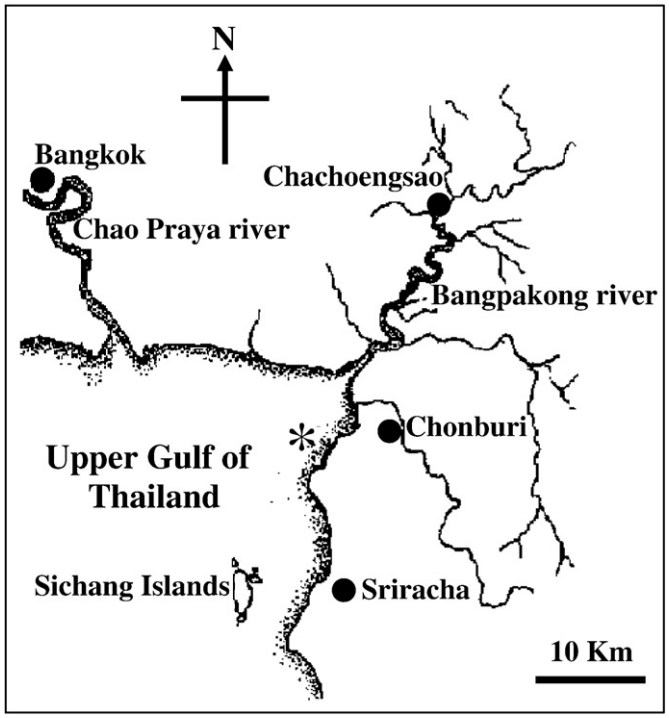

Fig. 1. Green mussel farms where samples were taken at Ang-Sila fishing village located at 13° 19.97¢ N, 100° 54.94¢ E (*) Chonburi Province of Thailand.

and harvest cycle. Small mussels were those smaller than 3 cm shell length (SL) while medium sized mussels were those between 3 and 5.99 cm SL, large mussels were of 6 cm SL upwards. In Chonburi, green mussels are first harvested after 8–9 months of growout. Harvesting of the annual crop usually lasts for 2–3 months. Infection intensity was analyzed in relation to size and season of sampling. All mussel samples were collected from farms within a period of several hours, taken to the laboratory and immediately processed.

The mussels were opened and a 16 mm^2 piece of gill tissue from each individual was excised from the inner and outer demibranch on the left-hand side of the bivalve. They were squashed between pre-weighed glass coverslips, observed under a compound microscope and the total number of oocysts was enumerated utilizing a counting grid. After counting, the coverslips along with excised gill tissue were dried at 75 °C overnight in a hot air oven and re-weighed to estimate dry excised gill tissue weight. In conjunction, the remaining tissue from the inner and outer demibranch also on the left-hand side of each mussel was dissected, dried and weighed on pieces of pre-weighed aluminum foil also after drying at 75 °C for 24 h. Oocyst infection intensity and dry weight of excised tissue was then compared with total gill dry weight expressed in milligrams.

An analysis of variance (ANOVA) was performed on the relationship between sizes of *P. viridis* and number of oocyst using collecting months as a blocking factor. Finally, the Duncan's multiple range test at 5% probability level was calculated for each size class when a significant difference among sizes was found.

2.1. Microscopic observation and transmission electron microscopy (TEM)

The inner and outer demibranch on the right-hand side of the mussels were dissected and prepared for histological observation and TEM. For light microscopy, the gills were placed in 10% neutral formalin at room temperature for 24 h and subsequently embedded for histological analysis following a standard protocol (Luna, 1960). Tissue sections were subsequently prepared by standard histological methods.

For TEM, small pieces of heavily infected gill tissues were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.8, for 24 h at 4 °C. They were later washed for 2 h at 4 °C in the same buffer and left overnight. Afterwards, the tissues were post-fixed in 1% O_sO_4 in 0.1 M sodium cacodylate buffer, pH 7.8 for 2 h at 4 °C. After washing, the samples were dehydrated through a serial concentration of ethanol and infiltrated with propylene oxide for 20 min (twice) followed by mixtures of propylene oxide and Araldite 502 resin (2:1) for 1 h and 1:2 overnight. The infiltrated samples were then embedded in a flat mold filled with pure Araldite 502 resin and polymerized in an incubator at 45 °C and 60 °C for 48 h each for gradual and even polymerization (Meepool et al., 2006).

The specimen blocks were cut at 60 to 90 nm thicknesses using an ultramicrotome (Leica, Model Ultracut R) and placed on a meshed copper grid. The ultrathin sections were first stained in saturated alcoholic uranyl acetate followed by counterstaining in 0.1% aqueous lead citrate and observed in a TEM (TECNAI 20, FEI) operated at 70 kV. Additional semithin sections (500–1,000 µm) were also stained with 1% aqueous methylene blue for light microscope observation.

3. Results

The mean water temperature and salinity during the sampling period were 29.81 °C and 30.46 psu, respectively while the mean pH was 8.09 (Fig. 2). The mean shell length (SL) of the small, medium and large green mussels were 2.3±0.2 cm, n=130, 5.6±0.3 cm, n=130 and 8.6±0.4 cm, n=130 respectively. A high prevalence of infection was found in all size classes from November 2003 to June 2004. Prevalence appears to have dropped sharply from July 2004. During the last month of sampling in November 2004 the prevalence in all size classes had increased to levels comparable to November 2003 (Fig. 3). From statistical analysis the prevalence profile of the *Nematopsis* parasite in the three size categories were not significantly different. However, infection intensity was found to be lowest in the smallest size category (Tables 1 and 2).

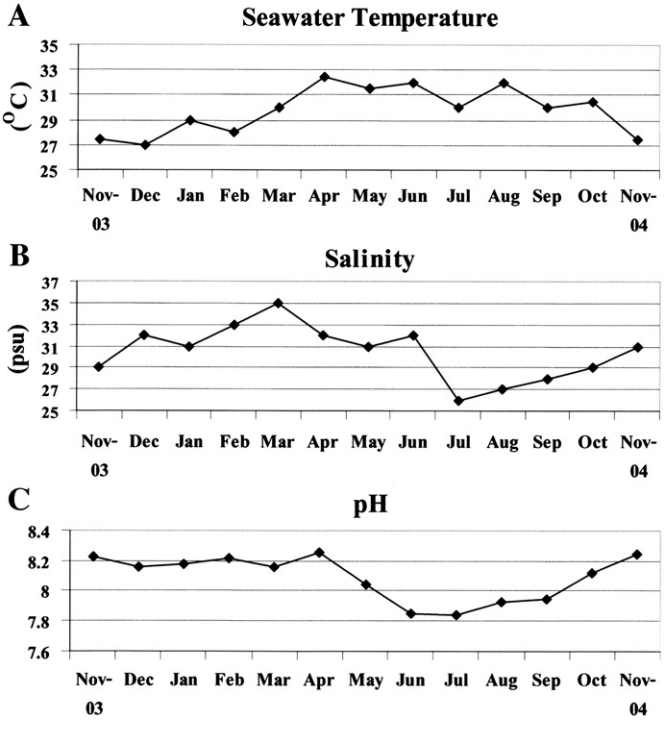

Fig. 2. Monthly variation of seawater temperature (A), salinity (B), and pH (C) from the sampling station.