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Rapid Communication

First record of the nonindigenous *Penaeus monodon* Fabricius, 1798 (Penaeidae) in the Caribbean Sea of Costa Rica, Central America, with observations on selected aspects of its reproductive biology

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Abstract

This contribution reports, for the first time, the presence of the giant tiger shrimp *Penaeus monodon* Fabricius, 1798 on the northern Caribbean coast of Costa Rica. This closed-thelycum penaeid species is originally from the Indo-West Pacific, and it has been reported from other regions of the Western Central Atlantic; however, the reproductive biology of this species has not been examined in its non-native range. Adult females and adult males were captured by local fishermen near the Colorado River mouth during September and October 2014; animals were preserved in ethanol for further identification, and an evaluation of reproductive condition was conducted based on visual and histological staging of ovaries. Terminal ampoules of males were analyzed histologically to document spermatogenic activity. The specimens examined exhibited ovarian maturation, spermatophore production, and evidence of successful mating. The presence of this species can affect food chains and disturb ecosystem function and structure and locations where it is not native, which can, in turn, threaten ecosystem services to people dependent on this ecosystem for their livelihood.

Key words: giant tiger shrimp, invasive species, ovarian maturation, spermatophores, histology

Introduction

Six species of the family Penaeidae occur along the Caribbean coast of Costa Rica: *Farfantepenaeus subtilis* Pérez-Farfante, 1967, *Farfantepenaeus brasiliensis* Latreille, 1817, *Metapenaeopsis smithi* Schmitt, 1924, *Metapenaeopsis martinella* Pérez-Farfante, 1971, *Litopenaeus schmitti* Burkenroad, 1936 and *Xiphopenaeus kroyeri* Heller, 1862 (Vargas and Cortés 1999; Wehrtmann and Cortés 2009). In the Wildlife Refuge Barra del Colorado and the National Park Tortuguero (northern Costa Rica), the most abundant species are *L. schmitti* and *X. kroyeri* (Keto-SINAC 2013). A non-native penaeid species has now colonized the area.

Penaeus monodon Fabricius, 1798, is a closed-thelycum penaeid shrimp from the Indo-West Pacific with a wide distribution from South Africa to Australia (Motoh 1985). A worldwide interest in the culture of *P. monodon* was established many years ago, stimulating its introduction in captivity outside the natural range. In the United States, the first wild captures were reported in 1988 following the accidental release from culture ponds in South Carolina (Fuller et al. 2014). Elsewhere in the Western Atlantic, *P. monodon* was first reported from Brazil (Coelho et al. 2001; Silva et al. 2002); then the Gulf of Mexico, United States (in 2006; Fuller et al. 2014); followed by Venezuela (Aguado and Sayegh 2007), Colombia (Gómez-Lemos and Campos 2008), Mexico (Wakida-Kusunoki et al. 2013), and Cuba (Giménez Hurtado et al. 2013).

According to the Convention on Biological Diversity (CBD 2012), invasive alien species represent one of the primary threats to biodiversity,

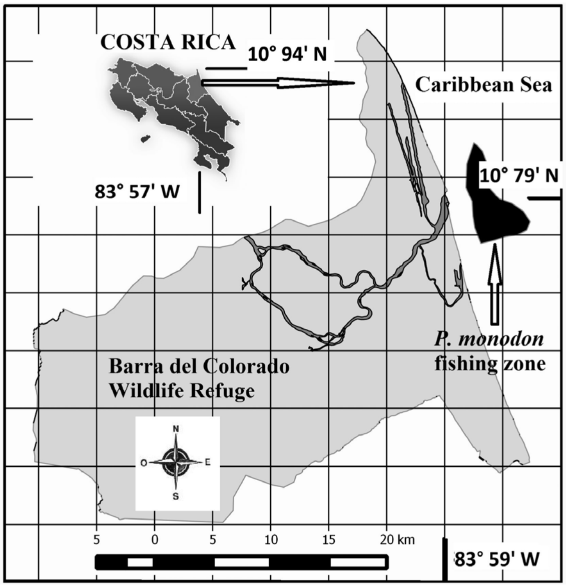

Figure 1. Sampling site of *Penaeus monodon* in the Caribbean Sea of Costa Rica.

especially in geographically and evolutionary isolated ecosystems. Therefore, early detection, rapid response, possible eradication, and long-term management should be undertaken when prevention fails (Simberloff et al. 2013). Based on the published literature, *P. monodon* clearly is spreading in the Western Atlantic, including the Caribbean Sea. However, there is no information on basic biology, including reproduction, of the species in these newly invaded regions. This contribution reports the detection of *P. monodon* in the Caribbean coast of Costa Rica, and provides preliminary information on selected aspects of the biology of the species.

Materials and methods

Shrimp capture

Specimens of *Penaeus monodon* were captured during September and October 2014 by artisanal fishermen trawling for shrimp in water 12–14 m deep off the mouth of Colorado River (Figure 1; 10°79′ N; 83°59′ W). All specimens retained were immediately injected with ethanol (96%) and stored in ethanol for later analysis. Carapace length (CL, to 0.1 mm accuracy) of specimens was measured with calipers from the base of the eyestalk to the posterior margin of the carapace, and total body weight (BW in g) was also recorded. Five adult females and five adult males were retained during the sampling trips. Of these, two females and two males were deposited in the Museo de Zoología at Universidad de Costa Rica (accession number MZUCR-3343-01).

Identification

Adult females and males of *Penaeus monodon* were identified using the criteria provided by Chan (1998) and Tavares (2002). The distinctive characters of *P. monodon*, as compared to native species, are: rostrum with 6 to 8 upper teeth and 3 lower teeth; adrostral crest short; gastrofrontal crest absent; hepatic crest nearly horizontal; fifth leg without exopod; and body with dark transverse cross bands on the abdomen (Figure 2).

Reproductive evaluation

Ovarian maturation was examined for the three adult females. The individuals were dissected and ovarian maturation stages were evaluated based on ovarian size and color as described by Tan-Fermin and Pudadera (1989), which associates the practical arbitrary stages (0-V) to the histological condition. The stages used were:

Stage 0: Previtellogenic. The ovary is colorless or white.

Stages I-II: Vitellogenic. Ovary is thick and the color is grayish green to blue green. Ovarian width at anterior abdominal region is 5 to 15 mm.

Stages III-IV: Cortical rod. The ovary is thick, solid, with granular texture and the color is olive or dark green. Ovarian width at anterior abdominal region is 20 to 30 mm.

Stage V: Spent. Ovaries are thin, similar to stage 0, or they may have dark patches.

According to Tan-Fermin and Pudadera (1989), ovarian maturity in wild *P. monodon,* is similar among regions of the ovary. Therefore, to corroborate the visual scale and vitellogenic stages, abdominal ovarian lobules from ethanol-preserved females were post-fixed in Davidson' solution for 24 h and stored in 50% ethyl alcohol (following Bell and Lightner 1988); embedded in paraffin; cross sectioned; and stained in hematoxylin plus eosin. This work was done at a private laboratory (Laboratorio de Patología Andrómeda, S.A., Guadalupe, San José, Costa Rica). Spermatophore maturity was examined for the three adult males. The terminal ampoules from both sides of the reproductive system were dissected and post-fixed in Davidson' solution. Ampoules were processed for histological examination as for the ovaries except that longitudinal sections were taken.

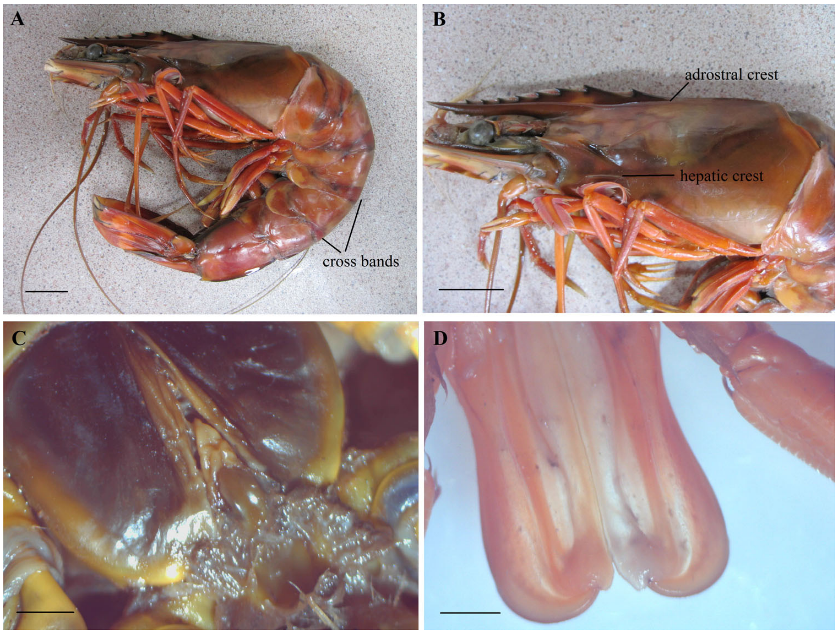

Figure 2. *Penaeus monodon* captured in the Northern Caribbean of Costa Rica. A–B: diagnostic characteristics. Sexual dimorphism in *Penaeus monodon,* C: female´s thelycum, D: male´s petasma. Scale bars = 2 cm (A–B), 2 mm (C–D). Photographs by J. Alfaro-Montoya.

Mating activity and stomach content

To demonstrate that this species had mated in the study area, the thelyca of the three adult females were dissected and tissue sections were transferred to Eppendorf tubes (2 mL) with ethanol, and gently homogenized with a plastic grinder to release spermatozoa (following Pongtippatee et al. 2007). The tissue debris was allowed to settle and the supernatant examined under a light microscope for transferred spermatozoa. The stomachs (foreguts) of the three females and three males were removed and the contents examined under a stereo microscope to identify major food groups.

Results and discussion

Shrimp fishing in the study area (Figure 1) mostly targets *Litopenaeus schmitti* and *Xiphopenaeus kroyeri*; however, the local fishermen report *Penaeus monodon* now represents around 2% of the catch (as biomass). This artisanal fishery at the mouth of the Colorado river represents 15% of the total catch from the region (Bolaños and Segura 2004), representing about 230 kg in recent years (Keto-SINAC 2013).

The adult females ranged in size from 61.3 to 80.7 mm CL (BW: 107 – 187 g) and males were from 51.4 – 63.6 mm CL (BW: 59 – 119 g). This is similar to the maximum size of *P. monodon* recorded from Asia, which is 71 and 81 mm CL (270 mm total length, TL) for males and females, respectively (Motoh 1985). Elsewhere, females up to 330 mm TL have been reported (Primavera et al. 1998). In the Western Central Atlantic, maximum sizes were reported as: 500 g from Colombia (Sandoval et al. 2014): 298 mm TL and about 200 g in the USA (Fuller et al. 2014); 290 mm TL in Cuba (Giménez Hurtado et al. 2013); and 317 mm TL in Mexico (Wakida-Kusunoki et al. 2013).

Based on visual evaluation of the ovaries, one female was in development stage 0 and two were in stages III-IV; however, the histological analysis demonstrated that the apparent stage 0 female was in a spent condition (stage V). The spent condition was characterized by the presence of remaining oocytes with yolk (oocyte diameter (OD): > 100 µm) or with cortical rods (OD: 138 µm) in addition to previtellogenic oocytes (Figure 3). The histological evaluation of maturing ovaries (stages III-IV) demonstrated the massive presence of oocytes in advanced stages of vitellogenesis without the formation of cortical rods (OD: > 130 µm). Ovarian width at anterior abdominal region measured 7 and 8 mm, for these maturing females.

Interestingly, the color of ovaries from both females was orange instead of green as is reported

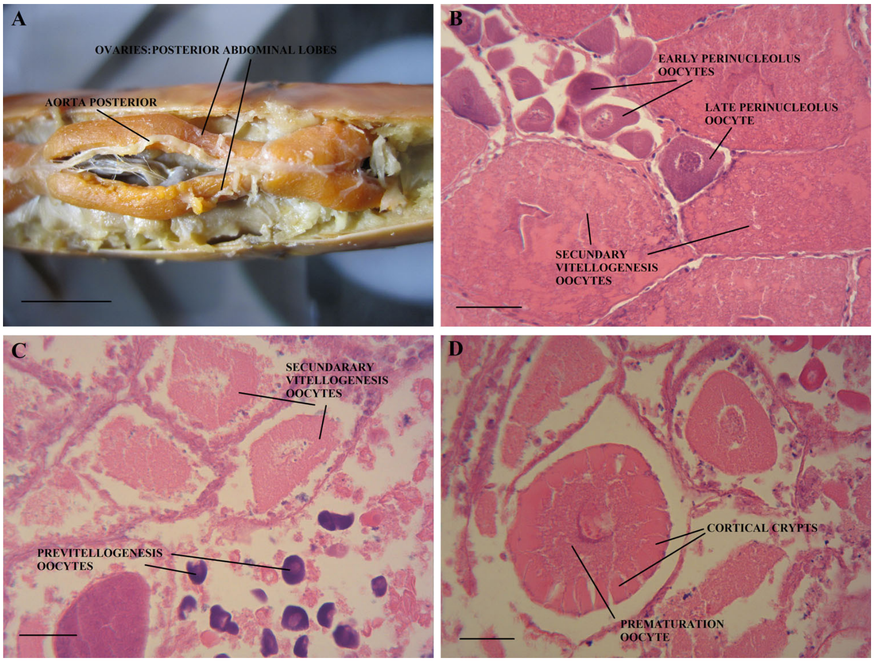

Figure 3. Ovarian maturation in wild *Penaeus monodon* captured in the Caribbean of Costa Rica. A: stage III ovary, scale bar = 1 cm. B: ovary in secondary vitellogenesis, C: spent stage with remaining yolky oocytes, D: spent stage with a prematuration oocyte (cortical crypts). Scale bars = 50 µm. Photomicrographs by J. Alfaro-Montoya.

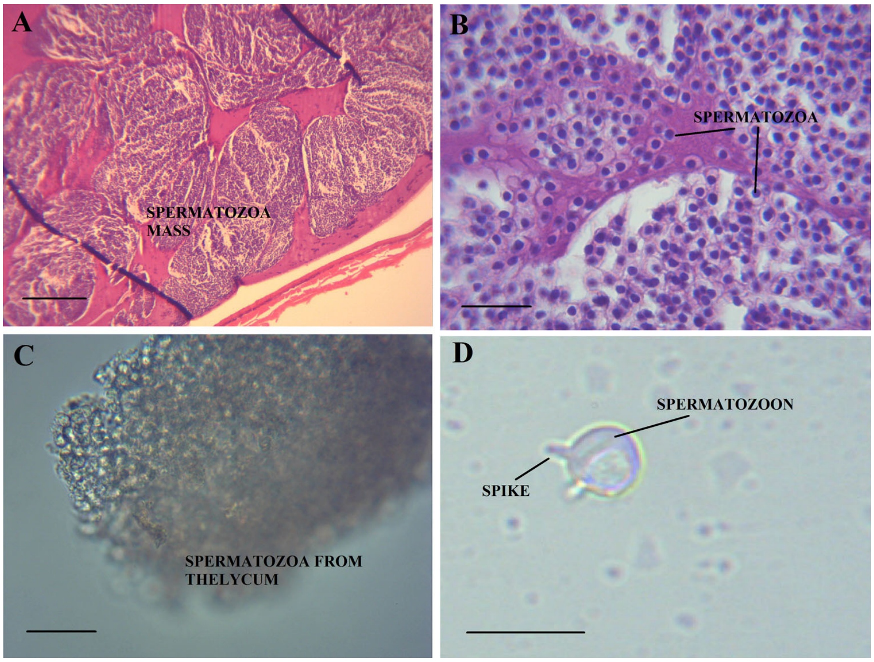

Figure 4. Spermatophore maturation of wild *Penaeus monodon* captured in the Caribbean of Costa Rica. A: masses of spermatozoa from terminal ampoule, scale bar = 200 µm. B: spermatozoa from terminal ampoule, scale bar = 20 µm. C: spermatozoa from female´s thelycum, scale bar = 20 µm. D: transferred spermatozoon from thelycum, scale bar = 10 µm. Photomicrographs by J. Alfaro-Montoya.

for Asian specimens (Tan-Fermin and Pudadera 1989; Uawisetwathana et al. 2011). This is a novel observation for this species; however, variations in color for maturing ovaries from yellow to orange and green occur in *Litopenaeus setiferus* Linnaeus, 1767 and *Farfantepenaeus aztecus* Ives, 1891 (Chamberlain and Lawrence 1983). Although both species develop these color variations, *F. aztecus* shows more profound variations. Our color observations in *P. monodon* were made in ethanol preserved females, and this condition could alter the natural coloration of ovaries. Similar procedures applied to the native species, *Rimapenaeus byrdi*

Burkenroad, 1934, which develops only green ovaries, indicated they maintain their color after preservation.

The histology of terminal ampoules of the male *P. monodon* revealed the presence of masses of spermatozoa, filling completely the corresponding chamber (Figure 4). Since the females´ thelyca also contained masses of spermatozoa (Figure 4), the shrimp in this population clearly have mated successfully.

The reproductive pattern of closed-thelycum penaeid shrimps consists of reproductive molting, mating, ovarian maturation, and spawning (Primavera 1985). In their native range, sub-adult *P. monodon* occur offshore in waters > 20 m deep, and adults inhabit sand or muddy-sand bottoms at depths of 160 m (Motoh 1985); however, migration and mating patterns in the Western Atlantic may be different. Our observations, admittedly from only a few adult females, indicate that ovarian maturation has taken place in the coastal waters of the Northern Caribbean of Costa Rica at depths of 12–14 m. Moreover, the observation of a spent female suggests that spawning activity also occurred in the shallow waters of the study area.

In the mariculture of *P. monodon*, the industry is confronting major problems related to disease outbreaks, slow growth, shrimp prices, and male fertility (Pongtippatee et al. 2007). After mating, spermatozoa are physiologically capacitated, as a pre-requisite for fertilization, in the female thelycum (Vanichviriyakit et al. 2004). However, spermatogenesis, and therefore spermatophore quality, is negatively compromised under culture conditions. Our limited evidence suggests normal male fertility in the natural environment of the Caribbean Sea. The histology of terminal ampoules indicates that *P. monodon* males are experiencing spermatogenic processes and producing abundant, and apparently normal, spermatozoa. However, demonstration of successful fertilization by means such as laboratory spawning and nauplii evaluation is still required. Moreover, detection of large numbers of postlarvae in protective nursery areas (i.e., estuaries) is needed to confirm that this exotic species established in the Caribbean.

The examination of a small number of *P. monodon* stomachs revealed mainly gastropod shell fragments and small amounts of crustacean exoskeleton. This finding is consistent with published reports that *P. monodon* is a predator of slow-moving benthic microinvertebrates (Motoh 1985); however, a more extensive evaluation of the feeding habits of the population is required to assess possible effects on the non-native ecosystem.

This is the first report on the presence of this invasive species in the Caribbean of Costa Rica. Additionally, reproductive activity has occurred in the study site near the Colorado River mouth, suggesting establishment could occur in this new environment. Reproductive condition was only evaluated in a few animals and cannot be assumed to represent the sexual activities of the population throughout the year. However, these observations do confirm that this nonindigenous shrimp is physiologically capable of activating the reproductive endocrine mechanisms in this new environment.

The presence of this species may alter the food web, thereby affecting ecosystem functioning and ultimately the ecosystem services that support fishing families on the northern Caribbean coast. As in dealing with other invasive species in the wider Caribbean (Gómez Lozano et al. 2013) and in Costa Rica (Sandel et al. 2015), it is urgently recommended to initiate a local inter-institutional strategy developed in collaboration with stakeholders from the environmental and fisheries sectors, universities, and local communities, in order to reduce negative effects and provide a framework to facilitate response to the invasion.

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