

Journal of Coastal Life Medicine

journal homepage: www.jclmm.com



Original article <https://doi.org/10.12980/jclm.5.2017J7-99>

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Composition of population and abundance of *Penaeus semisulcatus* (De Haan, 1844) in relation to microbial population from Al-Faw, Basrah, Iraq

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ARTICLE INFO

Article history:

Received 6 Jun 2017

Received in revised form 3 Jul, 2nd revised form 19 Jul, 3rd revised form 8 Aug 2017

Accepted 20 Aug 2017

Available online 30 Aug 2017

Keywords:

Penaeus semisulcatus

Microbial

Al-Faw City

ABSTRACT

Objective: To evaluate the composition of population and abundance of green tiger shrimp *Penaeus semisulcatus* (*P. semisulcatus*) in relation to presence of main groups of pathogenic bacteria isolated from tissues of different stages of shrimps in Al-Faw City, Basrah, Iraq.

Methods: The specimens of shrimp *P. semisulcatus* were collected from December 2016 to February 2017 by commercial trawl net. Pathogenic bacteria were isolated from muscle tissues of *P. semisulcatus*. Microbial species were characterized based on morphological and biochemical tests.

Results: Monthly changes in the composition of population of *P. semisulcatus* during three months of the present study were observed; the highest abundance of males and females was recorded in February 2017. The sex ratio indicated a preponderance of females over males in study area. The average total numbers of isolated bacteria were 14.31×10^3 CFU/100 mL from shrimp in young and larval stages, while from adult shrimps average total number of bacteria was 18.46×10^2 CFU/100 mL. Pathogenic bacteria isolated presumably belonged to three species, namely, *Escherichia coli*, *Vibrio cholerae* and *Pseudomonas aeruginosa*.

Conclusions: The current study showed that the total bacteria number in young and larvae specimens of *P. semisulcatus* was greater than that in adult specimens, and exceeded the allowed maximum bacteria number.

1. Introduction

The green tiger shrimp *Penaeus semisulcatus* (*P. semisulcatus*) is a prominent marine species with high economic value in markets of East Asian countries and in countries of Europe, Australia and Arabian Gulf (Persian Gulf)[1,2]. Many researchers studied and evaluated size structure, abundance and shrimp fishery of green tiger shrimp (*P. semisulcatus*) in various water bodies around the

world[2-7]. Bacterial infection is one of the major diseases in shrimp aquaculture[8].

Infectious diseases by microbes and parasites are not only a major obstacle to breeding, but also inflict tremendous economic losses to the aquaculture industry[8]. Fishery products are generally regarded as high risk commodity in respect of pathogens, natural toxins and other possible contaminants and adulterants, although there has been a significant increase in the knowledge of shrimp diseases[9,10]. In this context, this study aimed to evaluate the composition of population and abundance of green tiger shrimp (*P. semisulcatus*) in relation to presence of main groups of pathogenic bacteria isolated from tissues of different stages of shrimps in Al-Faw City, Basrah, Iraq.

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

2. Materials and methods

Specimens of *P. semisulcatus* were collected during three months from December 2016 to February 2017 by commercial trawl net for half an hour each time, at Al-Faw City, Basrah, Iraq. To avoid further contamination, during transportation from the collection site to laboratory, all the specimens obtained were identified, stored in ice boxes and transported to Laboratory of Marine Science Center, Basrah University and were frozen for other analysis. A total of 625 specimens of adult stage and 80 specimens of young and larval stage were analyzed during the study period.

2.1. Composition of population

The carapace length (CL, mm) was measured and defined as distance from the posterior margin of orbit to the posterior edge of the carapace (Figure 1). For the population study, the values of CL were grouped into size classes, which were analyzed monthly.



Figure 1. Specimens of *P. semisulcatus*.

2.2. Microbial medium

The medium was prepared by dissolving nutrient agar (NA) (Merck) in distilled water and was autoclaved.

2.3. Isolation of bacteria

Bacterial species were isolated by serial dilution technique using nutrient agar medium[11].

The muscles of samples of *P. semisulcatus* were dissected under a sterile condition and homogenized tissue was used for bacterial isolation tests. Each homogenized tissue sample was serially diluted and spread on nutrient agar medium separately, with one plate maintained as a control without sample. The plates were incubated at 37 °C for 24 to 48 h for observation of the bacterial colonies.

After incubation, the total number of colony forming unit (CFU) was determined and representative colonies were sub-cultured for identification. Bacterial numbers were calculated as the mean for each set of duplicates and expressed as CFU/100 mL of homogenized tissue. The bacteria were isolated from a random group of colonies of agar plates. Colonies were repeatedly cleared by sub-culture on agar[12].

2.4. Identification of isolated bacteria

The isolated bacterial species were identified by the following procedures. The morphological and biochemical characteristics of the individual colony was recorded. The individual colony was transferred to nutrient agar. The isolates were subjected to different biochemical tests, for example, Gram staining, motility test, indole test, methyl red test, Voges Proskauer test, catalase test, nitrate test and carbohydrate fermentation test as described by Buchanan and Gibbons[13].

2.5. Statistical analysis

Statistical analysis of the data was performed using the Excel (Microsoft) and significant differences were determined using ANOVA by Minitab statistical software version 18.2 and a probability level at 0.05 indicates significant difference.

3. Results

3.1. Composition of population

A total of 625 specimens of *P. semisulcatus* of adult stage, 302 males and 323 females, and 80 specimens of young and larval stage were collected from December 2016 to February 2017. The CL of males ranged from 15.30 to 61.60 mm with the mean length of (35.660 ± 0.056) mm, and the CL of females ranged between 17.80 and 65.70 mm with the mean length of (41.280 ± 0.074) mm (Figures 2–4).

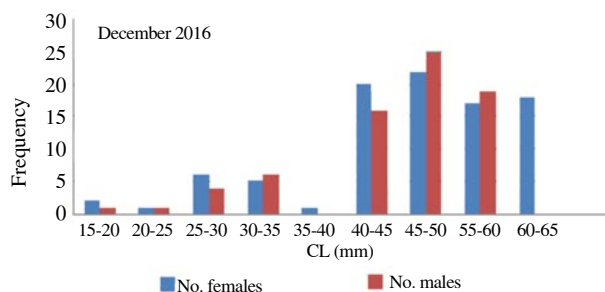


Figure 2. Size structure of males and females of *P. semisulcatus* in December 2016.

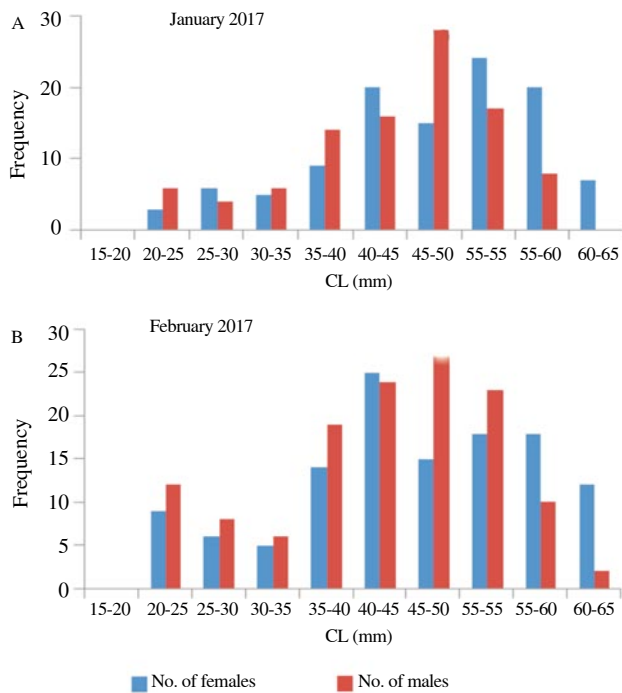


Figure 3. Size structure of males and females of *P. semisulcatus* in January (A) and February (B) 2017.

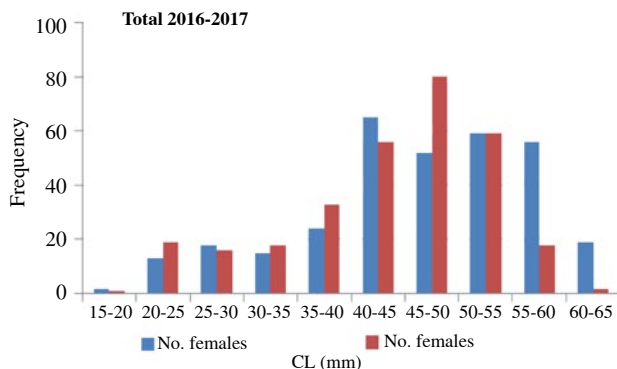


Figure 4. Total frequency of CL of males and females of *P. semisulcatus*.

Statistical analysis showed that there were significant differences in size structure between males and females of *P. semisulcatus* during the study period ($P < 0.05$), with females reaching larger sizes than males.

Table 1 shows the number of specimens for males and females in each month. The highest numbers of females (122 specimens) and males (131 specimens) were recorded in February 2017.

Table 1

Shrimp abundance, number of females and males in the study area from December 2016 to February 2017.

| Months | No. of females | No. of males |
|---------------|----------------|--------------|
| December 2016 | 92 | 72 |
| January 2017 | 109 | 99 |
| February 2017 | 122 | 131 |
| Total number | 323 | 302 |

The sex ratio indicated a preponderance of females over males in the study area. Out of the specimens, 323 (51.7%) were females

and 302 (48.3%) males. Male to female ratio in the sampling area was 1:1.14.

3.2. Pathogenic bacteria isolated from *P. semisulcatus*

The present study deals with the bacteria suspected to be the major reason in causing mortality of *P. semisulcatus* young and larval specimens. The average total numbers of isolated bacteria were 14.31×10^3 CFU/100 mL from shrimp young and larval specimens and 18.46×10^2 CFU/100 mL from adult specimens as shown in Table 2. This bacterial number was very important to note since one of the first criteria of microbiological test is that the maximum total bacteria count should be 1.0×10^3 CFU/100 mL for evaluating quality of shrimp larvae and adult macerated in agar, of which more than 90% of the colonies should be yellow in color. The present study showed that the total bacteria in *P. semisulcatus* larvae exceeded the allowed maximum number and therefore the mortality of larvae was mainly due to bacteria. Furthermore, most of the colonies on NA plate had a yellow color.

Table 2

The number of bacterial isolates from *P. semisulcatus* (CFU/100 mL).

| Bacterial colonies | Bacterial genera isolated | No. of bacteria isolates in shrimps | |
|-----------------------------------|---------------------------|-------------------------------------|---------------------|
| | | Young and larvae | Adult |
| S1 | <i>Escherichia</i> | 13.67×10^3 | 17.14×10^2 |
| S2 | <i>Vibrio</i> | 16.82×10^3 | 22.26×10^2 |
| S3 | <i>Pseudomonas</i> | 12.46×10^3 | 15.81×10^2 |
| Average total number (CFU/100 mL) | | 14.31×10^3 | 18.46×10^2 |

3.3. Identification of bacteria isolated from shrimps

All bacterial isolates including pathogens antagonistic isolates were identified using biochemical and morphological tests. Three species of bacteria including *Escherichia coli* (*E. coli*), *Vibrio cholerae* (*V. cholerae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (Table 3) were identified by the morphological and biochemical tests.

Table 3

Biochemical testes for bacterial isolates from tissues of *P. semisulcatus*.

| Test | Colonies | | |
|-------------------------------------|----------------|--------------------|----------------------|
| | S1 | S2 | S3 |
| Color of colony | Y | Y | Y |
| Gram staining | + | + | - |
| Motility | - | + | + |
| Shape | Rod | Rod | Rod |
| Methyl red | + | - | - |
| Indole | + | - | - |
| Voges Proskauer test | + | + | - |
| Citrate | + | - | + |
| Urease | + | + | + |
| Tribile sugar iron test | + | + | + |
| Catalase | + | + | - |
| Oxidase | + | + | + |
| Nitrate | + | + | - |
| Carbohydrate fermentation | + | + | + |
| Identification of bacterial species | <i>E. coli</i> | <i>V. cholerae</i> | <i>P. aeruginosa</i> |

4. Discussion

In the present work, monthly variations of length of *P. semisulcatus* were used to determine and estimate size structure of population in males and females and other population dynamic parameters. The results of morphometric measurements in the present study clearly showed that female shrimps had larger size than males. The size structure and abundance is common in other species of genus *Penaeus*[5-7].

Identification of bacteria was done based on the morphological and biochemical characteristics and the comparison, and the bacteria were confirmed as *E. coli*, *V. cholerae* and *P. aeruginosa*. Other researchers have reported that diseases in shrimp are caused by bacteria of the genus *Aeromonas*, *Pseudomonas*, and *Vibrio*[14-16].

In general, the results of this study indicate that the bacterial load in shrimp in marine water and sediment increases with increase in organic load, which is in agreement with previous reports by other researchers[17,18]. Based on the findings of the present study, it is concluded that the pathogenic bacteria are perhaps the most important pathogens in shrimp. There is also a need to control the pathogenic microbes.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We would like to thank D. TalibKhalaf, Marine Sciences Center, Basrah University, for reading the manuscript and for their valuable advice and suggestions.

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