The investigation of bacteria, parasite and fungi in blue crabs (*callinectes sapidus*, rathbun 1896) caught from Akyatan lagoon in east Mediterranean Sea

Research Article

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Abstract

Bacteria, parasites and fungus in blue crabs (*Callinectes sapidus*) caught from Akyatan Lagoon in East Mediterranean Sea, Adana, Turkey were investigated. Total 501 crab samples were used and average length and weight were 13.1-14.4cm and 141.2-293.8g, respectively. Total 21 bacteria belonging to 14 different genera which are *Acinetobacter baumannii*, *Acinetobacter lwoffii*, *Aeromonas cavaie*, *Aeromonas hydrophila*, *Serratia rubidea*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio mimicus*, *Citrobacter frendii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Esherichia vulneris*, *Klebsiella phenmonaie*, *Klebsiella oxytoca*, *Moraxella* sp., *Proteus mirabilis*, *Pseudomonas* sp., *Micrococcus* sp., *Staphylococcus aureus*, *Bacillus* sp., were isolated from 301 craps samples. Parasites that *are Ichthyophthiris multifilis*, *Cryptobia* sp., *Trypanosoma* sp., *Cloronorchis* sp.'s metacerceria, amoeboid trofont to belong *Hematodinium* sp., Metacestoda, spore of *Microsporidans*, *Ameson* sp., Trematodes metacerceria were identified whereas fungus that are Oospore and *Lagenidium* sp. Zoospore were found in the blue crabs samples.

Key Words: Bacteria, *Callinectes sapidus*, Fungus, Parasite

Introduction

The rapid development in aquaculture industry is mainly based on the amount of fish production. In addition to the fish species cultured, the amount of cultured aquatic invertabrates (crab, shrimp, bivalviae, molluscs etc.) also plays an important role in this rapid development of aquaculture. Among the cultured invertebrates, approximately 22 species of crabs have an important place in the production of shellfish (Siddiquie, et al, 1987).

Edible meat is important due to proteins and mineral content of waste parts pf blue crabs (*Callinectes sapidus*) since the waste products are used as feed additives that allows the assessment as an economic inputs. One of the products obtained from blue crab is chitin, which is used in textiles, inks, construction adhesives and cosmetic industry (Enzenross, et al, 1995).

The main source of blue crabs (*Callinectes sapidus*), which has an increasing consumption in the world, is the northern shores of Northern America.

This species has also been reported from the Western Mediterranean Coasts since the beginning of the 20th century and subsequently widely distributed through the eastern Mediterranean. In Turkey, it was first reported in the eastern Mediterranean waters off the Hatay province and in the Iskenderun Bay. The blue crabs (*Callinectes sapidus*) is also distributed along the Turkish Mediterranean coastal line starting from Finike, around Anamur, Taşucu, Kapızlı, Tuzla, Karataş, Yumurtalık ve Iskenderun (Gönül, 1997; Gelibolu, 2006). Serious populations of blue crab have also been reported in Mersin-Silifke, Akyatan and Yumurtalık lagoons existing in the Turkish Mediterranean Coast and this population has been attributed to nutrient enrichment (Anonim, 1997; Türeli, 1999; Gelibolu, et al., 2009).

In Turkey, there are many factors that cause problems for sales and marketing of these crabs unless the value of the waste product is not understood. However there are some risks such as bacteria, parasites and fungus in blue crab in order to use in the industry (Andersen, 2000; Flowers, et al., 2000; Krol, 2002). There are not enough studies related to bacteria, parasites and fungi contamination in blue crab caught from the Akyatan Lagoon, Adana, Turkey.

Therefore, the purpose of this study was to investigate contamination level of bacteria, parasites and fungi in blue crabs. For this, totally 501 crab samples were used for isolation and identification of the bacteria, parasites and fungi.

Materials and Methods

Callinectes sapidus belongs to Arthropoda member of Portunidae family that is used as research material. Blue crab was caught from Akyatan Lagoons, Karatas, Adana in the coast of the Mediterranean Sea. Sampling was carried out every month between October 2011 and October 2012 and also summer season in 2013-2014. The average length of ranged from 13.1 to 14.4cm and average weight ranged from 141.2 to 293.8g. total of 501 crabs were caught in the vicinity of the lagoon and sea links using one way of trap systems. After the crab was caught, they brought to the laboratory as live. After being sterilized using absolute ethanol, hemolymph samples for bacteriological studies were inoculated into on blood agar, endo agar, marine agar medium, applause – Mansur and Brain A-B- Media (Camdali and Ildir, 1976; Mims, et al., 2004; Ruangpan and Tendencia, 2004). Amount of hemolymph sample also stored making DNA extraction at -20^{0}C for determining Dinoflagellate *Hematodinium perezi*. Reproducing

colonies in the media used for bacteriological examination have been used to identify conventional methods after morphological examination (gram staining, oxidase, catalase test, etc.) (Roberts, 2001; Christopher and Bruno, 2003; Zoletti, et al, 2006). Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germany), according to the manufacturer's instructions.

Parasitological examination of crabs was determined in the hemolymph samples using Giemsa staining technique under a microscope after being examined macroscopically. In Fungal examination, abdominal area where the eggs of crabs after being examined macroscopically was carried out by scraping samples on slides fixing with methanol and dyeing Giemsa stain that was examined under the microscope (Maas, et al., 1999; Shields, 2003).

In parasitical identification we used as an identification key; (Couch, 1942; Markevic, 1951; Yamaguti, 1958;Yamaguti, 1961; Yamaguti, 1963; Gussev, 1985; Gussev, 1985; Gusev, et al., 1987; Roberts, 1989; Moravec, 1994; Shields and Overstreet, 2003) articles.

In fungal identification was used as an identification key; (Couch, 1942; Bland and Amerson, 1973; Gotelli, 1974; Bian, et al., 1979; Gil-Turnes, et al., 1989; Nakamura and Hatai, 1995; Ramasamy, et al., 1996; Kitancharoen, et al., 1997; Maas, et al., 1999; Leaño, 2002) articles.

Result and Discussion

Total 501 blue crabs have been examined under the laboratory conditions. A total of bacteria species (Table 1) was isolated from 301 crabs, which all bacteria genus belongs to 14 different genera and 337 bacterial sampling density was observed in December 2011, he highest density of bacteria was observed in August 2014 sampling period.

In parasitical examination, *Ichthyophthiris multifilis*, *Cryptobia* sp., *Trypanosoma* sp., *Cloronorchi*s sp. metacercaria, *Hematodinium* sp. amoeboid trofont, Metacestod, *Microsporidan* spore, *Ameson* sp., Trematod metacerceria belong to 9 genera were isolated and identified (Table 2). In fungal examination, Thraustochytrid oospore and *Lagenidium* sp. zoospore were found in the samples (Table 2).

Table 1. Bacteria Species Isolated From Blue Crab (*Callinectes sapidus*)

Gr (-) Bacteria	Number of Blue Crab	Gr (+) Bacteria	Number of Blue Crab
Acinetobacter baumannii	19	*Micrococcus* sp.	10
Acinetobacter lwoffii	11	*Staphylococcus aureus*	24
Aeromonas cavaie	9	*Bacillus* sp.	13
Aeromonas hydrophila	34		
Serratia rubidea	5		
Vibrio alginolyticus	26		
Vibrio parahaemolyticus	42		
Vibrio vulnificus	14		
Vibrio mimicus	12		
Citrobacter freundii	7		
Enterobacter aerogenes	12		
Enterobacter cloacae	10		
Escherichia coli	20		
Klebsiella phenmonaie	8		
Klebsiella oxytoca	15		
Moraxella sp.	13		
Proteus mirabilis	22		
Pseudomonas sp.	11		

Table 2. Parasitical and Fungal Agents Isolated from Blue Crab (*Callinectes sapidus*)

Parasitical Agents	Fungal Agents
Ichthyophthiris multifilis	Thraustochytrid oospore
Cryptobia sp.	*Lagenidium* sp. zoospore
Trypanasoma sp.	
Cloronorchis sp. Metacercaria	
Hematodinium sp. amoeboid trofont	
Metacestod	
Microsporidian spore	
Ameson sp.	
Tematod metacercaria	

Result obtained from this study showed that contamination with bacteria, parasites and fungi was seen most frequently in the summer season that is an agreement with other studies (Dawes, 1968; Leglise and Raguenes, 1975; Xu and Xu, 2002; Shields, 2003). This is the common factors in the vicinity of the Akyatan Lagoon since drainage channels and other formations that provide environmental pollution (chemicals used in the fields, fertilizers, waste of boat used for hunting, etc.) are polluted it. Also this situation is a large part of the diet of the crab is thought to be due to the creation of fish in the lagoon. The genus of Cryptobia of individuals fish parasites and belongs to Trypanosoma genus of individuals some periods use crabs as an intermediate host, but has been reported in some studies demonstrate no pathological phenomenon (Kozloff, 2004; Woo, 2002; Alvarez-Pellitero et al., 2004; Abowei et al., 2011). The blue crab observations made so far, fungal agents such as *Lagenidium callinectes*, *Haliphthoros milfordensis*, *Fusarium solani* and *Leptolegnia marina* (oomycetes) were found (Fisher, 1983; Gil-Turnes, et al., 1992; Shields, 2003). In our study, *Lagenidium* genera zoospore and Thraustochytrid oospore were found. During the summer, incidence of agents with an increasing salinity and temperature has been similar to other studies. It also similar the other studies due to regions where the other lagoons. The biggest difference from other areas of the region is associated with two the drainage channel. On the subject as, has carried out some research in Chesapeak Bay and Charliston Harbor a large number of pathogens have been reported (NOAA Chart 11524, 2014). The biggest difference from our study of these areas are used as port. Pathogens are transported with water inputs.

In addition, pathogen infection by ship from the harbor is spread. Improvement of flowing drainage channels with no pollutants into the lagoon, he control of waste of boats used in fishing, control of environmental pollutions and favorable conditions for hunting and eliminating stress factor by moving the crab are expected to reduce of the presence of pathogens.

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