

# First Episode of Shell Disease Syndrome in *Carcinus aestuarii* (Crustacea: Decapoda: Portunidae) in the Volturno River

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## Abstract

In this study is reported the first episode of a Shell Disease Syndrome (SDS) in wild specimens of *Carcinus aestuarii* from the polluted Volturno River estuary. Collected crabs showed erosive and ulcerative “black spot” lesions on the exoskeleton; histologically the lesions ranged from a mild to extensive and severe damages and intense haemocyte infiltration. Bacterial isolation confirmed the presence of some marine bacterial species with chitinolytic activity. The authors suggest that the detection of sds in wild crabs living in polluted waters could be used to monitor the health status of this environment.

**Keywords:** Shell disease syndrome; Histopathology; Bacteriology; *Carcinus aestuarii*; Biomonitor

## Introduction

Shell Disease Syndrome (SDS) is a term that describes a broad range of lesions in the exoskeleton of crustaceans supported by different species of chitinolytic opportunistic bacteria, belonging to different genera: *Vibrio*, *Listonella*, *Aeromonas*, *Pseudomonas*, *Alteromonas*, *Flavobacterium*, *Spirillum*, *Moraxella*, *Pasteurella* and *Photobacterium* [1-3].

In stressed and/or wounded specimens these bacteria can cause erosions on the exoskeleton and septicemia; moreover systemic tissues damages can occur if the bacteria complex penetrates internally [2]. SDS affects various species of crustaceans all over the world 35 i.e.: *Callinectes sapidus*, *Cancer magister*, *Cancer pagurus*, *Cancer irroratus*, *Cancer borealis* [4-8].

The disease is common in aquaculture facilities or in polluted waters [9-13]. In the past years the prevalence of the disease in natural populations has increased and the lack of baseline data on its distribution in the Mediterranean area represents a limit toward a better understanding of this syndrome Sindermann [14].

The aim of the present paper is to describe, for the first time, the presence of SDS in a wild population of *Carcinus aestuarii*. *C. aestuarii* is a common crab, belonging to the Portunidae Family; it has an extensive distribution in estuarine and lagoon waters of the Mediterranean Sea and plays an important ecological role in lagoon ecosystems as a component of the diet of fish Stentiford [15].

The examined specimens were collected from the Volturno River estuary. This area is a heavily polluted area of the Campania region (Southern Italy). The inorganic and organic compounds are the major contaminants [16,17]. The presence of high levels of nitrate indicating a severe contamination related to human activities (application of nitrogen fertilizers and wastewater disposal) was also reported Amodio [18] in a pollution risk map of the Volturno River. Degraded environmental conditions are generally considered to be associated with shell disease in crabs [1]. Low water quality might suppress immune-defence of residing animals leading to a weakened physiological state [4].

## Materials and Methods

In the spring of 2007, 50 adults *C. aestuarii* were collected from the

Volturno River estuary (Campania, Italy) with lobsterpots, in a single sampling, and submitted to our laboratory. All crabs showed “black spots” lesions typical of shell disease; each specimen was measured (carapace width) and the sex was determined. Lesions and disease signs (i.e.: limbs losses, lesions size, shape, color, distribution and position) 3 were 61 recorded and photographed.

Histological and microbiological assays were performed. For histological examination the diseased specimens were sacrificed and fixed with neutral buffered 10% formalin. The exoskeleton (decalcified in 5% formic acid), and the internal organs were processed for routine histopathology: dehydrated in graded alcohols, embedded in paraffin-wax, sectioned at 4-5 µm and stained with Haematoxylin-Eosin (H & E). Special staining as Periodic Acid Schiff (PAS) and Gram were also carried out.

Carapace lesions prevalence and severity was categorized following the *Stages Disease Index* [19]: in the 1<sup>st</sup> 69 stages the mild lesions consisted in small erosion of the epicuticle and calcified endocuticle; more extensive and profound erosion were characterized by endocuticle lesions with haemocytes infiltration and melanin deposition (2<sup>nd</sup> and 3<sup>rd</sup> stages). For microbiological assays, exoskeleton surface was sterilized with 70% ethanol and successively the hemolymph was withdrawn at joint of carapace and 5<sup>th</sup> pereopod into a sterile 1 ml syringe. Moreover, the lesions were scraped with a 0.1 ml disposable plastic loop. Hemolymph and scraped material were then spread on Marine Agar (Microbial Diagnostics) and TCBS agar (Thiosulfate Citrate Bile Sucrose-Oxoid). Cultures were incubated at 24 ± 1°C for 24-48 hours. Suspected colonies were isolated in pure culture and the strains were identified by morphological and biochemical tests [10].

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**Received** June 24, 2013; **Accepted** July 25, 2013; **Published** July 29, 2013

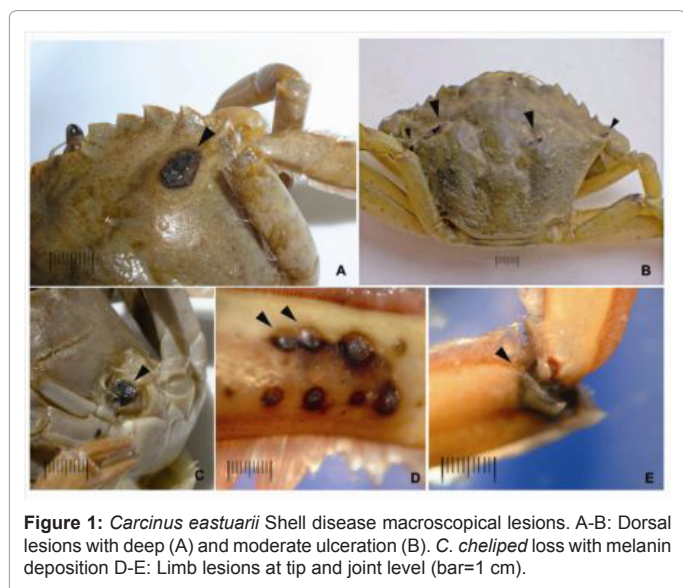
**Citation:** Mancuso M, Zaccone R, Carella F, Maiolino P, Vico GD (2013) First Episode of Shell Disease Syndrome in *Carcinus aestuarii* (Crustacea: Decapoda: Portunidae) in the Volturno River. J Aquac Res Development 4: 191 doi:10.4172/2155-9546.1000191

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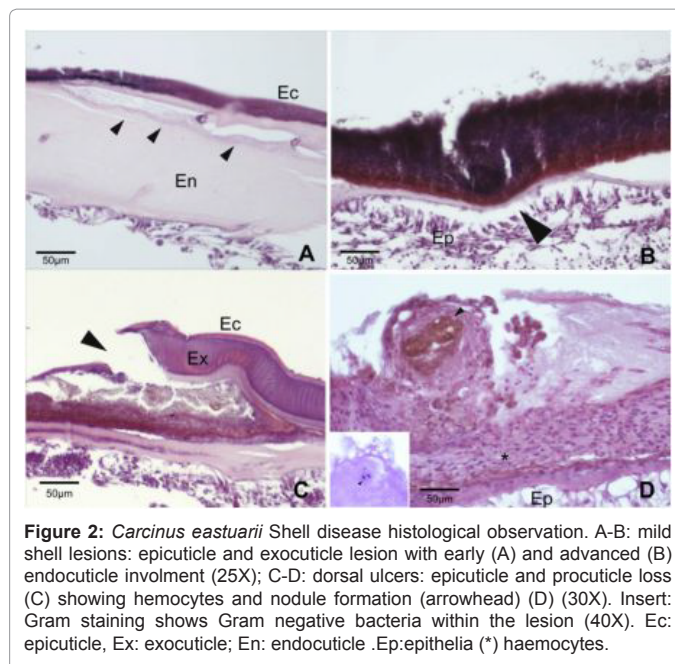
## Results

The specimens of *C. aestuarii* were all adults, the sex ratio was 0.20 (40 males and 10 females). The carapace width ranged from 18.84 to 37.70 mm. 18% of the animals was in moulting state. All animals were diseased, 74 % showed eroded or ulcerated lesions variable in shape (circular, oval, linear), in dimension (ranging from 1 to 7 mm) and colour (pale, brown, black) the so-called black spot lesions (Figure 1). Lesions were single or multiple and some coalesced leading to largest ones. The majority (56%) of the lesions occurred at the unsclerotized area of limbs, at the dactylus, at the 4propods, at the carpus, at leg tips and at the ventral 87 level, extending to the abdomen and sternum (Figure 2). Many animals showed necrotic areas at the point of the limb loss.

The males, that presented a greater size than females, displayed the highest number of outer lesions. Under microscopic observation, the lesions ranged from small erosion of the epicuticle and calcified endocuticle to more extensive and deeper endocuticle lesions with haemocytes infiltration and melanin presence (Figure 2). Mild lesions were characterized by endocuticular involvement at dactylus resulting in its complete loss with a moderate hyperplasia/hypertrophy of the epithelium. In other cases carapace ulcerations on the dorsal side were recorded measuring 5-6 mm. Ulcers were represented by the total loss of epicuticle, exocuticle and outer calcified endocuticle with melanin deposition and prominent accumulations of haemocytes nodules. At times lesions also involved muscular cells accompanied by a severe interstitial haemocytes infiltration and degenerative changes. Melanin depositions with haemocytes aggregates and PAS positive fibrinous-like material (so called pseudo membrane) were observed in the central and deeper portion of the lesion. On carapace, the gram staining underlined the presence of Gram negative bacteria within the lesion (Figure 2). In mild and most advanced lesions necrotic gills and degenerative changes in the hepatopancreas were frequently accompanied by inflammatory process. Interestingly gill's nephrocytes the analogous of fixed macrophages of Vertebrates involved in the uptake of toxic and/or foreign elements in the hemolymph were increased in number and size. The majority of cultured bacteria from the hemolymph and lesion pad were Gram negative. Identification showed that bacterial strains belonged to: *Vibrio alginolyticus* (12 strains), *Listonella anguillarum* (6),



**Figure 1:** *Carcinus aestuarii* Shell disease macroscopical lesions. A-B: Dorsal lesions with deep (A) and moderate ulceration (B). C. *cheliped* loss with melanin deposition D-E: Limb lesions at tip and joint level (bar=1 cm).



**Figure 2:** *Carcinus eastuarii* Shell disease histological observation. A-B: mild shell lesions: epicuticle and exocuticle lesion with early (A) and advanced (B) endocuticle involment (25X); C-D: dorsal ulcers: epicuticle and procuticle loss (C) showing hemocytes and nodule formation (arrowhead) (D) (30X). Insert: Gram staining shows Gram negative bacteria within the lesion (40X). Ec: epicuticle, Ex: exocuticle; En: endocuticle .Ep:epithelia (\*) haemocytes.

*Vibrio fluvialis* (6), *Vibrio sp* (4) and *Aeromonas hydrophila* (4).

## Discussion

In the *C. aestuarii* specimens the evolution of the disease seemed to follow the same pattern described by Martin et al. [20]. The males, greater in size than females, displayed a higher percentage 113 of deep lesions and limb loss, according to other authors [21] which reported a higher prevalence and infection intensity in males of *Cancer pagurus*.

The crabs with more severe disease displayed histopathological alterations of several organ and tissue systems, including systemic melanised haemocyte nodules, with a chitinolytic activity in the hemolymph as previously reported [22].

Moreover many of the specimens were in moult state and therefore more susceptible to bacterial attack [23]. The presence of Gram negative chitinolytic bacteria in the lesions and hemolymph of diseased animals showed that these bacteria are involved in the pathogenesis of the shell disease as opportunistic microorganisms [24,25]. The diseased animals were all adults; this observation is supported by previous studies [26] that noted the prevalence of the disease in populations of older crabs.

Involvement of internal organs has been reported during shell disease; gill necrosis and hepatopancreas degeneration are the most frequently recorded lesions [22-27]. The increased nephrocytes number and dimension in crustaceans, along with necrotic and degenerative changes in the gills and hepatopancreas, respectively, have been frequently observed as a consequence of polluted environment exposure [28]. In this context, it is well known that the Volturno River has been identified as a critical point for the pollution of adjacent marine waters, carrying pollutants from domestic, industrial and agricultural sources [16-17]. Furthermore, many anthropogenic factors are known to exacerbate the SDS: heavy metals could suppress the immune system [29-30], enhance chitinase activity [31] while some insecticides (methoprene) have also been found to inhibit chitin synthesis [32-33].

*C. aestuarii* is a more resistant to the pollution compared to the other species of Decapoda. Ricciardi et al. [34] demonstrated that

*C. aestuarii* caught in the polluted Lagoon of Venice, can be used as biomarkers with respect to the organic pollutants. In conclusion, as supported by the work reported above, even in our 139 case the presence of SDS in a wild population of *C. aestuarii*, could be suggested as biomarker of the environmental stress in the Volturno River. To our knowledge this is the first description of the disease in the wild population of *Carcinus aestuarii*, which could be used as a useful tool to unravel the relationship between environmental stressful conditions and the occurrence of the disease.

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