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NOTE

Tissue changes in the shore crab *Carcinus maenas* as a result of infection by the parasitic barnacle *Sacculina carcini*

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ABSTRACT: We studied the effect of parasitic invasion by the barnacle *Sacculina carcini* on shore crabs *Carcinus maenas* collected from the Menai Straits in North Wales, UK. A significant reduction in serum protein and circulating granular, semi-granular and hyaline blood cells was observed in parasitised individuals, while serum ammonium and glucose concentrations were significantly increased. No difference in hepatopancreatic glycogen concentration was found between healthy and parasitised crabs. Histological analysis showed the apparent removal of fibrillar protein from infected muscle by the parasite. Hepatopancreas tubule necrosis was also routinely observed in infected individuals. Parasitisation by *S. carcini* dramatically affects the haemocyte population and serum chemistry of infected crabs.

KEY WORDS: Haemolymph chemistry · Histopathology · Shore crab · Parasite · Barnacle · *Carcinus maenas* · *Sacculina carcini*

INTRODUCTION

The shore crab *Carcinus maenas* is ubiquitous in European waters and occupies an important ecological niche throughout inshore environments (Little & Kitching 1996). Furthermore, shore crabs are proven indicator species to monitor the health of estuarine ecosystems (Stentiford & Feist 2005). The species is harvested for recreational angling bait and directly for human consumption in continental Europe (Vale & Sampayo 2002). Shore crabs are also invasive pests in the USA and Australia because of damage to bivalve fisheries by predation (Goddard et al. 2005). Therefore, there are several economical and ecological incentives to study disease processes in this species of crab.

A common parasite of the shore crab is the rhizocephalan barnacle *Sacculina carcini* (Hoeg 1995, Walker 2001, Stentiford & Feist 2005). This highly specialised cirripede consists of an extensive rootlet system (termed interna) that penetrates the haemocoele of the host. Histological analysis has demonstrated rootlet penetration of the host digestive system, allowing removal, sequestration and presumably utilisation of host metabolites (Bresciani & Hoeg 2001). On maturation, the parasite forms an external egg sac (externa), which protrudes from the abdomen of chronically infected crabs. No host immune response to *S. carcini* has been observed in *Carcinus maenas*, and biological control methods are being actively studied in areas where the crab has been introduced artificially (Goddard et al. 2005).

Invasion by macrobial or microbial agents causes changes in the haemolymph of host crabs. However, the measured parameter can increase, decrease or remain unchanged, depending on host or parasite species (Shirley et al. 1986, Shields et al. 2003). For example, Sanviti et al. (1981) observed a 23% decrease of total serum protein in *Carcinus mediterraneus* infected

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by *Sacculina carcini,* while the same infection in *Pachygrapsus marmoratus* resulted in a significant increase in this parameter.

Overall, there has been scant comparison of haemolymph parameters between *Sacculina carcini* parasitized and healthy *Carcinus maenas*. The aim of this study was to compare haemocyte counts and tissue chemistry (serum protein, glucose, ammonium and hepatopancreatic glycogen) between apparently uninfected crabs and *S. carcini*-infected individuals. Histopathological studies of healthy and infected individuals were also performed on muscle, gill and hepatopancreatic tissue.

MATERIALS AND METHODS

Crabs. Adult *Carcinus maenas* were obtained during a 10 mo health study of stock at a local pilot crab farm (JW Aquaculture [Research], Queen's Dock, Swansea, UK). The crabs originated and were transported from the Menai Straits, North Wales, UK. Individuals infected with *Sacculina carcini,* which showed the ovoid sac of the parasite extruding from the abdomen, were taken from the farm on delivery of crabs and maintained in seawater aquaria with recirculation at 15°C.

Serum and tissue chemistry. Haemolymph samples (400 µl) were taken from the unsclerotised membranes using a 21-gauge needle and syringe, and were left for 2 h at 4°C to clot. After centrifugation (5000 × *g*; 5 min, 4°C) the supernatant (serum) was split into 100 µl fractions and stored at –80°C until required. For total haemocyte counts, 200 µl haemolymph was bled into a syringe containing an equal volume of ice-cold sterile marine anticoagulant (0.45 M sodium chloride, 0.1 M glucose, 30 mM trisodium citrate, 26 mM citric acid, 10 mM EDTA, pH 4.8) and used immediately. For glycogen, crabs were sacrificed by placing at –15°C for ca. 40 min until moribund. Individuals were opened along the breakage plane between the ventral sternal cuticle and dorsal carapace, and hepatopancreas samples (0.2 g wet weight in triplicate) were removed for immediate use. Total protein, glucose and ammonium content of serum and hepatopancreatic glycogen were calibrated against an appropriate standard curve. Assays were performed as described in detail previously (Eddy et al. 2007, Powell & Rowley 2007).

Histopathology. Control and diseased crabs (n = 10 in both cases) were selected for histology. Individuals were sacrificed by the injection of ca. 5 ml of Bouin's seawater fixative through unsclerotised membranes. Tissues were processed, stained and examined as described previously (Eddy et al. 2007).

Data analysis. Tissue chemistry data were checked for equal variance and subsequently analysed using Student's unpaired *t*-test. All data are shown as mean values ± SE.

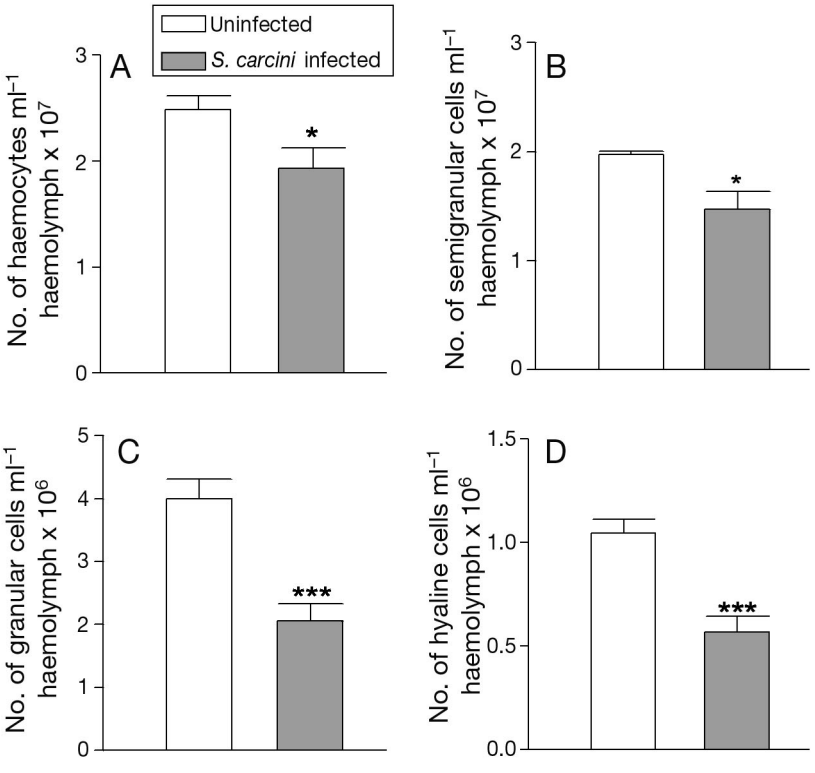

Fig. 1. *Sacculina carcini* infecting *Carcinus maenas.* Total and differential haemocyte counts of shore crabs infected with *S. carcini* compared with uninfected crabs. (A) Total haemocyte count. (B) Number of semigranular cells. (C) Number of granular cells. (D) Number of hyaline cells. *, ***Significantly different to uninfected individuals at $p < 0.05$ and $p < 0.001$ respectively (Student's *t*-test). n = 9 (infected crabs) and 20 (uninfected crabs)

RESULTS

Total (THC) and differential (DHC) haemocyte counts of *Sacculina carcini* parasitised crabs showed significantly depressed cell numbers (Fig. 1). Both THC (Fig. 1A) and semi-granular haemocyte (Fig. 1B) counts were significantly lower than in apparently healthy (control) individuals (Student's *t*-tests, $p < 0.05$). Granular (Fig. 1C) and hyaline (Fig. 1D) cell counts were also reduced in parasitised crabs (Student's *t*-tests, $p < 0.001$).

The serum protein concentration of control crabs was significantly higher than that of diseased crabs (Student's *t*-test, $p < 0.001$) (Fig. 2A). In contrast, serum ammonium concentration was significantly elevated in *Sacculina carcini*-affected crabs compared to controls (Student's *t*-test, $p < 0.01$) (Fig. 2B). Similarly, serum glucose was significantly greater in parasitised crabs (Student's *t*-test, $p < 0.001$) (Fig. 2C). However, the glycogen concentration of hepatopancreatic tissue was not significantly different between diseased and healthy crabs (Fig. 2D).

Histological changes were routinely observed in the structure of muscle and hepatopancreas of infected crabs while gills showed no apparent changes from parasitisation. The hepatopancreas from apparently healthy crabs consists of tubules separated by haemal spaces, which contain connective tissue and circulating haemocytes (Fig. 3A). Infected crabs showed the typical rootlet system of *Sacculina carcini* occupying these haemal spaces (Fig. 3B). Furthermore, the hepatopancreatic tubules adjacent to the rootlets became necrotic, sometimes with a lack of definition between cells. Cell debris was also apparent in the lumen of the tubules in some cases (Fig. 3B) and small foci of haemocyte-like cells were also occasionally observed in the intra-tubular spaces. Healthy muscle tissue from the base of the chelae and pereiopods appeared as homogeneous bundles of fibres, and stained a dense red colour. Muscle fibres adjacent to *S. carcini* rootlets became necrotic, were often displaced and stained a pale purple-lilac colour (Fig. 3C). This altered muscle still showed the nuclei and outline of the muscle fibres, but had apparently lost some fibrillar protein, resulting in a change in staining capacity (Fig. 3D). No obvious haemocytic reaction, such as infiltration and encapsulation, was observed in response to the altered muscle.

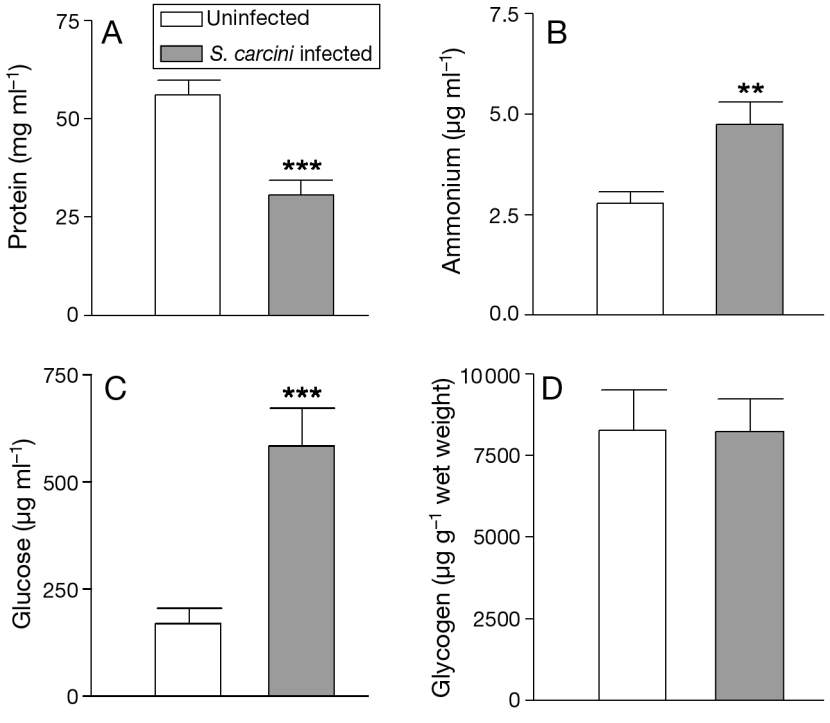

Fig. 2. *Sacculina carcini* infecting *Carcinus maenas*. Effect of *S. carcini* infection on (A) serum protein, (B) ammonium, (C) glucose and (D) hepatopancreatic glycogen *, ***Significantly different to uninfected individuals at $p < 0.01$ and $p < 0.001$ respectively (Student's *t*-test). n = 10 (infected crabs) and 20 (uninfected crabs)

DISCUSSION

The current study demonstrated a reduction in the number of circulating haemocytes in *Carcinus maenas* infected with *Sacculina carcini*. This is a common occurrence in diseased crustaceans and has been observed previously in *C. maenas* (e.g. Smith & Ratcliffe 1980). In bacterial infections, the observed decrease may involve accumulation of haemocytes in other organs, including the gills and hepatopancreas. This study did not show any significant morphological changes in gill tissue. Minor accumulation of haemocytes in the hepatopancreas may only partially explain the observed reduction of haemocyte number in the haemolymph. There have been few investigations into changes in host metabolite chemistry during *S. carcini* parasitisation, and those that exist are contradictory. The current study found a significant reduction of serum protein in parasitised *C. maenas*. The apparent loss of fibrillar protein from muscle suggests that the parasite rootlet system is also capable of utilising muscle-associated protein. Andrieux et al. (1980) observed the disappearance of a protein fraction in the haemolymph of *S. carcini*-parasitised crabs. However, Uglow (1969) found that the total serum protein in control shore crabs was not significantly different to that of *S. carcini*-parasitised crabs, while earlier studies have suggested that parasitised individuals had significantly higher blood protein than uninfected counterparts (e.g. Drilhon 1936). The reason for these apparent differences is currently unclear.

Increased serum ammonia concentrations have been previously observed in

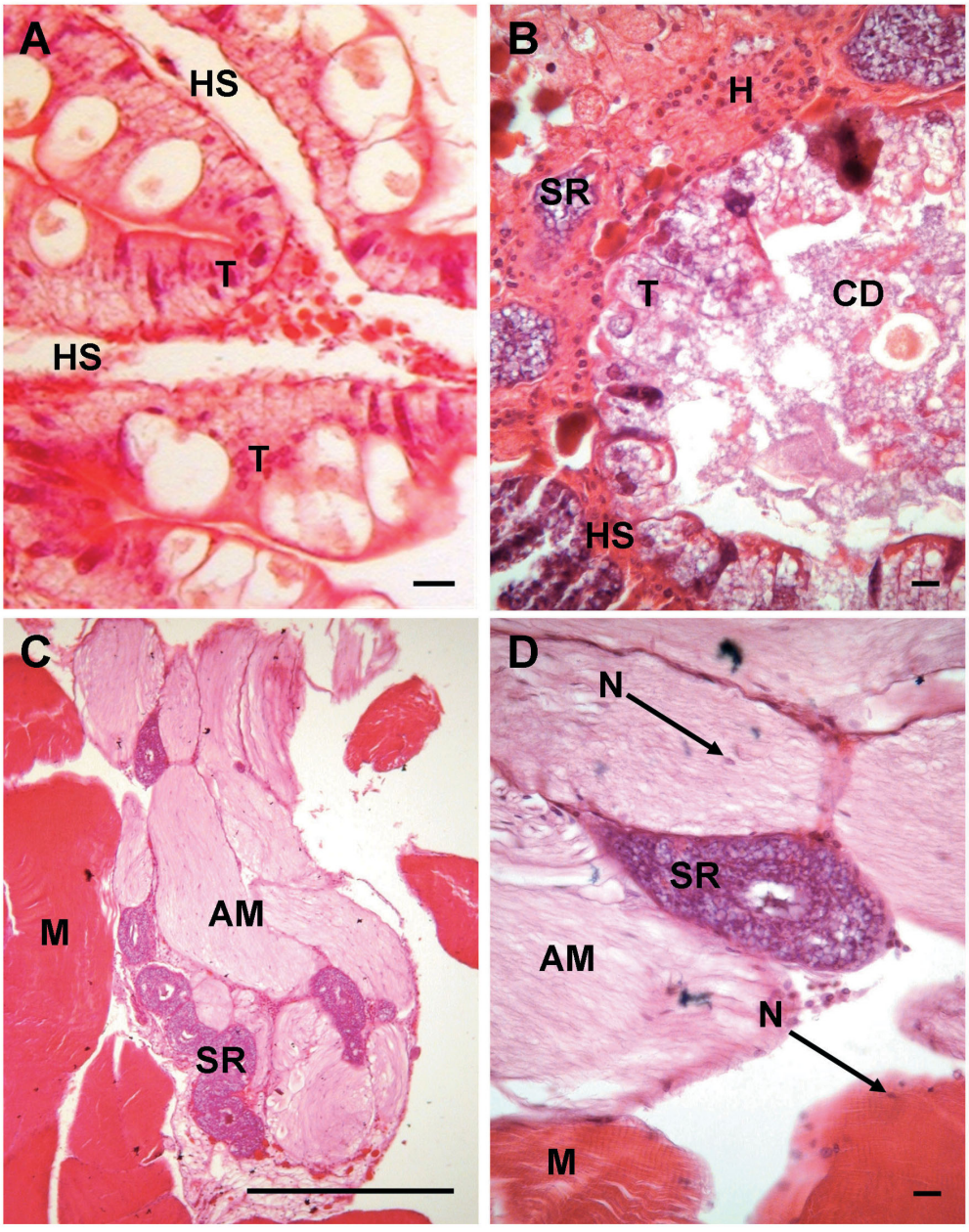

Fig. 3. *Sacculina carcini* infecting *Carcinus maenas*. Effect of *S. carcini* on the structure of the (A,B) hepatopancreas and (C,D) muscle. (A) Healthy hepatopancreatic tubule (T). HS: haemal space. (B) Necrotic tubule from crab infected with *S. carcini*; note necrosis in tubule with cell debris (CD) in the lumen. H: apparent haemocytes; HS: haemal space; SR: *Sacculina* rootlet; T: tubule. Scale bars = 20 µm. (C) Low power micrograph showing changes in the staining pattern of the atrophied (damaged) muscle (AM) directly adjacent to the rootlets of *S. carcini* (SR) compared with normal muscle fibres (M). Scale bar = 100 µm. (D) High power micrograph of affected area of muscle (M). Nuclei (N) are still present in healthy and parasitised muscle fibres although parasitised muscle has been apparently stripped of fibrillar protein. AM: atrophied (damaged) muscle; SR: *S. carcini* rootlet. Scale bar = 10 µm. Haematoxylin and eosin stain

diseased crustaceans. In crabs, ammonia excretion occurs across the gills (Taylor & Taylor 1992). *Sacculina carcini* parasitised individuals showed no obvious gill damage, suggesting that this was not an explanation for elevated serum ammonium. An alternative explanation is that serum ammonium increase could result from the added metabolic load of the parasite. No excretory system is present in *S. carcini*, and nitrogenous waste could be excreted directly into the host's haemocoele. Increased serum glucose concentration is often considered a response to stress in crustaceans (Hall & van Ham 1998) and has been observed

previously in crabs infected by rhizocephalan barnacles (Sanviti et al. 1981, Shirley et al. 1986). In the current study, an increase in serum glucose could result from the translocation of glucose from either hepatopancreas or muscle tissue. However, no decrease of hepatopancreatic glycogen was observed in infected animals. Perhaps glucose may be mobilised from other tissues that store glycogen which were not assayed in this study. Muscle glycogen content was significantly depleted in *Nephrops norvegicus* (L.) infected with the dinoflagellate *Hematodinium* sp. (Stentiford et al. 2000). Change in carbohydrate dynamics in infected crustaceans can also result from alterations in the endocrine system. Stentiford et al. (2001) found that *N. norvegicus* infected with *Hematodinium* sp. suffered depleted glucose and hepatopancreatic glycogen reserves, as infection disrupted the feedback loop for crustacean hyperglycaemic hormone.

In conclusion, infection by *Sacculina carcini* dramatically alters serum metabolite concentrations, haemocyte counts and tissue structure in shore crabs. The reduction of haemocytes and necrosis of key tissues may also provide further evidence that the parasite effectively utilises the organ systems of *Carcinus maenas*.

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LITERATURE CITED

Andrieux N, Herberts C, Frescheville JD (1980) Protein metabolism and parasitism in the crab *Carcinus maenas*—effects of *Sacculina carcini* (Crustacea, Rhizocephala) on serum and epidermal proteins of host. Can J Zool 58:580–585

Bresciani J, Hoeg JT (2001) Comparative ultrastructure of the root system in rhizocephalan barnacles (Crustacea: Cirrepedia: Rhizocephala). J Morphol 249:9–42

Drilhon A (1936) Quelques constants chimiques et physicochimiques du milieu interieur du crabe sacculine (*Carcinus maenas*). CR Hebdomaires Séances Acad Sci 202: 981–982

Eddy F, Powell A, Gregory S, Nunan LM and others (2007) A novel disease of the European shore crab, *Carcinus maenas* – molecular pathology and epidemiology. Microbiology 153:2839–2849

Goddard JHR, Torchin ME, Kuris AM, Lafferty KD (2005) Host specificity of *Sacculina carcini*, a potential biological control agent of introduced European green crab *Carcinus maenas* in California. Biol Invasions 7:895–912

Hall MR, van Ham EH (1998) The effect of different types of stress on blood glucose in the giant tiger prawn *Penaeus monodon.* J World Aquacult Soc 29:290–299

Hoeg JT (1995) The biology and life cycle of the Cirrepedia Rhizocephala. J Mar Biol Assoc UK 75:517–550

Little C, Kitching JA (1996) The biology of rocky shores. Oxford University Press, Oxford

Powell A, Rowley AF (2007) The effect of dietary chitin supplementation on the survival and immune reactivity of the shore crab, *Carcinus maenas.* Comp Biochem Physiol 147A:122–128

Sanviti G, Romestand B, Trilles JP (1981) Les sacculines (*Sacculina carcini* Thompson, 1836) de *Carcinus mediterraneus* et *Pachygraspus marmoratus*: comparaison immunochemique: étude comparée de leur influence sur la composition protéique de l'hémolymphe des deux hôtes. Parasitol Res 64:243–251

Shields JD, Scanlon C, Volety A (2003) Aspects of the pathobiology of the blue crabs, *Callinectes sapidus,* infected with the parasitic dinoflagellate *Hematodinium perezi.* Bull Mar Sci 72:519–535

Shirley SM, Shirley TC, Meyers TR (1986) Haemolymph responses of Alaskan king crabs to rhizocephalan parasitism. Can J Zool 64:1774–1781

Smith VJ, Ratcliffe NA (1980) Host defence reactions in the shore crab, *Carcinus maenas* (L.): clearance and distribution of test particles. J Mar Biol Assoc UK 60:89–102

Stentiford GD, Feist SW (2005) A histopathological survey of shore crab (*Carcinus maenas*) and brown shrimp (*Crangon crangon*) from six estuaries in the United Kingdom. J Invertebr Pathol 88:136–146

Stentiford GD, Neil DM, Coombs GH (2000) Alterations in the biochemistry and ultrastructure of the deep abdominal flexor muscle of the Norway lobster *Nephrops norvegicus* during infection by a parasitic dinoflagellate of the genus *Hematodinium.* Dis Aquat Org 42:133–141

Stentiford GD, Chang ES, Chang SA, Neil DM (2001) Carbohydrate dynamics and the crustacean hyperglycaemic hormone (CHH): effects of parasitic infection in Norway lobsters (*Nephrops norvegicus*). Gen Comp Endocrinol 121:13–22

Taylor HH, Taylor EW (1992) Gills and lungs: the exchange of gases and ions. In: Harrison FW, Humes AG (eds) Microscopic anatomy of invertebrates, Vol 10. Wiley-Liss, New York, p 203–293

Uglow RF (1969) Haemolymph protein concentrations in portunid crabs – III. The effect of *Sacculina.* Comp Biochem Physiol 31:969–973

Vale P, Sampayo MAD (2002) First confirmation of human diarrhoeic poisonings by okadaic acid esters after ingestion of razor clams (*Solen marginartus*) and green crabs (*Carcinus maenas*) in Aveiro lagoon, Portugal and detection of okadoic acid esters in phytoplankton. Toxicon 40:989–996

Walker G (2001) Introduction to the Rhizocephala (Crustacea: Cirrepedia). J Morphol 249:1–8

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