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Hemolymph Responses of The Xanthid Crab *Actaea hirsutissima* to Rhizocephalan *Sacculina ignorata* From Hurghada, Red Sea

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ABSTRACT

The effect of parasitic invasion by the Rhizocephala *Sacculina ignorata* on brachyuran crabs *Actaea hirsutissima* was studied. The organic constituents (glucose, total proteins, albumin, triglycerides and cholesterol) of the haemolymph of the infected and normal crab *Actaea hirsutissima* were estimated. A significant reduction in serum protein and albumin was observed in parasitized individuals; while serum glucose, cholesterol and triglycerides concentrations were significantly increased. Parasitism by *S. ignorata* dramatically affects the serum chemistry of infected crabs. T-test values were statistically highly significant between healthy and infected specimens.

INTRODUCTION

A common parasite of the Xanthid crab *Actaea hirsutissima* is the Rhizocephalan barnacle, *Sacculina ignorata* (Boschma, 1947b). This highly specialized cirriped consists of an extensive rootlet system termed (interna) that penetrates the haemocoel of the host. Histological analysis has demonstrated rootlet penetration of the host digestive system, allowing removal, sequestration and presumably utilization 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).

Stress responses occur in all animals when regulated physiological systems are extended beyond their normal range by external stressors. Failure of all or part of the integrated homeostatic response may lead to increasing physiological disturbance and ultimately death (Morris and Airriess, 1998). Indicators of such stress responses may therefore be useful in assessing the short-term well-being or long-term health status of an animal (Fossi *et al.,* 1997; Paterson and Spanoghe, 1997) and such indicators have received considerable attention in commercially important decapod crustacean species (Paterson and Spanoghe, 1997; Chang *et al.,* 1999). An important stressor is the infection of an animal by parasites (Thompson, 1983).

levels in their haemolymph (Krzynowek and Panunzio 1989; King *et al.,* 1990; O, Leary and Matthews 1990; Muriana *et al.,* 1993; Omar *et al.,* 1995). In pathogenic cases, Hudson (1995) studied the changes in biochemical parameters of haemolymph induced by parasites in crabs.

Overall, there has been scant information on haemolymph parameters of *A. hirsutissima* parasitized and healthy individuals. This study aims to evaluate the effect of parasitism on organic constituents (serum protein, albumin, cholesterol, triglycerides and glucose) of apparently uninfected crabs and infected individuals.

MATERIALS AND METHODS

Collection of crabs:

In the present study, adult crabs of *A. hirsutissima* were collected from Hurghada City (6.5 km North, beside Balm Beach Resort; Fig. 1), Red Sea (27°17'35.02" N and 33°45'37.68" E) during the period from February 2013 to June 2014. The specimens were collected by hand from the intertidal zone where they were hidden underneath small stones, coral rubbles and gravel. Subtidal inhabiting specimens were collected by snorkeling.

Invasion by macrobial or microbial agents causes changes in the haemolymph of host crabs. However, the measured parameters can increase, decrease or remain unchanged, depending on host or parasite species (Shirley *et al.,* 1986; Shields *et al.,* 2003). For example, a 23% decrease of total serum protein was observed in *Carcinus mediterraneus* infected by *Sacculina carcini*; while the same infection in *Pachygrapsus marmoratus* has a significant increase in these parameters. (Sanviti *et al.* 1981)

A number of researchers have suggested different methods for quantifying the stress reactions in crustaceans; these include the measurement of different hemocyte types in the hemolymph (Jussila *et al.,* 1997), physiological, biochemical (Paterson and Spanoghe, 1997; Stentiford *et al.,* 1999), molecular changes in tissue and hemolymph (Fossi *et al.,* 1997), and the elevated transcription of heat shock proteins during periods of stress (Chang *et al.,* 1999).

In crabs, studies on the levels of glucose, total proteins, total lipids, cholesterol, and albumin are still far from complete (Tsai *et al.,* 1984; Akpan, 1997). In contrast, these parameters were extensively studied in shrimps and recorded variable

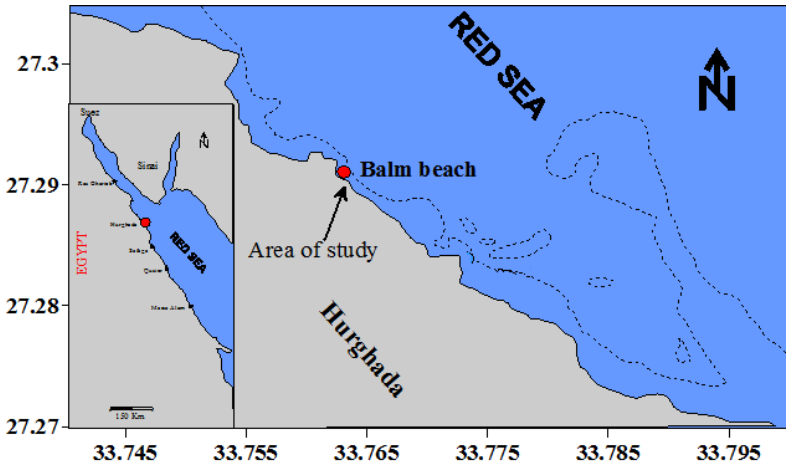

Fig. 1: A map showing the investigated site close to Balm Beach Resort. 6.5km North Hurghada, Red Sea, Egypt.

Parasite survey and identification:

For the parasite, it was identified according to Guerin-Ganivet (1911) and Boschma, (1947a, b; 1948).

Serum chemistry:

For biochemical analyses, 20 specimens of *A. hirsutissima* were collected,

sorted and measured to the nearest millimeter. The haemolymph was collected by heart puncture at the cardiac region of the carapace using a syringe. The collected haemolymph samples were put in marked vials, labeled, and then transported in ice – box to the laboratory, after that frozen till analysis.

At the laboratory, all the biochemical measurements were carried out using Schimadzu spectrophotometer (UV – Vis. 1201), Japan. Determination of organic constituentstotal proteins, albumin, glucose, cholesterol and triglycerides) were determined according to Hennery, 1968; Johnson *et al.,* 1999; Bergmeyer, 1974; Thomas, 1992; Cole *et al.,* 1997, respectively. All determinations were done colorimetrically using Bicon Burbach kits Germany.

Data analysis: The data of these parameters were statistically analyzed using the student T- test.

RESULTS

Total proteins:

The values of total proteins in normal *A. hirsutissima* were higher than those infected specimens (Table 1) and were graphically represented in Figure (2). The higher values in normal crabs averaged, 5.80±0.30 g/dl for females and 5.60±0.39 g/dl for males with mean value (5.70± 0.23).These values slightly decreased to 4.03± 0.14 and 4.37± 0.31 g/dl in infected females and males, respectively, with mean value (4.20±0.17).

Table 1: Mean values of (glucose, total proteins, albumin, triglycerides and cholesterol) in the haemolymph of *Actaea hirsutissima* Hurghada City from the Red Sea during the period from February 2013 to June 2014

Crabs status \ Parameters			Total proteins (g\dl)	Albumin (g\dl)	Triglycerides (mg\dl)	Cholesterol (mg\dl)	Glucose (mg\dl)
normal	♂	X±SE	5.60±0.39	2.72±0.13	147.84±12.14	154.54±4.97	24.42±2.44
		Range	4.80-6.97	2.29-3.03	112.90-172.40	145.70-173.70	17.70-29.90
	♀	X±SE	5.80±0.30	3.09±3.27	152.40±24.90	174.30±7.99	30.20±6.67
		Range	4.72-6.44	2.31-3.94	102.00-246.00	157.00-204.00	18.70-54.10
	♂&♀	X±SE	5.70± 0.23	2.91±0.16	150.12±13.08	164.42±5.53	27.31±3.49
infected	♂	X±SE	4.37± 0.31	2.02±0.12	195.62±18.28	220.66±16.02	49.48±1.63
		Range	3.61-5.15	1.60-2.30	144.40-258.70	187.10-273.00	45.00-53.20
	♀	X±SE	4.03± 0.14	1.90±0.27	231.34±13.11	210.60±4.03	38.40±2.05
		Range	3.70-4.50	1.30-2.60	185.00-261.10	199.00-220.00	31.50-41.90
	♂&♀	X±SE	4.20±0.17	1.96±0.14	213.48±12.16	215.63±7.97	43.94±2.22

The difference in protein levels was statistically highly significant between normal and parasitized crabs, as well as between normal and infected females. While the difference in protein levels were statistically significant between normal and infected males, as well as, between normal males and infected females (Table 2).

Albumin:

The haemolymph albumin of *A. hirsutissima* has the same pattern of protein for both normal and infected specimens of crabs. The albumin values were relatively higher in normal crabs than infected ones, (Table 1) and Figure (2). The albumin values were 3.09±3.27 and 2.72±0.13 g/dl for normal females and males, respectively, with mean value (2.91±0.16). Its values declined remarkably to 1.90±0.27g/dl and 2.02±0.12 g/dl in infected females and males respectively with mean value averaged 1.96±0.14. The differences in albumin levels were statistically highly significant between normal and parasitized crabs, as well as between normal females and infected males. While the differences in albumin levels were statistically significant between normal and infected males. Also, between normal males and infected females (Table 2).

Cholesterol:

In contrast to total proteins and albumin, the cholesterol values for *A. hirsutissima* (Table 1 and Figure 2) were lower in normal crabs of both sexes than

infected ones. Cholesterol values were 154.54±4.97 mg/dl and 174.30±7.99 mg/dl for males and females of normal crabs, respectively, with mean value beings 164.42±5.53.

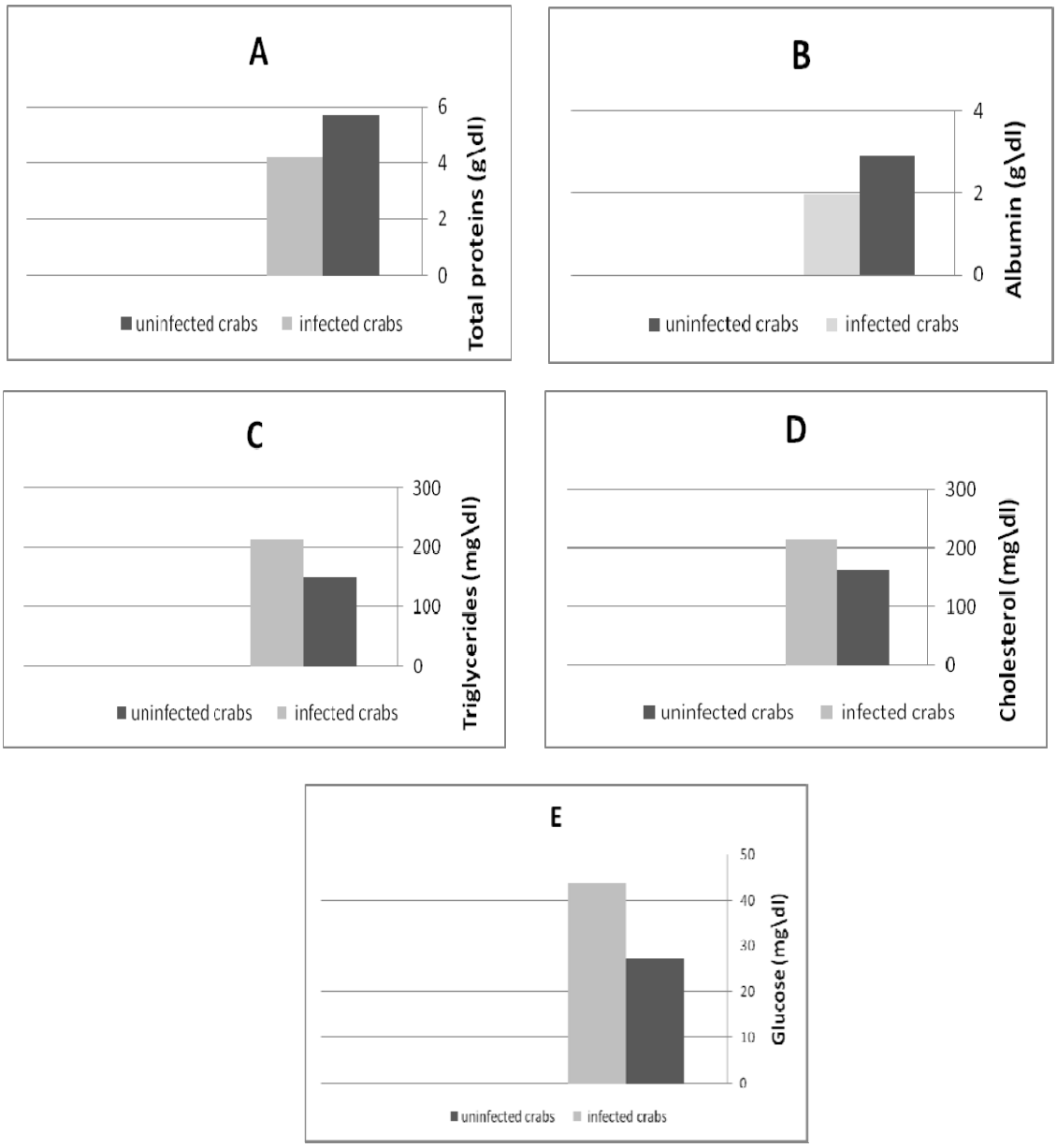

Fig. 2: Effect of *Saccculina ignorata* infection on *Actaea hirsutissima* organic constituents: (A) Total proteins (B) Albumin (C) Triglycerides (D) Cholesterol (E) Glucose.

The cholesterol levels are increased remarkably in infected crabs, to 210.60±4.03 mg/dl and 220.66±16.02 mg/dl for females and males respectively, with mean value beings 215.63±7.97. With only exception infected males and females, all statistical analyses were significant (Table 2).

Triglycerides:

The triglycerides values for *A. hirsutissima* (Table 1 and Figure 2) were lower in normal crabs than infected ones, with lower values in males than females. It was 147.84±12.14 mg/dl and 152.40±24.90 mg/dl for males and females of normal crabs, respectively, with mean value beings 150.12±13.08. The triglycerides levels are increased remarkably to 231.34±29.32 mg/dl and 195.62±18.28 mg/dl for infected females and males respectively, with mean value being 213.48±12.16. The statistical analyses

(t-test) showed that the difference between infected and normal females were significant, while those between normal males and infected females as well as healthy and infected crabs were highly significant (Table 2).

Table 2: Students t-test values for organic constituents (glucose, total proteins, albumin, triglycerides and cholesterol) in the haemolymph of healthy and parasitized *Actaea hirsutissima* with *Sacculina ignorata*.

Parameters / Crabs & sex	Total protein	Albumin	Triglycerides	Cholesterol	Glucose
Normal ♂ and ♀	0.449 NS	1.161NS	0.257NS	5.008**	0.972 NS
Normal and infected ♂	2.928*	3.373*	1.944 NS	5.470**	7.036 **
Normal ♂ and infected ♀	3.563*	3.420*	4.708**	6.775**	5.532**
Normal ♀ and infected ♂	2.641 NS	6.653**	1.269NS	3.719*	2.470 NS
Normal and infected ♀	4.168**	2.521 NS	3.349*	3.239*	1.144 NS
Infected ♂&♀	1.077 NS	0.342 NS	1.586 NS	0.519 NS	3,851*
Normal and infected crabs	5.070**	3.682**	3.755**	5.552**	3.535**

*: significant (P<0.05).
**: highly significant (P<0.01).
N.S: non significant (P>0.05).

Glucose:

A remarkable increase in glucose level for males than females in infected crabs was noticed (Table and Figure 2). The glucose levels were 49.48±1.63 and 38.40±2.05 mg/dl for infected males and females, respectively, with mean value averaged 43.94±2.22. However, a reverse was noticed in normal ones. Their values were 24.42±2.44 mg/dl in normal males and 30.20±6.67 mg/dl in normal females with mean value was 27.31±3.49. The statistical analyses (t-test) showed that the difference between infected females and males were significant (Table 2), while those between normal males and infected males as well as normal males and infected females, in addition to healthy and parasitized crabs were highly significant (Table 2).

DISCUSSION

Crustaceans particularly those of commerical values, have high ratios of total protein than carbohydrates and total lipids (Warner, 1977; Amer *et al.*, 1991; Hashem, 1992; Abd El-Reheem, *et al* 2003).

The current study found a significant reduction of serum protein and albumin in parasitised *A. hirsutissima*. This agrees with Powell and Rowley (2008) who found a significant reduction of serum protein in parasitized *Carcinus maenas*, infected with *Sacculina carcini* and also noticed that the apparent loss of fibrillar protein from muscles. The parasite rootlet system is also capable of utilizing muscle-associated protein. Andrieux *et al.* (1980) observed the disappearance of a protein fraction in the haemolymph of *S. carcini*-parasitized crabs. However, the present results disagrees with Uglow (1969) who found that the total serum protein in control shore crabs was not significantly different to that of *S.carcini*-parasitized crabs, while earlier studies have suggested that parasitized individuals had significantly higher blood protein than uninfected counterparts (Drilhon, 1936).

Albumin is produced entirely in the liver and is of great importance in regulating the flow of water between the plasma and tissue fluid by its effect on colloid osmotic pressure as well as it is a negative acute phase protein and its concentration is decreased during inflammatory and parasitic infection (Mojabi, 2000, Stockham and Scott, 2002 and Kaneko, *et al.,* 2008). With an increase in load of gastrointestinal nematodes infection, the concentrations of total protein and albumin were decreased. (Chaichisemsari, *et al.,* 2011).

A drop in serum albumin level is usually associated with a decrease protein synthesis by the liver or increase protein loss through the gut or the kidney. Other possible cause of decrease in albumin may include malabsorption and increased protein need

secondary to infection (Halsted and Halsted, 1981; Cheesbrough, 1998). While El-Sayed *et al.* **(**2003) suggested that, the increase in the level of total proteins and albumin in the haemolymph of *Leptodius exaratus* individuals, infected with *Sacculina sp.* resulted from the destruction of both gonads and others tissues by the rootlets of the parasite within its host **.**

In Crustacea, the increase in haemolymph glucose levels is considered an important response of animals to stress (Jussila *et al.,* 1997; Hall and van Ham, 1998; Lorenzon, *et al*., 2005). Hyperglycemia has been recorded following exposure of Crustacea to different stressors, such as emersion (Durand *et al.,* 2000), cold shock (Kuo and Yang, 1999), anoxia (Hall and van Ham, 1998) and pollutants (Lorenzon *et al.,* 2000) 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 recorded in the parasitized crabs. This agrees with Powell and Rowley (2008). They showed that an increase in serum glucose recorded in the parasitized crabs could result from the translocation of glucose from either hepatopancreas or muscle tissues. But they noticed that, no decrease of hepatopancreatic glycogen was observed in infected animals. So, they suggest that glucose may be mobilized from other tissues that store glycogen which were not assayed in their study. Muscle glycogen content was significantly depleted in *Nephrops norvegicus* 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) concluded that the release of glucose into the haemolymph is mediated by the crustacean hyperglycemic hormone through the mobilisation of intracellular glycogen stores. Whereas, they found that *N. norvegicus* infected with *Hematodinium sp*. suffered depleted glucose and hepatopancreatic glycogen reserves, as infection disrupted the feedback loop for crustacean hyperglycemic hormone. In contradictory with the present result, El-Sayed *et al.,* (2003) reported that the sharp decline in infected specimens of *L. .exaratus* infected with *Sacculina sp.* indicate sharp depletion in haemolymph glucose which may be consumed by the parasites.

The total lipid is generally low in crustaceans (Warner, 1977; Amer *et al.,* 1991; Hashem, 1992; El-Zawahry *et al.,* 1997; Abd El-Reheem, *et al* 2003). Cholesterol is one of the most important constituents of lipids in haemolymph of crustaceans and is the basic constituent of sex hormones (Highnam & Hill, 1979). During this study, the cholesterol level was high in the haemolymph of the parasitized crabs than healthy ones. El-Sayed *et al.,* (2003) mentioned that remarkable changes were evident and closely correlated with the morphological (moulting) and physiological (reproductive) changes of the crabs of the two sexes for two different species. A remarkable decrease was recorded in infected males and females of *L. exaratus* as a result of infection with *Sacculina sp.* (El-Sayed *et al.,* 2003). As shown herein, infected crabs have high circulating levels of triglycerides and cholesterol. These results are in agreement with those published by Robson (1911) who explain the effect of the Rhizocephalan parasite *Sacculina neglecta* upon the sexual physiology of its host, the spider-crab, *Inachus mauritanicus.* He reported that the increased fat in the blood of the infected crabs, *Inachus mauritanicvis* parasitized with *Sacculina neglecta* is the result of an increased activating of fat that would otherwise have been stored up or is due to an increased initial supply.

In the light of the previous facts, it is obvious that parasitism by *Sacculina ignorata* dramatically affects the serum chemistry of infected crab *A. hirsutissima*. Whereas, it causes a significant reduction in serum protein and albumin and increases serum glucose, cholesterol and triglycerides concentrations in parasitized individuals.

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