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Effect of some biological factors on the chitin yield of two crustacean species inhabiting the Egyptian waters

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ABSTRACT

Objective: To investigate the chitin yield of two commercial crustacean species that are exploited in the Suez Canal region, the Red Sea crab *Charybdis natator* (*C. natator*) and the Mediterranean mantis shrimp *Erugosquilla massavensis* (*E. massavensis*), and to assess the effect of some biological factors such as sex, size and maturity stages of females' ovaries on this yield.

Methods: A total of 64 specimens of crabs were collected from the Red Sea and 1 377 mantis shrimps were collected from the Mediterranean Sea. Chitin was obtained after the de-proteinization, de-mineralization and de-colorization of 5 g oven dried exoskeletons and values were expressed as g/5 g and percentages.

Results: Chitin yield was significantly higher in *E. massavensis* than *C. natator* (22.1%, 14.22%, respectively). No significant difference in the yield was recorded between males and females of *C. natator* (12.9%, 14.9%, respectively), while the yield in *E. massavensis* males was significantly higher than females (25.3%, 21.2%, respectively). Significant variations in the chitin yield were observed between the different sizes of *E. massavensis* with the maximum being from the individuals falling in the size range 90–130 mm body length. The yield was at its lowest in the immature stage of *C. natator* females' ovaries (9.29%). However, the values increased and remained constant for the remaining stages ($\geqslant$ 18%).

Conclusions: The study recommends the use of the mantis shrimp for the production of chitin on commercial scale particularly medium sized males.

1. Introduction

Chitin and cellulose are the most abundant natural biopolymers present in nature. Carapaces of large crustaceans especially crabs, shrimps and mantis shrimps contain chitin as a main component[1]. Many authors reported the use of chitin and its derivatives in several fields such as agriculture, treatment of wastewater, drug transportation, tissue engineering, molecular imprinting, cosmetics and food preservation[2-5]. Chitin based studies are progressively abundant, and new possible ways of utilization have been reported[6-9].

Several crustacean species are commercially harvested from the Mediterranean and Red Seas which include shrimps, crabs and mantis shrimps. However, this industry generates large volumes of waste materials that are discarded on a daily basis and represents a disposal problem. An improved commercial way to use this ample waste material is by converting them to forms with added value such as nutrients, (*e.g.*, proteins and minerals) as well as other useful biochemical compounds like chitin and

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chitosan[10,11].

No studies have been carried out on the production of chitin from any crustacean species inhabiting the Egyptian waters. The impact of the biological factors affecting chitin production in these crustaceans such as size, sex and females' maturation stage has not been investigated. This study aimed to investigate the chitin yield of two of the commercial crustacean species that have a fishery potential and a considerable popularity in the Egyptian markets, the brachyuran crab *Charybdis natator* (Herbst, 1794) (*C. natator*) and the mantis shrimp *Erugosquilla massavensis* (Kossman, 1888) (*E. massavensis*). It also aimed to assess the effect of some biological factors on this yield.

2. Materials and methods

2.1. Sample collection

During the period 2013–2014, a total of 1 377 specimens of *E. massavensis* were collected from the Mediterranean Sea at Port Said, and 64 of *C. natator* collected from the Red Sea at the Gulf of Suez (Figure 1). Fresh samples were obtained from the fishing ports and immediately stored in the freezer at –20 ℃ for long time preservation. After thawing, specimens were sorted according to sex, then measured by means of a vernier caliper with an accuracy of 0.1 mm. The crab was measured for its carapace width while the mantis shrimp for its body length (BL) (Figure 2). Individuals were then divided into size groups with 10 mm interval for *C. natator* and 20 mm for *E. massavensis*. Females of each species were dissected to judge the gonad maturity stages and divided into 4 groups (immature, maturing, mature and ripe)[12]. Three replicate sub-samples were taken from each group for chitin extraction. The integuments were separated from specimens by means of a forcep.

2.2. Chitin extraction

For chitin extraction, integuments were digested by boiling for 10 min and dried at 40 ℃ in an oven until constant weight. Dried integuments were ground using a Grinder Rotor (Retch RM200, Germany) until a fine powder was obtained. Three consecutive processes were then applied, de-proteinization, de-mineralization and de-coloration[13-15].

2.2.1. De-proteinization

In a conical flask, 5 g of dried integument powder was added to 50 mL of 4% NaOH solution at a ratio of 10:1 (v/w). The mixture was treated under standard autoclaving conditions (15 psi/121 ℃) for 10 min (to decompose the albumen into soluble amino acids) and then filtered. The solid residue was washed to neutrality in running tap water to remove any traces of chemicals and soluble impurities. The residue was rinsed with de-ionized water and then oven-dried at 40 ℃ over night. The obtained substance was weighed and the weight of the protein was calculated.

Figure 1. Sampling areas in the Egyptian Mediterranean coast at Port Said and the Gulf of Suez, Red Sea, Egypt.

2.2.2. De-mineralization

1 mol/L HCl was added to the obtained de-proteinized sample at a ratio of 20:1 (v/w) and stirred for 15–30 min to remove existing minerals (mainly calcium carbonate). The duration was dependent on the species, being longer in case of the rock crab due to its relatively hard exoskeleton. The mixture was filtered and the solid residue was washed and oven-dried as described in the previous step. The obtained substance was weighed and the weight of the minerals was calculated.

2.2.3. De-coloration

After de-mineralization, 25 mL of acetone was added to the

sample and left for 10 min to remove any impurities[16]. The mixture was then filtered and dried for 2 h at room temperature. Sodium hypochlorite solution was added to the mixture at ratio of 1:10 (w/v) and kept for 5 min at room temperature with constant stirring, then filtered. Sodium hypochlorite was used to reduce the odor of the material and remove pigments[17]. The solid residue was washed with tap water for 30 min, oven-dried and weighed. The chitin content was determined from the weight differences between the raw material and that of the chitin obtained after treatment and calculated as g/5 g.

Data was presented as mean percentages. The Two-way ANOVA test was performed using the software package SPSS version 15.0 (SPSS Inc., USA).

Figure 2. Dorsal view of *C. natator* (A) and *E. massavensis* (B).

3. Results

Dried exoskeleton of the two species consisted of protein, minerals, pigments and chitin with minerals constituting more than 50% of the skeleton dry weight (Figure 3). In general, proteins and minerals were significantly less in the mantis shrimp (19.62%, 55.81%, respectively) than the crab (21.33%, 62.28%, respectively) ($F_{(1, 133)} = 4$, $P < 0.05$ for protein and $F_{(1, 133)} = 50.56$, $P < 0.01$ for minerals) (Figure 3). In terms of weight (g/5 g), chitin yield was significantly higher in *E. massavensis* (1.16 g) than *C. natator* (0.69 g) ($F_{(1,133)} = 97.38$, $P < 0.01$) (Tables 1 and 2). On the other hand, chitin yield was significantly higher in *E. massavensis* males (average 1.27, 25.3%) than females (average 1.05, 21.2%) ($F_{(1, 60)} = 30.51$, $P < 0.01$) while in *C. natator*, females showed insignificant slightly higher values of chitin (average 0.73 g, 14.9%) than males (average 0.65 g, 12.9%) (Tables 1 and 2, Figure 3).

Body size ranged between 70 and 170 mm in both sexes of *E. massavensis* while in *C. natator* the carapace width ranged between 70–130 mm in females and 70–140 mm in males (Tables 1 and 2). Insignificant variations in chitin content were noticed among different sizes of *C. natator* for both sexes, with irregular values ranged between 0.49 g (9.9%) and 0.88 g (17.7%) (Figure 4A). Conversely, significant variations in chitin yield were observed between the different sizes of *E. massavensis* with the maximum (33%) being in the size range 90–130 mm BL ($F_{(4, 16)} = 3.61$, $P < 0.05$, $F_{(4, 16)} = 7.17$, $P < 0.01$) (Figure 4B).

With regard to maturity stage, chitin yield in *C. natator* was at its lowest values in the immature stage of females (9.29%) then values increased and remained constant for the remaining stages (≥ 18%). In contrast, values were almost equal in all stages of *E. massavensis* (Figure 5).

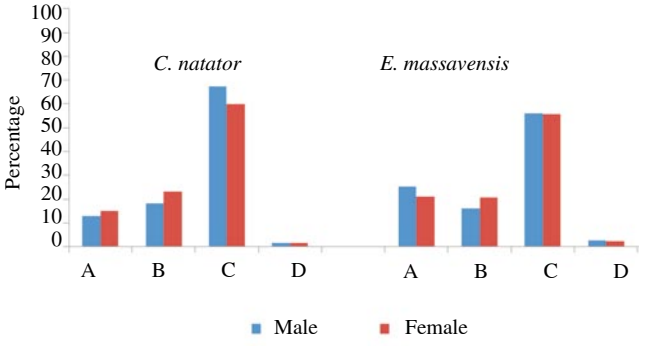

Figure 3. Percentage of exoskeleton components of *C. natator* and *E. massavensis*.
A: Chitin; B: Proteins; C: Minerals; D: Pigments.

Table 1
Mean values of chitin (g/5 g) obtained from *C. natator* in relation to size.

Size range (mm)	Females	Males
70–80	0.62	0.63
80–90	0.60	0.77
90–100	0.87	0.51
100–110	0.74	0.49
110–120	0.65	0.54
120–130	0.88	0.84
130–140		0.74
Average	0.73	0.65
Total mean	0.69	

Table 2
Mean values of chitin (g/5 g) obtained from *E. massavensis* in relation to size.

Size range (mm)	Females	Males
70–90	0.92	0.88
90–110	1.03	1.65
110–130	1.10	1.49
130–150	1.18	1.25
150–170	1.02	1.07
Average	1.05	1.27
Total mean	1.16	

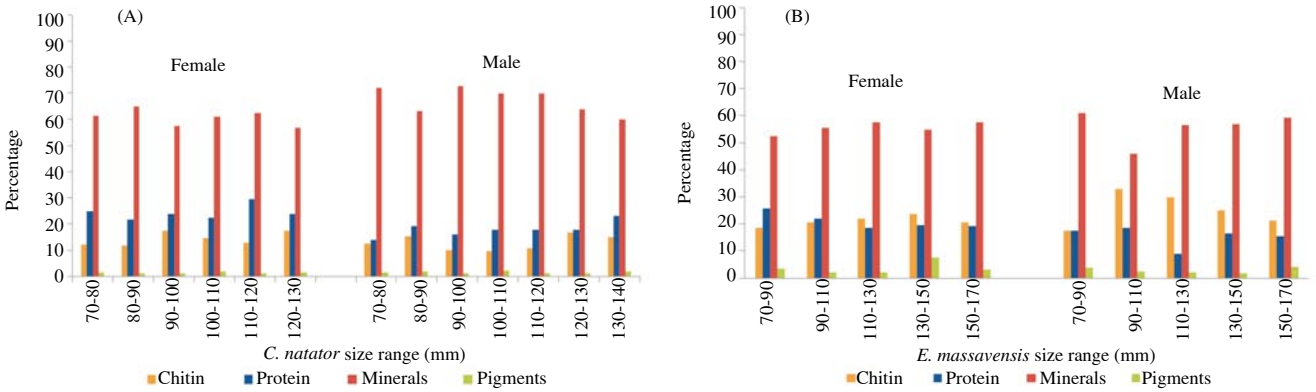

Figure 4. Percentage of exoskeleton components of *C. natator* (A) and *E. massavensis* (B), with regard to the size ranges of both sexes.

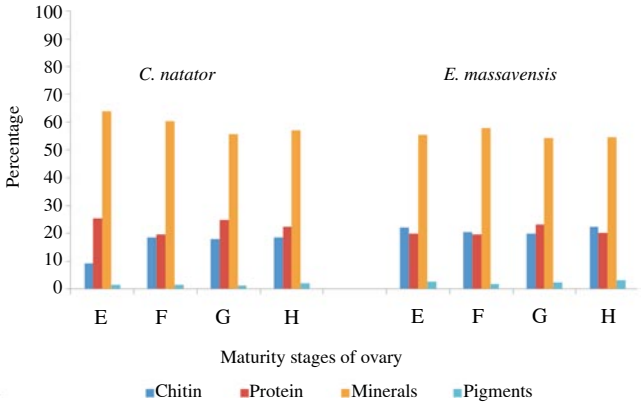

Figure 5. Variations in the chitin yield of *C. natator* and *E. massavensis* with regard to females' maturity stages.
E: Immature; F: Maturing; G: Mature; H: Ripe.

4. Discussion

Crustaceans' exoskeleton has been described as "a bio-mineralized structure which consists of an organic matrix together with an inorganic mineral"[18]. The present study was an attempt to estimate the yield of chitin from two crustacean species inhabiting Egyptian waters, the Red Sea crab *C. natator* and the Mediterranean mantis shrimp *E. massavensis*, and stand on the effect of size, sex and maturity stage on this yield.

The percentages of exoskeleton components (*e.g.* chitin, proteins, minerals and pigments) obtained for the two species in the present work were in agreement with previous studies on other crustaceans[17,19]. Differences in the levels of those components with high proportion of minerals in two species (more than 50%) could be referred to the mechanical requirements as well as the difference in the biological escape behavior between the two animals. *E. massavensis* showed lower percentages of minerals than *C. natator* where lobsters are known to have a lighter, more elastic cuticle than crabs since they are motile, fast-swimming animals, therefore, they are able to escape from predators and seek shelter between rocks[18]. Crabs, on the other hand, need a hard, highly mineralized shell in order to hold tightly to the ground and burrow into the sand during any attack. Moreover, mantis shrimps are known as very aggressive predators with high swimming and predation abilities[20]. Accordingly, the shell of *E. massavensis* is less mineralized and therefore lighter and less hard than that of *C. natator*, a fact that explains the lower levels of both protein and minerals recorded between these two species in the present study.

Previous studies reported chitin values close to those measured in the present study for the mantis shrimp[20-22] and crab[11,20,22] (Table 3). The variability in content of chitin in shells according to species has been documented[20-22]. Also, several factors were found to influence chitin values in crustacean shells such as season, nutritional and geographic condition[23,24].

Table 3
Chitin yield (g/20 g) of the studied species in comparison with other crustaceans studied elsewhere.

Species		Reference	Chitin (g/20 g)	Preparation method
Mantis shrimp	*Oratosquilla quinquedentata* (Kemp, 1911)	[21]	2.13	C1
	Oratosquilla nepa (Latreille, 1825)	[21]	2.15	C1
	Squilla spp.	[22]	4.83*	C1
	Squilla mantis (Linnaeus, 1758)	[20]	4.80*	C2
	E. massavensis (Kossmann, 1880)	Present study	4.42*	C3
Crab	*Podophthalmus vigil* (Fabricius, 1798)	[11]	5.50	C1
	Calappa lophos (Herbst, 1782)	[22]	7.42*	C1
	Dromia dehaani (Rathbun, 1923)	[22]	5.54*	C1
	Dorippe facchino (Herbst, 1785)	[22]	1.79*	C1
	Portunus puber (Linnaeus, 1767)	[20]	2.00*	C2
	C. natator (De Haan, 1833)	Present study	2.84*	C3

C1: Conventional 1[22]; C2: Conventional 2[20]; C3: Conventional 3[15].
*: Values have been calculated on the basis of 20 g initial weight for comparative reasons.

The maximum chitin yield for *E. massavensis* was noticed in the size range 90–130 mm BL. It is proposed that at this size range, maturity takes place and therefore the intermolt intervals lengthen, thus individuals keep the chitin levels in their shells high. Increased size or reproductive maturity may stop molting in crustaceans[25,26].

Increasing chitin yield in *E. massavensis* males than females is reasonable since reproduction in females is known to deplete all resources from the body organs to help with ovarian development. The immature stage of the ovary is the onset of the reproductive process where chitin starts to deposit in the ova during development. Crabs are more likely to be less active prior to the reproduction and consequently protein and minerals are not consumed excessively and could therefore be found in their shells with high levels[23]. On the other hand, increasing in chitin levels had been reported in the shell waste of the Norway lobster *Nephrops norvegicus* from Northern Ireland and was attributed to the decrease in protein[27]. In this context, other small crustaceans have been reported as an alternative chitin source. Of these, *Artemia* cyst was suggested as a new source

of chitin[28], in addition to the resting eggs of the fresh water flea *Daphnia longispina* that was reported to have about 23%–25% chitin content[9].

Conclusively, the present study shows the significance of the two studied species' integuments in the production of chitin on a commercial scale and recommends the sustainable exploitation of this resource. Special attention can be drawn to middle size males' mantis shrimps in order to obtain the maximum possible yield.

Conflict of interest statement

We declare that we have no conflict of interest.

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