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Shell disease does not affect biochemical profiles of the North Sea brown shrimp *Crangon crangon*

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ABSTRACT: The brown shrimp *Crangon crangon* is a key component of the North Atlantic coastal food web and an important target species for the fishery economy. As the brown shrimp contains large amounts of protein and essential fatty acids, its consumption makes it a beneficial choice for humans. Commercially harvested crustaceans like *C. crangon* are frequently affected by bacterial shell disease, with necrotizing erosions and ulcerations of the cuticle. To determine whether shell disease influences the nutritional value of *C. crangon*, total protein and lipid contents, as well as fatty acid compositions of muscle tissue and hepatopancreas, together with the hepatosomatic index, were examined in healthy and affected individuals. The biochemical composition of the tissues did not differ significantly between the 2 groups. Also, the hepatosomatic index, as an indicator of energy reserves in shrimps, was similar between healthy and affected animals. Our results indicate that the nutritional value of *C. crangon* is not affected by shell disease, as long as it remains superficial as in the present study.

KEY WORDS: Black spot disease · Lipids · Fatty acids · Omega-3 · EPA · DHA · Hepatosomatic index

1. INTRODUCTION

The brown shrimp *Crangon crangon* (Linnaeus, 1758) is one of the most ecologically and economically important invertebrate species along the North Atlantic coast of Europe. It forms a key component in the marine food web and is commercially harvested throughout most of its distribution range, with a yield of more than 35 000 t yr^{-1} (Revill & Holst 2004, Beukema & Dekker 2005, ICES 2015). Several exploited crustaceans, such as the brown shrimp, the edible crab *Cancer pagurus*, or the American lobster *Homarus americanus*, can be affected by shell disease (Smolowitz et al. 2005, Shields 2013), with significant commercial implications. For example, for the US fishing industries, shell disease in the American lobster has caused economic losses reaching millions of dollars, since it directly renders affected individuals unmarketable as a live product and indirectly affects reproduction and recruitment (Ayres & Edwards 1982, Getchell 1991, Wahle et al. 2009, Lafferty et al. 2015).

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The term 'shell disease' includes both enzootic and epizootic shell disease and describes different forms of necrotic lesions and disintegrations of the cuticle of crustaceans resulting from distinct etiologies and causative agents (Dyrynda 1998, Vogan & Rowley 2002, Shields 2013). Enzootic or classical shell disease induces irregularly shaped lesions of the cuticle that are commonly observed in lobsters, crabs, and shrimps (Smolowitz et al. 1992, Vogan et al. 1999). In contrast, the more aggressive epizootic shell disease progresses inwards through the carapace and occurs mainly in the American lobster (Castro & Angell 2000, Chistoserdov et al. 2005, Smolowitz et al. 2005). In both disease forms, the characteristic black ulcerations may be caused by a pathogenic shift in the bacterial shell community. In this context, a multitude of different chitinolytic and lipolytic bacteria strains have been observed, including species in genera such as *Aquimarina*, *Pseudomonas*, and *Vibrio* (Rosen 1967, Meres et al. 2012, Feinman et al. 2017).

Some of these chitinolytic and lipolytic bacteria are also present on the cuticle of healthy crustaceans (Meres et al. 2012, Kraemer et al. 2020). Before the symbiosis becomes pathogenic, and chitin degradation by bacteria can occur, a portal of entry is required in the form of destruction of the outermost non-chitinous epicuticle by mechanical (Vogan et al. 1999) or chemical abrasion (Schlotfeldt 1972), or predatory or cannibalistic attacks (Dyrynda 1998). In addition, toxins, pollution, and higher temperatures may further accelerate bacterial growth and reduce the resistance of the host (Young & Pearce 1975, Glenn & Pugh 2006, Laufer et al. 2012).

Superficial early stages of shell disease are usually not fatal, and the affected individual may overcome the condition by molting (Smolowitz et al. 1992, Barris et al. 2018, Segelken-Voigt et al. 2018). Nevertheless, shell disease can also cause death, either directly through invading pathogens into the underlying soft tissues, leading to internal infections, or indirectly, through impaired feeding function or locomotion, due to lesions on the appendages (Smolowitz et al. 1992, Vogan et al. 2001). Additionally, shell disease can interfere with immunocompetence (Chen et al. 1995, Tlusty et al. 2007) and thus reduce the survival chance of affected crustaceans (Smolowitz et al. 2005, Hoenig et al. 2017, Segelken-Voigt et al. 2018). Likewise, affected crustaceans may be energetically compromised or produce elevated levels of ecdysone due to altered gene expressions and hormone levels, resulting in changes in the molting behavior and cellular stress response (Laufer et al. 2005, Castro et al. 2006, Tarrant et al. 2012).

In marine invertebrates, proteins and lipids play an important role as the main energy reserves or as cell membrane components (Kattner et al. 1994, García et al. 2002, Lee et al. 2006, Jimeńez & Kinsey 2015). During stressful environmental conditions (e.g. food scarcity, heavy metal or pathogen exposure), energy reserves can be mobilized to maintain physiological functions and increase the resilience of an organism (Floreto et al. 2000, Verslycke et al. 2003, Sacristán et al. 2017). In decapods, lipids are primarily stored in the hepatopancreas (midgut gland), which synthesizes enzymes and lipids (Chang & O'Connor 1983, Diaz et al. 2010, Martínez-Alarcón et al. 2019). Physiological impairments and diseases directly affect this organ (Johnson 1980), and a low hepatosomatic index (HSI) could indicate poor health or reduced food intake. For example, *H. americanus* affected by shell disease have significantly lower lipid levels, including omega-3 fatty acids, in their hepatopancreas compared to healthy specimens, as well as a lower HSI, indicating a reduced lipid-storage capacity (Floreto et al. 2000).

Our study organism, *C. crangon,* contributes important nutritional components to the human diet. It contains high amounts of essential omega-3 fatty acids such as eicosapentaenoic acid (EPA, 20:5(n-3)) and docosahexaenoic acid (DHA, 22:6(n-3)), which can improve human health status and reduce the risk of cancer, cardiovascular diseases, and diabetes (Heu et al. 2003, Harper & Jacobson 2005, Vaughan et al. 2013, Calder 2017). The biochemical composition (e.g. contents of proteins and lipids) of shrimp and other edible crustaceans may reflect their physiological state of health and, thus, their nutritional value for human consumption. To investigate a possible relationship between shell disease and the nutritional value of *C. crangon*, total protein and lipid contents and the fatty acid composition were analyzed in 2 tissue types of healthy and affected brown shrimp. The hepatopancreas served as an indicator of the metabolic state of the shrimp, whereas muscle tissue reflected the nutritional value of the animal.

2. MATERIALS AND METHODS

2.1. Sampling and preparation of *Crangon crangon*

In July 2017, *C. crangon* specimens were collected at depths of 8–10 m by a beam trawl of RV 'Uthörn' in

the Weser estuary in the German Bight, North Sea (53° 38.90' N, 8° 25.19' E) (Fig. 1). After sampling, the shrimp were immediately weighed (fresh mass) and measured (total length from end of the rostrum to end of the tail). In total, 41 adult males and non-egg-bearing females were analyzed for signs of shell disease (see Fig. 2). The affected individuals were photographed with a Nikon P7000, and the size of the black ulcerations on the shell was measured using ImageJ software (http://rsb.info.nih.gov/ij/) (Table 1). To calculate the relative size of black ulcerations in percentage coverage, the size of the necrotic lesions was normalized to the body length of the individual. In total, 18 healthy (0% shell-disease coverage) and 23 affected shrimp (>5% shell-disease coverage) were used for subsequent biochemical analyses. The individuals were snap-frozen in liquid nitrogen after measurement and dissected at a later stage for tissue sampling. The hepatopancreas and a piece of the muscle from the second abdominal segment were stored at −80°C until further biochemical analyses. As an indicator of the energetic status of the shrimp, the hepatopancreas was weighed to determine the HSI (% of fresh mass). This value was computed as HSI % = HW / BW × 100, where HW is the wet mass of the hepatopancreas (mg), and BW is the wet mass of the brown shrimp (mg). All animal work was conducted according to the German animal protection law (approval no. 33.19-42502-05-15A542).

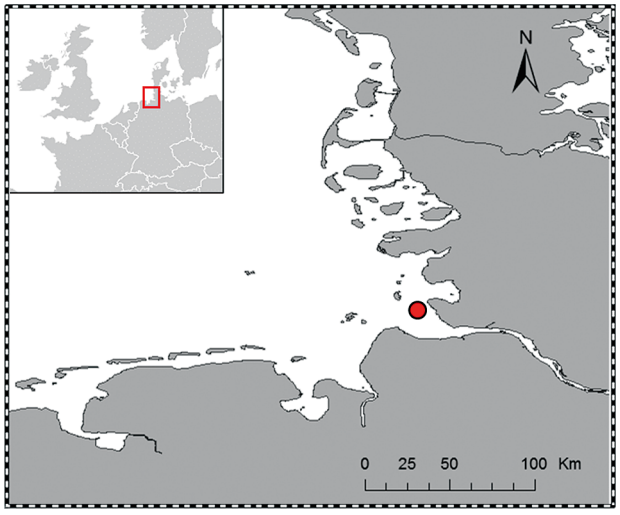

Fig. 1. Sampling location of brown shrimp *Crangon crangon* in the Weser estuary in the German Bight, North Sea (53° 38.90' N, 8° 25.19' E)

2.2. Total protein content

The total protein content was determined following the protocol of Lowry et al. (1951) and using the DC™ Protein Assay Kit (BIO RAD Laboratories) with bovine serum albumin (BSA) standard. The standard curve consisted of 5 dilution standards of known protein concentration (0, 0.19, 0.38, 0.76, 1.52 mg ml^{-1}) mixed in 1 M NaOH (sodium hydroxide). Tissue samples of muscle and hepatopancreas were diluted 1:200 (m:v) in 1 M NaOH. Small glass beads (∅ 0.1 mm) were added to the samples. Standards and samples were homogenized by vortexing (Vortex Genie 2, Scientific Industry) for 30 s and incubated on a rotating heating block (Thermomixer comfort, Eppendorf) for 20 min at 50°C and 200 × *g*. These steps were repeated 3 times. Samples were centrifuged for 10 min at room temperature and 1500 × *g*. Aliquots of 5 µl of the supernatants and the protein standards, respectively, were transferred (in duplicate) into individual wells of a 96-well microtiter plate. Aliquots of 25 µl alkaline copper tartrate solution (Bio-Rad DC Reagent A) and 200 µl Folin reagent (Bio-Rad DC Reagent B) were added to each sample and standard. After 15 min of incubation in the dark, the samples were measured spectrophotometrically in a microplate reader (Infinite 200 Pro, Tecan) at a wavelength of 750 nm. Total protein content was calculated based on the standard curve and expressed in % of dry mass (% DM).

2.3. Total lipid content

The total lipid content was determined following the methods of Folch et al. (1957) and Hagen et al. (2000). Muscle and hepatopancreas samples were lyophilized for 48 h and transferred to an evacuated desiccator for 30 min. The pulverized tissue samples were weighed and homogenized with Potter homogenization and ultrasonic disruption, and then total lipids were extracted with dichloromethane/methanol (DCM/MeOH, 2:1 per volume). This procedure was repeated, and each time the supernatant was transferred into a clean test tube. After adding 2 ml KCl (potassium chloride) solution (0.88%), the samples were centrifuged at 1495 × *g* (10 min at 2°C). After phase separation, the lipid phase was pipetted into a clean glass vial and the solvents evaporated under a stream of nitrogen. The pure, extracted lipids were weighed and the total lipid contents of muscle and of hepatopancreas were calculated in % DM. For subsequent fatty acid analyses, the extracted lipids were redissolved in DCM/MeOH and stored in nitrogen at −80°C.

Table 1. List of *Crangon crangon* specimens sampled for biochemical analyses with total length, fresh masses of the individual and the hepatopancreas (HP), sex (F: female; M: male), and shell-disease coverage

Sample no.	Total length (mm)	Fresh mass (mg)	HP mass (mg)	Sex	Shell-disease cover (mm^2)
1	59	1530	50	F	0
2	55	1390	69	F	0
3	50	1000	57	F	0
4	52	1240	66	M	0
5	55	1150	61	F	0
6	50	890	46	F	0
7	53	1270	62	M	0
8	56	1680	63	F	0
9	55	1420	53	F	0
10	51	1230	78	F	0
11	47	890	49	F	0
12	47	730	47	M	0
13	55	1130	70	F	0
14	55	1500	60	F	0
15	53	1370	93	F	0
16	52	1300	59	F	0
17	50	1030	42	M	0
18	58	1930	90	F	0
19	50	1240	57	F	3.2
20	56	1760	61	F	3.3
21	60	1900	79	F	3.4
22	55	1360	77	F	3.4
23	54	1400	78	F	3.6
24	56	1650	96	F	3.8
25	51	1130	77	M	4.3
26	56	1540	76	F	4.4
27	53	2080	79	F	4.4
28	41	430	29	M	4.6
29	51	1010	64	F	4.8
30	50	1130	54	F	5.1
31	46	920	30	M	5.2
32	56	1530	77	F	5.3
33	50	1030	41	F	5.6
34	49	1030	59	F	5.6
35	54	1320	60	F	5.8
36	55	1540	96	F	5.9
37	50	990	48	M	6.8
38	54	1270	51	F	7.6
39	44	610	42	M	8.2
40	53	1070	46	F	8.9
41	55	1500	69	F	9.8

2.4. Fatty acid composition

A subsample of the redissolved lipid samples was hydrolyzed to convert the fatty acids to methylester derivatives (FAMEs). This trans-esterification was performed by heating the samples with 3% H_2SO_4 (sulfuric acid) in methanol and 250 µl hexane for 4 h at 80°C (Kattner & Fricke 1986). FAMEs were dissolved in 1 ml hexane and their composition analyzed in a gas chromatograph (HP 6890; Agilent Technologies) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) operating with an automated temperature vaporizer injector and with helium as carrier gas. Fatty acid peaks were identified by comparing their retention times with 2 standards of known composition (copepod lipids and menhaden fish oil). The quantities of the individual fatty acids were expressed as mass percentage of total fatty acids (% TFA).

2.5. Statistical analysis

Before running the statistical tests in SPSS 24, the data were checked for normality and homogeneity of variances. Initially, we separated the shrimp into 3 groups to examine potential differences between healthy (0% shell-disease coverage), moderately (5–10% shell-disease coverage, Fig. 2A,B), and severely affected shrimp (>10% shell-disease coverage, Fig. 2C–F).

The biochemical profiles (total lipid and protein levels plus fatty acid compositions) in these 3 groups, as well as separately in females and males, were then visualized by non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity, after a square-root-transformation of the data (Kruskal & Wish 1978) using PRIMER 6.1.6 (Clarke & Gorley 2006). Additionally, a permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) was performed to test for differences in biochemical composition (total amount of lipids and proteins as well as fatty acid composition) of muscle tissue and hepatopancreas with the factors 'shell-disease coverage' (fixed at 3 levels = healthy, moderately, and severely affected) and 'sex' (fixed at 2 levels = female and male) based on the Bray-Curtis similarity matrix of square-root-transformed data. The analysis was run using the permutation of residuals under a reduced model, with 9999 permutations and type III sums of squares.

As no differences were observed between moderately and severely affected shrimp, we pooled these individuals into 1 group (see also Fig. 2). To test for significant differences in the biochemical parameters

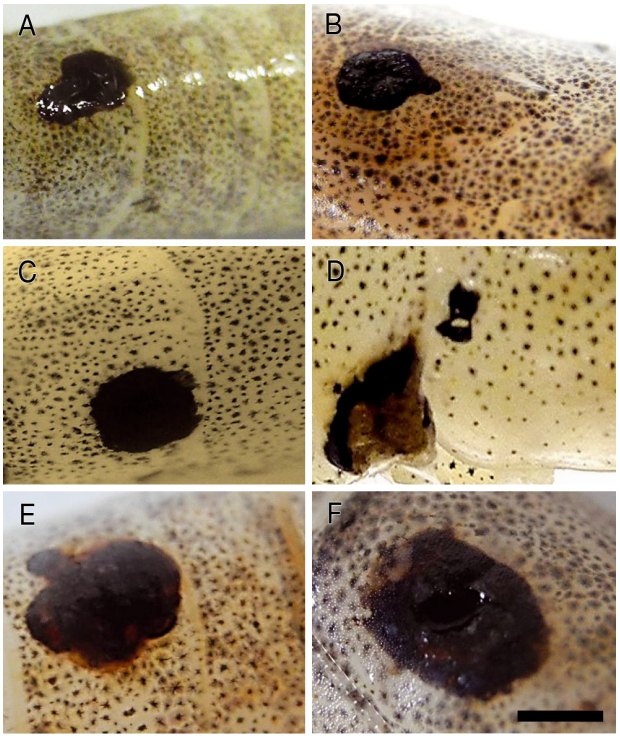

Fig. 2. Shell disease in *Crangon crangon* with varying lesion sizes: (A) 3.6, (B) 4.8, (C) 5.8, (D) 6.8, (E) 8.9, (F) 9.8 mm^2. Scale bar = 2.5 mm

and the HSI between healthy and shell-disease affected animals, a *t*-test or a 1-way ANOVA was applied (depending on the number of groups). The significance level was set to $\alpha = 0.05$.

3. RESULTS

There were no apparent differences in the biochemical profiles of the hepatopancreas and muscle of healthy vs. affected shrimp. The nMDS plot (Fig. 3) displaying the composition of fatty acids as well as the amounts of lipids and proteins in the muscle (Fig. 3A) and in hepatopancreas (Fig. 3B) revealed no clustering for healthy, moderately, and severely affected brown shrimp. In the muscle tissue and hepatopancreas (Table 2), the PERMANOVA showed neither specific differences between the 3 groups, nor between sexes. In the following, all individuals were therefore classified as either healthy or shell-disease affected.

Fresh mass, total length, and HSI did not differ significantly between healthy and shell-disease affected brown shrimp. Mean ± SD fresh mass and total length of *Crangon crangon* amounted to 1260 ± 300 mg and 53 ± 4 mm in healthy individuals and

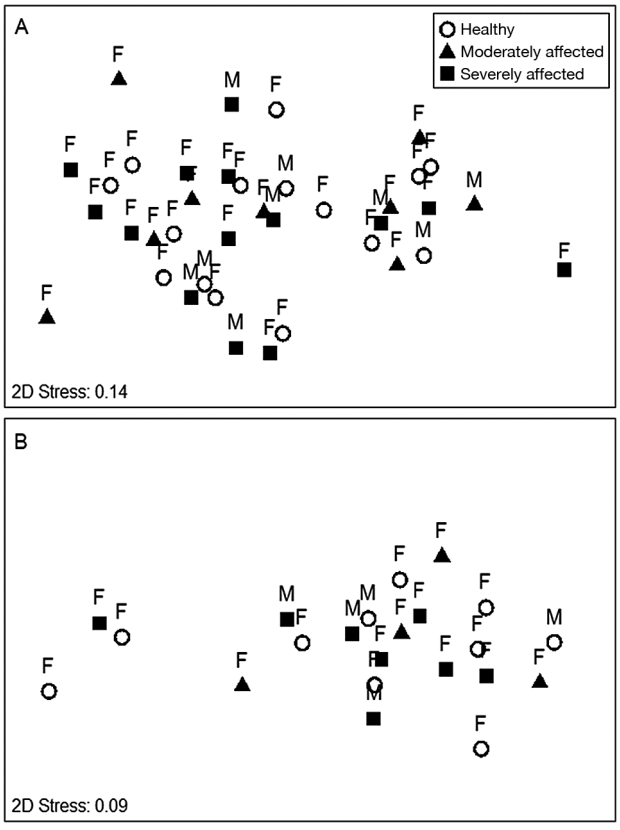

Fig. 3. Non-metric multidimensional scale ordination of Bray-Curtis similarity from square-root transformed data of biochemical compositions of (A) muscle and (B) hepatopancreas of *Crangon crangon* between the 3 health categories. Open circles represent healthy shrimp, black squares represent severely affected shrimp, and black triangles represent moderately affected shrimp. Individuals are further differentiated by sex (F: female; M: male)

Table 2. Results of PERMANOVA testing for differences in biochemical composition of muscle and hepatopancreas of *Crangon crangon* between the 3 health categories (healthy, moderate, and severely shell-disease affected), and between the 2 sexes (female and male); p (perm) refers to the significance level assessed by a permutation test

Source	df	Pseudo-*F*	p (perm)
Muscle			
Shell disease	2	0.8268	0.577
Sex	1	1.9516	0.102
Shell disease × sex	2	0.7159	0.701
Residuals	32		
Total	37		
Hepatopancreas			
Shell disease	2	0.4912	0.7849
Sex	1	0.6097	0.5567
Shell disease × sex	1	0.9694	0.3369
Residuals	17		
Total	21		

1280 ± 400 mg and 52 ± 5 mm in the affected animals. The mean hepatopancreas mass was 61 ± 18 mg in healthy and 62 ± 22 mg in affected shrimp. This resulted in an HSI of 5.0 ± 1.0% in healthy and 4.9 ± 1.0% in affected shrimp (t = −0.060, p = 0.953).

Mean total protein and lipid contents in muscle and in hepatopancreas tissue did not differ significantly between healthy and affected *C. crangon*. The total protein content in the muscle accounted for 65.9 ± 6.8% DM in the healthy and 65.3 ± 9.7% DM in the affected brown shrimp (t = −0.245, p = 0.808, Fig. 4A). In the hepatopancreas, the total protein content averaged 52.6 ± 11.3% DM in the healthy and 53.4 ± 7.1% DM in the affected shrimp (t = 0.188, p = 0.853, Fig. 4B). The total lipid content of the muscle was 6.3 ± 0.9% DM in healthy and 5.8 ± 1.0% DM in affected *C. crangon* (t = −1.486, p = 0.146, Fig. 4C). The hepatopancreas had a mean total lipid content of 20.9 ± 6.1% DM in healthy animals, and 20.5 ± 5.2% DM in affected shrimp (t = −0.158, p = 0.876, Fig. 4D).

In total, 16 fatty acids were identified, each exceeding 1% of TFAs (Table 3). Major fatty acids in both tissue types of healthy and affected animals were palmitic acid (16:0), oleic acid (18:1(n-9)), eicosapentaenoic acid (EPA, 20:5(n-3)), and docosahexaenoic acid (DHA, 22:6(n-3)). Fatty acid compositions of the muscle and of the hepatopancreas did not differ significantly between the 2 *C. crangon* groups. In the muscle, all omega-3 fatty acids (Σn-3) accounted for 46.2 ± 1.7% of TFAs in the healthy and 46.1 ± 2.6% of TFAs in the affected shrimp (t = 0.108, p = 0.914). The omega-3 fatty acids in the hepatopancreas amounted to 43.1 ± 5.8% in healthy and 43.5 ± 4.5% in affected individuals (t = −0.188, p = 0.853).

The compositions of saturated (SFAs), monounsaturated (MUFAs), and polyunsaturated fatty acids

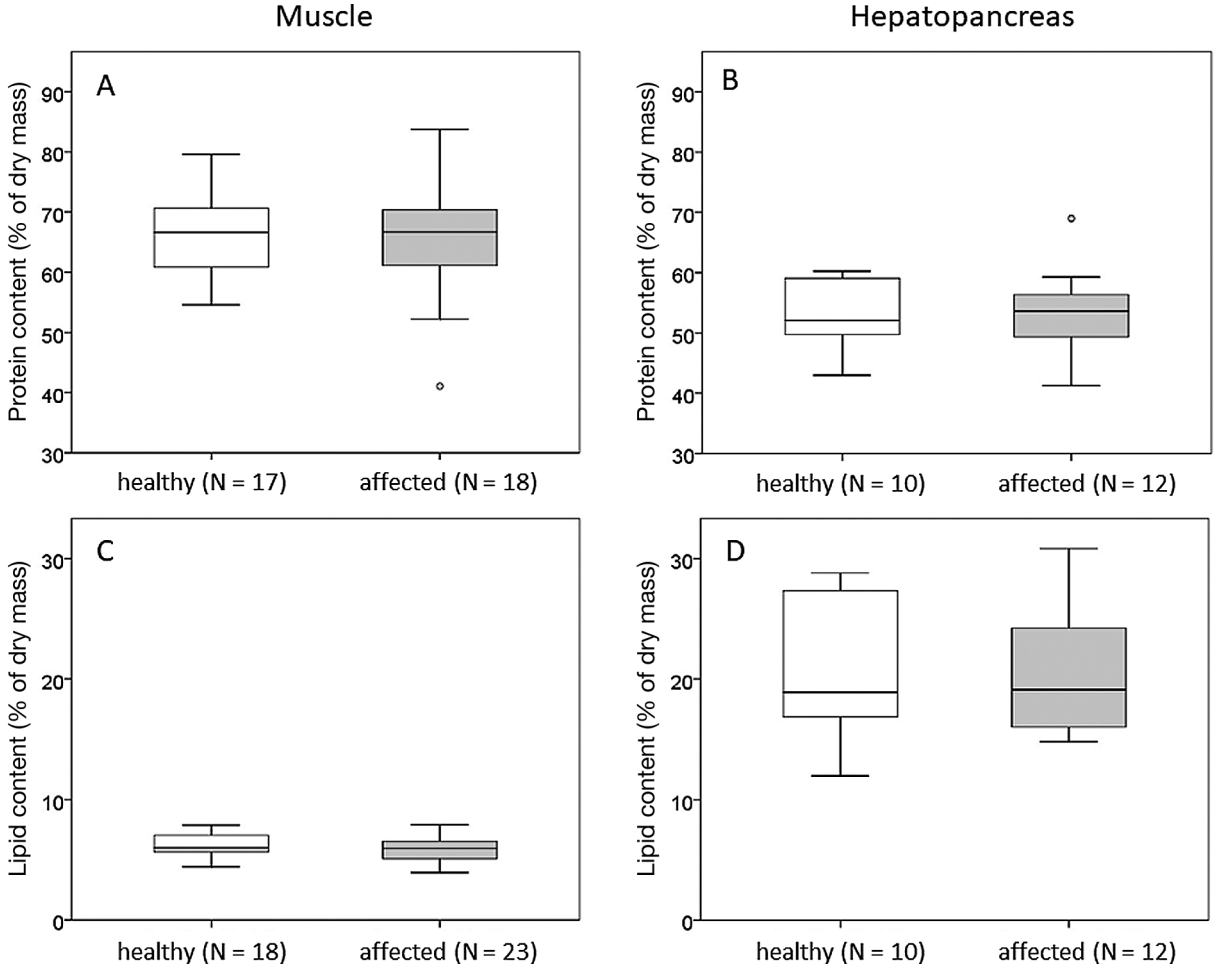

Fig. 4. Total protein contents in (A) muscle tissue and (B) hepatopancreas, and total lipid contents in (C) muscle and (D) hepatopancreas of healthy and shell-disease affected *Crangon crangon*. The center line in the box plot represents the median with upper and lower quartiles, whiskers indicate minima and maxima, and open circles are outliers

Table 3. Fatty acid (FA) compositions (mass % of total FAs greater than 1%) of muscle tissue and of hepatopancreas of healthy and shell-disease affected *Crangon crangon*. Values represent means ± SD. SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; n-3: omega-3 FA

FA	Muscle		Hepatopancreas	
	Healthy (N = 15)	Affected (N = 23)	Healthy (N = 10)	Affected (N = 12)
14:0	1.0 ± 0.1	1.1 ± 0.2	1.8 ± 0.7	1.8 ± 0.6
16:0	20.5 ± 0.9	20.2 ± 1.4	15.2 ± 1.9	15.0 ± 1.4
16:1(n-7)	4.2 ± 0.8	4.0 ± 0.7	5.7 ± 2.0	5.5 ± 1.5
16:3(n-4)	0.6 ± 0.2	0.7 ± 0.2	0.9 ± 0.3	0.9 ± 0.2
17:0	1.0 ± 0.1	1.0 ± 0.1	0.7 ± 0.2	0.8 ± 0.1
18:0	4.1 ± 0.7	4.0 ± 0.6	3.6 ± 0.7	3.5 ± 0.6
18:1(n-9)	9.3 ± 0.8	9.3 ± 1.2	10.0 ± 2.1	10.1 ± 1.8
18:1(n-7)	5.0 ± 0.5	5.1 ± 0.6	5.6 ± 0.6	5.6 ± 0.9
18:2(n-6)	1.2 ± 0.1	1.2 ± 0.2	1.2 ± 0.3	1.2 ± 0.2
20:1(n-11)	0.3 ± 0.1	0.2 ± 0.1	0.7 ± 0.5	0.9 ± 0.4
20:1(n-9)	0.3 ± 0.1	0.3 ± 0.3	1.0 ± 0.4	0.8 ± 0.3
20:1(n-7)	0.3 ± 0.1	0.4 ± 0.2	1.1 ± 0.6	1.2 ± 0.3
20:4(n-6)	3.6 ± 0.4	3.6 ± 0.4	3.8 ± 0.7	3.7 ± 0.8
20:5(n-3)	22.6 ± 1.0	23.0 ± 1.3	21.1 ± 3.1	21.3 ± 2.2
22:5(n-3)	1.2 ± 0.2	1.3 ± 0.3	1.6 ± 0.7	2.1 ± 0.7
22:6(n-3)	21.6 ± 2.0	21.2 ± 2.1	18.3 ± 4.6	18.4 ± 3.5
Σ SFA	28.1 ± 1.5	27.9 ± 2.1	23.6 ± 2.8	23.4 ± 2.4
Σ MUFA	19.7 ± 1.5	19.9 ± 1.8	25.1 ± 3.7	25.2 ± 2.9
Σ PUFA	52.1 ± 1.6	52.1 ± 2.7	50.9 ± 5.7	51.1 ± 4.7
Σ n-3	46.2 ± 1.7	46.1 ± 2.6	43.1 ± 5.8	43.5 ± 4.5

(PUFAs) between affected and healthy shrimp differed neither in the muscle (SFAs: t = 0.240, p = 0.811; MUFAs: t = –0.308, p = 0.760; PUFAs: t = 0.020, p = 0.984) nor in the hepatopancreas (SFAs: t = 0.221, p = 0.828; MUFAs: t = –0.047, p = 0.963; PUFAs: t = –0.090, p = 0.930) (Table 3).

4. DISCUSSION

In this study, we examined the biochemical profiles of healthy and shell-disease affected *Crangon crangon* to assess possible effects of the classical shell disease on the nutritional composition of this food product. We detected no differences in total protein or lipid contents, or in the fatty acid compositions, between healthy and affected brown shrimp, pointing to a similar nutritional value of both groups. The muscle tissue of all *C. crangon* was rich in PUFAs, especially in the omega-3 fatty acids EPA and DHA. These are the most common fatty acids found in seafood and have been associated with many health benefits for humans (Harper & Jacobson 2005, Vaughan et al. 2013, Calder 2017). The amounts of these dominant fatty acids are in accordance with previous findings for *C. crangon* and other decapods, such as the whiteleg shrimp *Litopenaeus vannamei* and the giant freshwater prawn *Macrobrachium rosenbergii* (Bragagnolo & Rodriguez-Amaya 2001, Perez-Velazquez et al. 2003, Martínez-Alarcón et al. 2019). Mika et al. (2014) and Turan et al. (2011) also reported similar compositions, with principal fatty acids such as 16:0, 18:1(n-9), 20:5(n-3), and 22:6(n-3) in *C. crangon* from the White Sea and Black Sea.

In contrast to our findings, muscle tissues of the American lobster *Homarus americanus* affected by shell disease revealed slightly higher ratios of 20:4(n-6) and 22:6(n-3) compared to healthy lobsters (Floreto et al. 2000). In addition, a lower HIS, as well as significantly lower lipid levels, were measured in the hepatopancreas of affected *H. americanus*. The authors concluded that lobsters affected by shell disease have either a reduced capability to store lipids, or exhibit a higher energetic requirement compared to healthy individuals. As in our study, the constitution and biochemistry of the lobsters may not be affected by large, superficial black ulcers, but rather by lesions that have already penetrated the cuticle into the soft tissue. In our study, in spite of shell disease, affected *C. crangon* do not use more of their energy stores when compared to healthy shrimp, pointing towards a stable physiological state in terms of biochemical composition. Hence, our results suggest that in *C. crangon,* classical shell disease predominantly generates superficial symptoms, as indicated by the black necrotic lesions on the cuticle. Other studies suggest that all layers of the cuticle, as well as the soft underlying tissue, of brown shrimp may be affected by shell disease (Schlotfeldt 1972, Segelken-Voigt et al. 2018).

In our study, no signs of necrotic lesions were observed on the epidermis of *C. crangon* specimens, thus the lesions were apparently not severe enough to penetrate the different shell layers and to impair the vitality of *C. crangon*. It has previously been stated for *C. crangon* that necrotic lesions remain superficial on the epicuticle or develop at the end of a molting cycle and that brown shrimp can diminish or even eliminate black spots via ecdysis (Smolowitz et al. 1992, Segelken-Voigt et al. 2018). Our data and literature studies suggest that the high molting frequencies in *C. crangon* minimize the progression of the disease symptoms into

deeper layers of the cuticle, internal organs, and tissues. Brown shrimp have a fast molting rate of approximately 3 wk (Hufnagl & Temming 2011) and a short intermolt period. Hence, these shrimp have little time to develop shell-disease-induced lesions compared to adult American lobsters that have a molting rate of approximately once per year (Hughes & Matthiessen 1962).

Despite the fact that brown shrimp can heal itself of the black necrotic lesions by molting, we cannot exclude an effect of shell disease on biochemical and physiological processes in *C. crangon.* For example, an infestation with black spots can reduce the respiratory performance of affected shrimp (Segelken-Voigt et al. 2018), and, in the worst case, the bacteria may cause a sepsis, possibly leading to death (Smolowitz et al. 1992, Vogan et al. 1999, Vogan & Rowley 2002). Consequently, we cannot exclude the possibility that the microbiological quality of shrimp as a consumer product will be affected by shell disease, while the nutritional value remains at a high level.

Shell disease can also cause significant alterations in the immune system of crustaceans, by a reduction of serum protein, via low concentrations of circulating hemocytes in the hemocoel, by destruction of blood cells through phagocytosis (Prince 1997), or by an enhancement of hemocytes in the hemolymph by increased immune activity in affected individuals (White & Ratcliffe 1982, Vogan & Rowley 2002). Therefore, future studies should address the immune-system responses of affected brown shrimp and examine the biochemical composition of *C. crangon* bearing lesions that have already penetrated the epidermis.

5. CONCLUSIONS

Our findings show that as long as the black ulcers are superficial, shell disease in *Crangon crangon* does not affect its energy stores or biochemical profiles. No significant alterations in the biochemical composition or the HSI were detected in shrimp affected by shell disease compared to healthy specimens. Total protein and lipid contents, as well as fatty acid compositions in muscle and hepatopancreas, remained similar between healthy and affected shrimp. We thus conclude that the nutritional value of superficially affected brown shrimp is maintained, including the levels of essential omega-3 fatty acids such as EPA and DHA.

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