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# The biochemical profiles of shell-diseased American lobsters, *Homarus americanus* Milne Edwards

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## Abstract

The proximate (% of dry weight), amino (% of total amino acids) and fatty acid (% of total fatty acids) profiles of tissues (muscle, hepatopancreas, hemolymph and exoskeleton) of American lobster, *Homarus americanus* (Milne Edwards) affected with shell-disease, were compared with those of healthy, unaffected animals. Muscle tissues of affected lobsters had significantly lower levels of carbohydrate and the protein profile had significantly lower ratios of arginine, threonine, serine and total essential amino acids. However, the ratio of glycine was about 50% higher than in muscle tissues of healthy lobsters. Muscle tissues of affected lobsters had slightly higher ratios of 20:4n – 6 and 22:6n – 3. Affected lobsters had significantly lower hepatosomatic indices. Their hepatopancreas contained significantly higher levels of protein, 35% less lipid and 266% higher levels of ash, than healthy lobsters. The protein profile had significantly lower ratios of phenylalanine, threonine, and proline, but significantly elevated ratios of arginine. The ratio of 20:5n – 3, was about half that of healthy lobster hepatopancreas. Hemolymph of affected lobsters contained about 40% less protein, about 35% higher levels of ash and significantly higher histidine ratios in its protein profile than corresponding ratios in healthy lobsters. Ratios of phenylalanine and threonine were slightly but significantly lower and ratios of 18:2n – 6 and 20:4n – 6 were significantly elevated than in healthy lobsters. The ulcerated exoskeleton of affected lobsters had significantly lower levels of total carotenoids and ash, and significantly higher moisture content and proportions of protein and lipid than the non-ulcerated parts, or the exoskeleton of healthy

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lobsters. The amino and fatty acid profiles of the exoskeleton of healthy lobsters and the non-ulcerated exoskeletal parts of affected lobsters were not significantly different from each other. However, the non-ulcerated exoskeleton of affected lobsters had slightly but significantly lower total carotenoid content than the exoskeleton of healthy lobsters. The ulcerated exoskeleton had higher ratios of  $20:4n - 6$  and  $22:6n - 3$ . There are differences in the biochemical profiles of tissues between healthy and shell-diseased lobsters. The higher accumulation of ash in the hepatopancreas and hemolymph of affected lobsters may indicate problems in the transport/deposition of minerals to the exoskeleton, or withdrawal of these nutrients from the shell. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** American lobster; *Homarus americanus*; Shell disease; Amino acid profile; Fatty acid profile

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## 1. Introduction

Lobstering is an important industry in several New England states of the USA and the Maritime Provinces of Canada. In the state of Maine, USA, about 20,000 metric tons of market-size American lobsters, *Homarus americanus* (Milne Edwards) were landed in 1999 (National Marine Fisheries Service), worth about US\$133 million. The season for lobster fishing is in the summer and fall and approximately 10% of the catch is soft-shelled or newly molted lobsters. These lobsters are temporarily held in tidal enclosures called “lobster pounds” for 3–6 months, during which time they harden their exoskeleton and gain weight. The crustaceans are then sold at a higher price from winter to spring, when the supply from the natural fishery is low.

Lobsters are often kept at very high densities in the pounds (up to 11 animals/m<sup>2</sup>) where the stress and potential deterioration of the water quality brought about by overcrowding or poor husbandry could contribute to disease. One of the more common afflictions in pounded lobsters is ‘shell disease’, a condition characterized by gross lesions, ulcers and erosion of the exoskeleton. The infection has been shown to be caused by bacterial invasion of a broken epicuticle (Malloy, 1978), but has also been associated with poor water quality (Wilk et al., 1996; Bullis, 1989), nutritional stress (Prince, 1997; Prince et al., 1995) and unfavorable environmental conditions (Martin and Hose, 1995). Lesions usually harbor naturally occurring, chitinoclastic bacteria such as *Vibrio*, *Pseudomonas*, and *Aeromonas* (Prince, 1997; Prince et al., 1993; Bayer et al., 1989). As severely affected animals are weak and unsightly, and therefore not suitable for the lucrative live market, they are processed for canned meat. The incidence of shell disease in the wild lobster catch of the eastern USA was estimated at < 2% (Wilk et al., 1996). This figure could increase from about 3–4% in well-managed lobster pounds to as high as a third of the pounded stock in some Nova Scotia farms (Prince, 1997), resulting in substantial economic loss.

A comparison of the common biochemical indices (proximate composition, and amino/fatty acid profiles) of the major lobster tissues such as muscle, hepatopancreas, hemolymph and exoskeleton in both healthy and affected animals, might give information on how shell disease develops and how it affects these tissues and overall lobster health. The information could be useful in developing therapy for the condition, in developing effective artificial diets for pounded lobsters, as formulated diets are usually

balanced to simulate the nutrient profiles of the target species (Deshimaru and Shigeno, 1972) or in developing therapeutic feed. Seafood, including lobster, is rich in the  $n - 3$  highly unsaturated fatty acids (HUFAs) such as 20:5 $n - 3$  (EPA) and 22:6 $n - 3$  (DHA), essential fatty acids in human nutrition associated with intelligence and decreased incidence of cardiovascular disease (Carroll and Woodward, 1989). Hence, investigating the fatty acid profiles of the edible tissues also provides information on the relative nutritional value of consuming healthy and shell-diseased lobsters.

## 2. Materials and methods

Locally pounded lobsters were brought to the lobster rearing facility at the University of Maine and were fasted for 3 days. All the lobsters chosen for study were in the intermolt stage. Staging was done according to the method of Aiken (1980). They were caught in Fishing District 34 of Nova Scotia, Canada and had been kept in a Maine lobster pound for 4 months on a diet of salted fish racks and herring. Four healthy lobsters that were free of any shell lesions, and possessing normal dark coloration and complete appendages, and four lobsters severely affected with shell disease, characterized by numerous lesions, were deliberately chosen for study. Lobster mean wet weight was  $478 \pm 20$  g for healthy animals, and  $490 \pm 42$  g for shell-diseased lobsters. Hemolymph, hepatopancreas, muscle and exoskeleton were sampled for biochemical analyses as explained below.

Before the lobsters were dissected, at least 10 ml of hemolymph sample from the 4 randomly chosen animals from each group were drawn from the hemocoel between the first abdominal segment and carapace by a sterile syringe. The lobsters were then euthanized by severing the ventral nerve at the level of the first abdominal segment with a pair of scissors and quickly making a dorsal longitudinal cut on the carapace all the way to the rostrum (to destroy the brain) and halfway across to expose the hepatopancreas. For computation of the hepatosomatic indices, 10 lobsters from each group were sampled. The hepatopancreas was completely removed and weighed. Hepatosomatic index (HSI %) was computed as

$$HSI \% = H/B \times 100,$$

Where  $H$  is the wet weight of the hepatopancreas (g) and  $B$  is the wet weight of the whole lobster (g).

For the muscle sample, a piece of the inner, unpigmented muscle was obtained from the first abdominal segment. The exoskeletal sample was obtained from either side of the carapace. For shell-diseased lobsters, the portion of the carapace with numerous lesions, as well as non-ulcerated parts were purposely sampled. The exoskeletal samples were thoroughly washed to remove all meat and membranes.

All tissues except hemolymph were washed with deionized water, lightly dried with paper towels, quickly frozen in tared vials immersed in a mixture of acetone and dry ice, freeze-dried at  $-50^{\circ}\text{C}$  for 50 h, and weighed (Mettler AE 240,  $\pm 0.01$  mg). The dry tissues were pulverized with a glass rod (In the case of the exoskeleton, tissues were

ground up with a mortar and pestle.) and stored at  $-20^{\circ}\text{C}$  in an atmosphere of nitrogen gas until further analyses.

### 2.1. Proximate analyses

Total protein was determined spectrophotometrically at 725 nm using a commercial microprotein determination kit (Sigma Diagnostics) based on the Lowry procedure. Soluble carbohydrate was determined using the phenol–sulfuric acid reaction (Robyt and White, 1990). This carbohydrate assay does not detect amino sugars such as chitin. Crude lipid was determined gravimetrically after extraction using the method of Bligh and Dyer (1959). Ash was determined using a muffle furnace at  $550^{\circ}\text{C}$ . For total carotenoid analysis, exoskeletal samples were first demineralized with 50% aqueous acetic acid at  $80^{\circ}\text{C}$  until the sample was white. After cooling, the dissolved pigments were repeatedly extracted with hexane until no color could be removed (Fox, 1973). The hexane was evaporated by nitrogen gas and the absorbance of the pigments at 475 nm was read in acetone using 1910 as the extinction coefficient (Guillou et al., 1995). Spectrophotometric analyses were all performed in duplicates.

### 2.2. Amino acid analyses

The tissue samples ( $2.0 \pm 0.5$  mg) were spiked with norleucine as an internal standard and hydrolyzed in vacuo with 4 N methanesulfonic acid for 22 h at  $110^{\circ}\text{C}$  (Simpson et al., 1976). Dabsyl amino acids were prepared from the hydrolysates (Stocchi et al., 1989) and assayed by HPLC (Hewlett Packard Series 1050 with automatic sampler) using a Supelcosil LC-DABS<sup>TM</sup> column (15 cm  $\times$  4.6 mm, 3  $\mu\text{m}$ ). A commercial amino acid mixture (Standard H, Pierce Chemical, Rockford, IL) and individual amino acids (Sigma, St. Louis, MO) were used to prepare the standard calibration mixtures. Each sample was analyzed in duplicate.

### 2.3. Fatty acid analysis

Preparation of fatty acid methyl esters (FAMES) from lipids was done as described previously (Floreto et al., 1996). Tricosanoic acid (23:0) was used as the internal standard. The FAMES were taken up in hexane and assayed by GC-FID (Hewlett Packard 5890A) using a capillary column (Omegawax<sup>TM</sup> 320; 30 m  $\times$  0.32 mm; film thickness, 0.25  $\mu\text{m}$ ). Operating parameters were: column temperature,  $210^{\circ}\text{C}$ ; FID temperature,  $250^{\circ}\text{C}$ ; and carrier gas He, 30 ml.min<sup>-1</sup>. The FAMES were identified by comparing relative retention times and equivalent chain lengths of the peaks with those of authentic standards (Sigma) and cod liver oil FAMES. Each sample was analyzed in duplicate.

### 2.4. Statistics

Date were analyzed by one-way ANOVA using the software package StatView SE + Graphics (Abacus Concepts, 1988), and reported as means of four replicates  $\pm$

standard error (SE) for all measured parameters. Probabilities of  $P < 0.05$  were considered significant.

### 3. Results

#### 3.1. Muscle

Muscle tissue from healthy lobsters contained significantly higher levels of carbohydrate than muscle tissue from affected lobsters (Table 1). Protein, lipid and ash levels were not significantly different between the two groups. The amino acid profiles of muscle tissues are shown in Table 2. Muscle from affected lobsters contained significantly lower ratios of arginine, threonine and serine. Arginine levels in affected lobsters were nearly half the level in muscle from healthy lobsters. On the other hand, muscle tissues of affected lobsters contained significantly higher ratios of phenylalanine and glycine. Glycine levels were 50% higher in muscle tissues of affected lobsters. Muscle tissues of healthy lobsters contained significantly higher ratios of total essential amino acids while muscle tissues of affected lobsters significantly contained higher ratios of total non-essential amino acids.

Table 1

A comparison of the moisture content and proximate composition (% of dry weight) of muscle, hepatopancreas and hemolymph of healthy and shell-diseased American lobsters. Mean  $\pm$  SE. Treatments with different letters are significantly different from each other ( $P < 0.05$ )

	Healthy	Shell-diseased
<i>Muscle</i>		
Moisture (%)	80.45 $\pm$ 1.43	79.08 $\pm$ 0.48
Proximate analysis (% of dry tissue)		
Crude protein	53.49 $\pm$ 3.47	51.30 $\pm$ 1.83
Carbohydrate	6.34 $\pm$ 0.37 <sup>a</sup>	4.27 $\pm$ 0.17 <sup>b</sup>
Crude lipid	6.06 $\pm$ 0.22	6.37 $\pm$ 0.45
Ash	34.11 $\pm$ 3.91	38.10 $\pm$ 1.71
<i>Hepatopancreas</i>		
Moisture (%)	78.48 $\pm$ 0.27 <sup>a</sup>	68.65 $\pm$ 1.94 <sup>b</sup>
Proximate analysis (% of dry tissue)		
Crude protein	32.06 $\pm$ 0.64 <sup>b</sup>	34.77 $\pm$ 0.93 <sup>a</sup>
Carbohydrate	16.78 $\pm$ 1.64	14.61 $\pm$ 1.91
Crude lipid	42.64 $\pm$ 2.45 <sup>a</sup>	27.96 $\pm$ 2.99 <sup>b</sup>
Ash	8.52 $\pm$ 2.37 <sup>b</sup>	22.67 $\pm$ 4.44 <sup>a</sup>
<i>Hemolymph</i>		
Moisture (%)	93.89 $\pm$ 0.67	92.34 $\pm$ 1.30
Proximate analysis (% of dry tissue)		
Crude Protein	37.56 $\pm$ 2.94 <sup>a</sup>	22.33 $\pm$ 2.29 <sup>b</sup>
Carbohydrate	1.79 $\pm$ 0.04	2.05 $\pm$ 0.15
Crude Lipid	6.90 $\pm$ 0.57	5.59 $\pm$ 1.11
Ash	53.75 $\pm$ 2.90 <sup>b</sup>	70.04 $\pm$ 3.44 <sup>a</sup>

Table 2

A comparison of the amino acid profiles (% of total) of muscle, hepatopancreas, hemolymph and exoskeleton of healthy and shell-diseased American lobsters. Mean  $\pm$  SE. Treatments with different letters are significantly different ( $P < 0.05$ )

	Muscle		Hepatopancreas		Hemolymph		Exoskeleton		
	Healthy	Shell-diseased	Healthy	Shell-diseased	Healthy	Shell-diseased	Healthy	Shell-diseased	
								Lesion-free parts	Ulcerated parts
<i>Essential</i>									
Arginine	7.45 ± 0.83 <sup>a</sup>	3.73 ± 0.51 <sup>b</sup>	5.77 ± 0.61 <sup>b</sup>	8.83 ± 0.65 <sup>a</sup>	7.83 ± 1.60	6.03 ± 0.19	4.96 ± 0.34 <sup>b</sup>	6.59 ± 0.90 <sup>b</sup>	20.31 ± 2.02 <sup>a</sup>
Histidine	4.53 ± 0.40	4.59 ± 0.39	5.00 ± 0.54	3.57 ± 0.90	14.63 ± 0.88 <sup>b</sup>	19.19 ± 0.54 <sup>a</sup>	7.21 ± 0.67 <sup>a</sup>	7.46 ± 1.18 <sup>a</sup>	3.66 ± 0.54 <sup>b</sup>
Isoleucine	3.12 ± 0.07	3.25 ± 0.22	3.07 ± 0.41	2.76 ± 0.29	4.48 ± 0.53	4.08 ± 0.60	3.82 ± 0.65	3.56 ± 0.59	2.98 ± 0.28
Leucine	9.06 ± 0.42	9.28 ± 0.38	8.05 ± 0.26	7.03 ± 0.38	8.64 ± 0.48	7.94 ± 0.14	5.40 ± 0.66	5.38 ± 0.66	6.60 ± 0.42
Lysine	5.99 ± 0.17	6.58 ± 0.23	5.01 ± 0.18	4.12 ± 0.45	4.33 ± 0.58	4.66 ± 0.16	2.63 ± 0.32	2.80 ± 0.44	3.46 ± 0.40
Methionine	3.78 ± 0.09	3.47 ± 0.21	3.03 ± 0.21	2.71 ± 0.16	2.69 ± 0.19	3.05 ± 0.25	3.73 ± 2.70	4.43 ± 3.28	2.17 ± 0.36
Cystine	0.50 ± 0.04	0.41 ± 0.03	2.51 ± 0.13	2.60 ± 0.46	0.54 ± 0.10	0.57 ± 0.08	1.12 ± 0.19 <sup>a</sup>	1.27 ± 0.21 <sup>a</sup>	0.56 ± 0.08 <sup>b</sup>
Phenylalanine	4.16 ± 0.17 <sup>b</sup>	4.85 ± 0.12 <sup>a</sup>	5.30 ± 0.25 <sup>a</sup>	4.23 ± 0.17 <sup>b</sup>	8.15 ± 0.03 <sup>a</sup>	7.68 ± 0.03 <sup>b</sup>	6.18 ± 0.49	5.91 ± 0.77	4.46 ± 0.24
Tyrosine	2.37 ± 0.04	2.66 ± 0.21	3.29 ± 0.30	2.56 ± 0.32	3.87 ± 0.40	3.53 ± 0.19	5.57 ± 0.28 <sup>a</sup>	5.22 ± 0.38 <sup>a</sup>	2.05 ± 0.21 <sup>b</sup>
Threonine	5.37 ± 0.09 <sup>a</sup>	4.87 ± 0.05 <sup>b</sup>	6.99 ± 0.12 <sup>a</sup>	6.08 ± 0.33 <sup>b</sup>	7.42 ± 0.24 <sup>a</sup>	6.49 ± 0.20 <sup>b</sup>	5.05 ± 0.39	5.02 ± 0.61	5.93 ± 0.32
Tryptophan	0.31 ± 0.07	0.33 ± 0.02	0.53 ± 0.10	0.43 ± 0.04	0.62 ± 0.09	0.55 ± 0.06	0.60 ± 0.12 <sup>a</sup>	0.73 ± 0.11 <sup>a</sup>	0.33 ± 0.03 <sup>b</sup>
Valine	3.43 ± 0.16	3.38 ± 0.11	3.77 ± 0.38	3.47 ± 0.40	5.04 ± 0.27	4.86 ± 0.23	6.75 ± 0.26 <sup>a</sup>	6.65 ± 0.46 <sup>a</sup>	3.98 ± 0.18 <sup>b</sup>
Total	50.07 ± 0.23 <sup>a</sup>	47.40 ± 0.75 <sup>b</sup>	52.32 ± 1.67	48.38 ± 2.22	68.26 ± 0.54	68.62 ± 0.38	52.04 ± 1.91	51.06 ± 1.75	56.49 ± 3.02
<i>Non-essential</i>									
Aspartic acid	4.22 ± 0.13	4.15 ± 0.14	5.39 ± 0.75	6.13 ± 0.51	4.21 ± 0.09 <sup>b</sup>	4.98 ± 0.19 <sup>a</sup>	11.74 ± 2.33 <sup>a</sup>	10.24 ± 2.09 <sup>a</sup>	4.21 ± 0.60 <sup>b</sup>
Glutamic acid	18.30 ± 0.11	17.50 ± 0.39	13.88 ± 2.28	15.79 ± 1.89	10.26 ± 0.43	10.12 ± 0.42	10.14 ± 1.26	10.16 ± 0.75	13.60 ± 1.69
Serine	5.68 ± 0.31 <sup>a</sup>	4.94 ± 0.06 <sup>b</sup>	5.64 ± 0.34	5.16 ± 0.26	3.55 ± 0.24	4.24 ± 0.25	5.22 ± 0.56	5.26 ± 0.31	5.36 ± 0.22
Glycine	10.89 ± 0.66 <sup>b</sup>	15.56 ± 0.36 <sup>a</sup>	10.02 ± 1.18 <sup>b</sup>	14.18 ± 0.51 <sup>a</sup>	4.41 ± 0.21	4.41 ± 0.04	8.09 ± 0.44	7.98 ± 0.67	8.10 ± 0.80
Alanine	5.06 ± 0.15	5.02 ± 0.18	4.97 ± 0.14	4.93 ± 0.26	2.77 ± 0.07	2.64 ± 0.10	3.72 ± 0.28	3.61 ± 0.45	4.39 ± 0.16
Proline	5.80 ± 0.45	5.28 ± 0.39	7.78 ± 0.73 <sup>a</sup>	5.56 ± 0.40 <sup>b</sup>	6.56 ± 0.56	4.99 ± 0.45	11.59 ± 1.47	11.66 ± 1.52	7.87 ± 0.42
Total	49.93 ± 0.23 <sup>b</sup>	52.60 ± 0.75 <sup>a</sup>	47.68 ± 1.67	51.62 ± 2.22	31.75 ± 0.54	31.38 ± 0.38	47.96 ± 1.91	48.94 ± 1.75	43.51 ± 3.02

The fatty acid composition of the muscle tissue is shown in Table 3. Total fatty acids (mg/g dry weight), as well as total  $n - 3$  fatty acids content (mg/g dry weight), did not significantly vary between healthy and affected lobsters. However, as a ratio of total fatty acids, muscle tissue from affected lobsters contained significantly lower proportions of 16:1 $n - 7$  and total monounsaturates, but significantly higher proportions of 18:2 $n - 6$ , 18:3 $n - 3$ , 20:4 $n - 6$ , 22:6 $n - 3$ , total polyunsaturated fatty acids (PUFA), total  $n - 3$  and total  $n - 6$  PUFAs.

### 3.2. Hepatopancreas

Hepatosomatic indices were significantly lower in affected ( $3.29 \pm 0.18$ ), than healthy lobsters ( $4.43 \pm 0.16$ ). Hepatopancreas of affected lobsters contained significantly lower moisture content than those of healthy lobsters (Table 1). Protein and ash contents of the hepatopancreas were significantly higher in affected than healthy lobsters. Levels of ash in the hepatopancreas of affected lobsters were nearly three times higher than levels in the hepatopancreas of healthy lobsters. Carbohydrate content did not significantly differ between the two sets of lobsters. However, affected lobster hepatopancreas significantly contained about 35% less lipid than those of unaffected lobsters.

The protein of the hepatopancreas of affected lobsters had significantly higher proportions of arginine and glycine, and significantly lower proportions of phenylalanine, threonine and proline (Table 2). The total essential amino acids content did not significantly differ between the two groups of lobsters.

Hepatopancreas of the healthy group contained nearly twice the amount of total fatty acids as the hepatopancreas of affected lobsters (Table 3). Proportions of several fatty acids were significantly lower in affected lobsters: 15:0, 16:0, 17:0, total saturates, 16:1 $n - 7$ , 17:1, 18:1 $n - 7$ , 18:4 $n - 3$ , 20:4 $n - 3$ , 20:5 $n - 3$  and total  $n - 3$  PUFAs. The fatty acids that were found in significantly higher proportions in affected lobsters were 20:1 $n - 9$ , 22:1 $n - 9$ , total monounsaturates, and 18:2 $n - 6$ . On a dry weight basis, hepatopancreas of healthy lobsters contained nearly three times more  $n - 3$  PUFAs than the hepatopancreas of affected lobsters.

### 3.3. Hemolymph

Protein content of the hemolymph of affected lobsters was about 40% lower than that of healthy lobsters (Table 1). Ash levels in the hemolymph of affected lobsters were about 30% higher than levels in the hemolymph of healthy lobsters. However, carbohydrate and lipid levels were not significantly different between the two groups of lobsters. The protein of the hemolymph of affected lobsters contained significantly lower ratios of phenylalanine and threonine, but significantly higher ratios of histidine and aspartic acid (Table 2). Histidine ratios were about 30% higher in the hemolymph of affected lobsters.

Total fatty acid content (dry weight basis) of the hemolymph of affected lobsters was less than half the total fatty acid content of hemolymph of healthy lobsters (Table 3). Total  $n - 3$  PUFA content of hemolymph of affected lobsters, on a dry weight basis, was only about 40% that of hemolymph of healthy lobsters. In the hemolymph of

Table 3

A comparison of the fatty acid profiles (% of total fatty acids) of muscle, hepatopancreas, hemolymph and exoskeleton of healthy and shell-diseased American lobsters. Mean  $\pm$  SE. Treatments with different letters are significantly different ( $P < 0.05$ ). Lowly occurring fatty acids ( $< 0.5\%$  of total) are not included for clarity

	Muscle		Hepatopancreas		Hemolymph		Exoskeleton		
	Healthy	Shell-diseased	Healthy	Shell-diseased	Healthy	Shell-diseased	Healthy	Shell-diseased	
								Lesion-free parts	Ulcerated parts
$\Sigma$ Fatty acids (mg/g dry weight)	22.33 $\pm$ 1.36	19.47 $\pm$ 0.88	398.20 $\pm$ 60.08 <sup>a</sup>	209.41 $\pm$ 27.63 <sup>b</sup>	5.86 $\pm$ 0.29 <sup>a</sup>	2.45 $\pm$ 0.39 <sup>b</sup>	0.70 $\pm$ 0.03 <sup>b</sup>	0.63 $\pm$ 0.10 <sup>b</sup>	1.58 $\pm$ 0.21 <sup>a</sup>
<i>Saturates</i>									
14:0	1.00 $\pm$ 0.10	0.75 $\pm$ 0.08	2.21 $\pm$ 0.24	2.06 $\pm$ 0.25	1.18 $\pm$ 0.11	1.19 $\pm$ 0.29	1.20 $\pm$ 0.15	1.45 $\pm$ 0.30	0.88 $\pm$ 0.11
15:0	0.85 $\pm$ 0.06	0.90 $\pm$ 0.10	0.73 $\pm$ 0.08 <sup>a</sup>	0.41 $\pm$ 0.06 <sup>b</sup>	0.61 $\pm$ 0.09	0.43 $\pm$ 0.08	0.69 $\pm$ 0.06	0.71 $\pm$ 0.05	0.64 $\pm$ 0.05
16:0	14.43 $\pm$ 0.83	14.05 $\pm$ 0.63	11.15 $\pm$ 0.44 <sup>a</sup>	8.86 $\pm$ 0.20 <sup>b</sup>	11.64 $\pm$ 0.54	10.43 $\pm$ 0.72	13.12 $\pm$ 0.42	14.24 $\pm$ 0.26	12.32 $\pm$ 0.92
17:0	0.78 $\pm$ 0.07	0.68 $\pm$ 0.07	0.60 $\pm$ 0.07 <sup>a</sup>	0.31 $\pm$ 0.06 <sup>b</sup>	0.57 $\pm$ 0.04	0.39 $\pm$ 0.09	0.71 $\pm$ 0.13	0.73 $\pm$ 0.12	0.53 $\pm$ 0.08
18:0	4.03 $\pm$ 0.32	3.58 $\pm$ 0.24	2.67 $\pm$ 0.33	2.06 $\pm$ 0.22	4.10 $\pm$ 0.32	4.03 $\pm$ 0.24	3.91 $\pm$ 0.24	4.53 $\pm$ 0.18	3.94 $\pm$ 0.38
22:0	–	–	–	–	–	–	2.55 $\pm$ 0.91	2.82 $\pm$ 0.97	0.79 $\pm$ 0.13
Total	21.85 $\pm$ 0.65	20.67 $\pm$ 0.64	18.31 $\pm$ 0.95 <sup>a</sup>	14.52 $\pm$ 0.53 <sup>b</sup>	19.70 $\pm$ 0.27 <sup>a</sup>	17.01 $\pm$ 1.15 <sup>b</sup>	22.35 $\pm$ 0.67 <sup>a</sup>	23.55 $\pm$ 0.81 <sup>a</sup>	19.45 $\pm$ 0.62 <sup>b</sup>
<i>Monounsaturates</i>									
16:1 <i>n</i> – 7	5.89 $\pm$ 0.20 <sup>a</sup>	5.10 $\pm$ 0.18 <sup>b</sup>	5.55 $\pm$ 0.28 <sup>a</sup>	4.26 $\pm$ 0.34 <sup>b</sup>	4.59 $\pm$ 0.43	3.52 $\pm$ 0.41	4.39 $\pm$ 0.09 <sup>b</sup>	4.54 $\pm$ 0.10 <sup>b</sup>	6.89 $\pm$ 0.80 <sup>a</sup>
16:1 <i>n</i> – 5	0.29 $\pm$ 0.07	0.48 $\pm$ 0.10	0.55 $\pm$ 0.17	0.26 $\pm$ 0.13	0.45 $\pm$ 0.08	0.19 $\pm$ 0.04	–	–	–
17:1	1.04 $\pm$ 0.03	0.97 $\pm$ 0.16	0.75 $\pm$ 0.05 <sup>a</sup>	0.50 $\pm$ 0.07 <sup>b</sup>	1.17 $\pm$ 0.18	1.90 $\pm$ 0.34	1.17 $\pm$ 0.08 <sup>a</sup>	1.21 $\pm$ 0.06 <sup>a</sup>	0.83 $\pm$ 0.11 <sup>b</sup>
18:1 <i>n</i> – 9	11.12 $\pm$ 0.09	11.73 $\pm$ 0.29	11.16 $\pm$ 0.42	11.71 $\pm$ 0.13	12.17 $\pm$ 0.42	11.78 $\pm$ 0.59	14.57 $\pm$ 0.72 <sup>a</sup>	15.28 $\pm$ 0.81 <sup>a</sup>	12.25 $\pm$ 0.64 <sup>b</sup>
18:1 <i>n</i> – 7	6.84 $\pm$ 0.40	5.90 $\pm$ 0.23	7.48 $\pm$ 0.65 <sup>a</sup>	5.39 $\pm$ 0.12 <sup>b</sup>	6.70 $\pm$ 0.49	4.97 $\pm$ 0.52	6.71 $\pm$ 0.42 <sup>b</sup>	6.92 $\pm$ 0.36 <sup>b</sup>	8.56 $\pm$ 0.41 <sup>a</sup>



19:1	–	–	–	–	0.73 ± 0.08 <sup>a</sup>	0 <sup>b</sup>	0.59 ± 0.21	0.59 ± 0.21	0.57 ± 0.17
20:1 <i>n</i> – 11	2.53 ± 0.98	3.32 ± 0.33	6.73 ± 2.43	2.67 ± –	6.66 ± 0.99	8.84 ± 0.76	5.39 ± 1.18	5.64 ± 1.35	6.35 ± 0.52
20:1 <i>n</i> – 9	1.42 ± 0.44	0.44 ± 0.07	3.49 ± 0.61 <sup>b</sup>	16.50 ± 1.53 <sup>a</sup>	1.27 ± 0.08 <sup>a</sup>	0.62 ± 0.06 <sup>b</sup>	1.52 ± 0.12 <sup>a</sup>	1.65 ± 0.13 <sup>a</sup>	0.74 ± 0.03 <sup>b</sup>
22:1 <i>n</i> – 9	–	–	3.29 ± 0.83 <sup>b</sup>	12.92 ± 1.26 <sup>a</sup>	0.83 ± 0.28	2.02 ± 0.50	–	–	–
Total	30.36 ± 0.26 <sup>a</sup>	28.53 ± 0.45 <sup>b</sup>	44.93 ± 3.16 <sup>b</sup>	56.62 ± 2.95 <sup>a</sup>	35.10 ± 0.74	33.86 ± 2.51	34.89 ± 1.08	36.32 ± 1.05	36.89 ± 1.13
<i>Polyunsaturates</i>									
16:4 <i>n</i> – 3	–	–	–	–	1.20 ± 0.24	1.96 ± 0.63	–	–	–
18:2 <i>n</i> – 6	0.79 ± 0.04 <sup>b</sup>	1.48 ± 0.17 <sup>a</sup>	0.75 ± 0.07 <sup>b</sup>	1.80 ± 0.34 <sup>a</sup>	0.96 ± 0.06 <sup>b</sup>	2.08 ± 0.50 <sup>a</sup>	1.40 ± 0.58	1.55 ± 0.67	1.46 ± 0.13
18:3 <i>n</i> – 3	0.28 ± 0.04 <sup>b</sup>	0.51 ± 0.04 <sup>a</sup>	0.32 ± 0.06	0.37 ± 0.05	0.32 ± 0.05	0.44 ± –	0.59 ± –	0.61 ± –	0.38 ± 0.01
18:4 <i>n</i> – 3	0.40 ± 0.06	0.25 ± 0.04	0.57 ± 0.09 <sup>a</sup>	0.15 ± 0.02 <sup>b</sup>	0.42 ± 0.20	0.56 ± –	–	–	–
20:4 <i>n</i> – 6	2.62 ± 0.05 <sup>b</sup>	3.82 ± 0.19 <sup>a</sup>	1.80 ± 0.15	1.87 ± 0.31	3.77 ± 0.10 <sup>b</sup>	5.23 ± 0.61 <sup>a</sup>	2.99 ± 0.06 <sup>b</sup>	3.02 ± 0.24 <sup>b</sup>	5.09 ± 0.62 <sup>a</sup>
20:4 <i>n</i> – 3	–	–	0.44 ± 0.02 <sup>a</sup>	0.32 ± 0.02 <sup>b</sup>	–	–	5.81 ± 1.13 <sup>a</sup>	6.06 ± 1.25 <sup>a</sup>	0 <sup>b</sup>
20:5 <i>n</i> – 3	25.80 ± 1.15	25.97 ± 0.33	13.30 ± 0.94 <sup>a</sup>	6.73 ± 0.79 <sup>b</sup>	19.25 ± 0.51	18.41 ± 0.67	18.53 ± 0.55	16.40 ± 0.68	19.03 ± 0.95
22:5 <i>n</i> – 3	–	–	1.21 ± 0.11	1.09 ± 0.03	1.79 ± 0.19	1.96 ± 0.38	2.32 ± 1.23	2.49 ± 1.39	0.88 ± –
22:6 <i>n</i> – 3	12.84 ± 0.56 <sup>b</sup>	14.47 ± 0.35 <sup>a</sup>	8.73 ± 0.64	7.64 ± 0.67	11.50 ± 0.53	14.28 ± 1.74	9.10 ± 0.19 <sup>b</sup>	8.00 ± 0.44 <sup>b</sup>	11.14 ± 0.60 <sup>a</sup>
Total	44.61 ± 0.42 <sup>b</sup>	48.36 ± 0.56 <sup>a</sup>	29.37 ± 1.74	23.19 ± 2.37	40.49 ± 0.91	42.52 ± 4.11	39.96 ± 1.38	37.25 ± 1.24	40.76 ± 1.32
Σ <i>n</i> – 3	40.28 ± 0.62 <sup>b</sup>	42.28 ± 0.60 <sup>a</sup>	24.66 ± 1.41 <sup>a</sup>	17.60 ± 1.89 <sup>b</sup>	34.68 ± 1.01	36.95 ± 3.14	35.17 ± 1.23	32.29 ± 1.15	32.76 ± 1.46
Σ <i>n</i> – 6	3.92 ± 0.24 <sup>b</sup>	5.55 ± 0.17 <sup>a</sup>	4.06 ± 0.27	4.26 ± 0.48	5.07 ± 0.11 <sup>b</sup>	7.69 ± 0.23 <sup>a</sup>	4.17 ± 0.50 <sup>b</sup>	4.32 ± 0.63 <sup>b</sup>	6.65 ± 0.73 <sup>a</sup>
Σ <i>n</i> – 3 (mg/g dry weight)	8.98 ± 0.49	8.23 ± 0.38	96.29 ± 10.60 <sup>a</sup>	35.33 ± 2.03 <sup>b</sup>	2.04 ± 0.15 <sup>a</sup>	0.83 ± 0.22 <sup>b</sup>	0.28 ± 0.04 <sup>b</sup>	0.20 ± 0.03 <sup>b</sup>	0.53 ± 0.06 <sup>a</sup>

affected lobsters, proportions of total saturates and 20:1  $n - 9$  were significantly lower; the monounsaturate, 19:1, was not detected; and the proportions of 18:2  $n - 6$ , 20:4  $n - 6$  and total  $n - 6$  PUFAs were significantly higher. Proportions of total monounsaturates and total PUFA did not significantly vary between the two groups of lobsters.

### 3.4. Exoskeleton

Moisture, protein and lipid contents (% of dry tissue) were significantly elevated in the ulcerated parts of the exoskeleton of affected lobsters than in the lesion-free parts of affected lobsters or the exoskeleton of healthy lobsters (Table 4). However, carbohydrate levels did not significantly differ between the two groups of lobsters. Ash content was slightly, but significantly lower in the ulcerated exoskeleton of affected lobsters. Proximate composition of the exoskeleton of healthy lobsters and the lesion-free portions of the exoskeleton of affected lobsters did not significantly differ from each other. As expected, total carotenoid content of the ulcerated parts of shell-disease affected lobsters was significantly lowest, followed by the lesion-free parts of the exoskeleton of affected lobsters and the exoskeleton of healthy lobster.

The ulcerated parts of the exoskeleton of affected lobsters had significantly lower levels of histidine, cystine, tyrosine, tryptophan, valine and aspartic acid; and significantly higher levels of arginine. Arginine ratios in the ulcerated parts were more than three times higher than the ratios in the lesion-free parts of the exoskeleton of affected lobsters and the exoskeleton of healthy lobsters. Levels of aspartic acid in the exoskeleton of affected lobsters were about 60% less than the levels in healthy lobster exoskeleton. The amino acid profiles of the lesion-free parts of the exoskeleton of affected lobsters and the exoskeleton of healthy lobsters did not significantly differ from each other.

The ulcerated parts of the exoskeleton of affected lobsters contained significantly higher total fatty acids and total  $n - 3$  PUFAs (both on dry weight basis); and significantly higher proportions of 16:1  $n - 7$ , 18:1  $n - 7$ , 20:4  $n - 6$  and 22:6  $n - 3$  (Table 3). The proportions of total saturates, 17:1, 18:1  $n - 9$ , and 20:1  $n - 9$  were significantly lower in the ulcerated parts. The fatty acid profiles of the lesion-free parts

Table 4

A comparison of the proximate composition and carotenoid content of the exoskeleton of healthy and shell-diseased American lobsters. Mean  $\pm$  SE. Treatments with different letters are significantly different from each other ( $P < 0.05$ )

	Healthy	Shell-diseased	
		Lesion-free parts	Ulcerated parts
Moisture % (% of dry weight)	52.92 $\pm$ 3.65 <sup>b</sup>	55.53 $\pm$ 0.85 <sup>b</sup>	67.00 $\pm$ 1.61 <sup>a</sup>
Crude protein	3.53 $\pm$ 0.37 <sup>b</sup>	3.70 $\pm$ 0.20 <sup>b</sup>	4.53 $\pm$ 0.13 <sup>a</sup>
Carbohydrate	1.42 $\pm$ 0.31	1.84 $\pm$ 0.10	1.88 $\pm$ 0.32
Crude lipid	0.92 $\pm$ 0.06 <sup>b</sup>	1.01 $\pm$ 0.03 <sup>b</sup>	3.46 $\pm$ 0.52 <sup>a</sup>
Ash	94.13 $\pm$ 0.01 <sup>a</sup>	93.45 $\pm$ 0.30 <sup>a</sup>	90.14 $\pm$ 0.91 <sup>b</sup>
Total carotenoid (mg/kg dry weight)	36.09 $\pm$ 1.77 <sup>a</sup>	27.33 $\pm$ 0.57 <sup>b</sup>	19.84 $\pm$ 2.87 <sup>c</sup>

of the exoskeleton of affected lobsters and the exoskeleton of healthy lobsters did not significantly differ from each other. The PUFA 20:4 $n$  – 3, which was found in the healthy and lesion-free exoskeletons, was not detected in the ulcerated exoskeleton.

Significant changes in the proportions of certain amino acids were common in several tissues: arginine, for muscle, hepatopancreas and exoskeleton; phenylalanine and threonine for muscle, hepatopancreas and hemolymph; histidine for hemolymph and exoskeleton; aspartic acid for hemolymph and exoskeleton; and glycine for muscle and hepatopancreas. A significant increase in the relative proportions of  $n$  – 6 PUFAs were noted in several tissues of affected lobsters: 18:2 $n$  – 6 for muscle, hepatopancreas and hemolymph; and 20:4 $n$  – 6 for muscle, hemolymph and exoskeleton. Significant changes in the HUFAs of lobster tissues were limited to specific tissues: the hepatopancreas for 20:5 $n$  – 3; and muscle and exoskeleton for 22:6 $n$  – 3.

#### 4. Discussion

Results showed significant differences in the biochemical profiles of the various tissues between healthy and shell-disease affected lobsters. However, the study does not ascertain if these differences were a direct result of shell disease, a consequence of physiological changes in the organs indirectly brought about by the disease or contributing factors to the disease.

The elevated levels of ash in the hepatopancreas and hemolymph of affected lobsters, and the lower protein content of the hemolymph implied impaired transport/deposition of minerals from the hepatopancreas and hemolymph to the poorly mineralized lesions of the exoskeleton; or early withdrawal of these nutrients from the shell. As the exoskeleton of affected lobsters are poorly mineralized and contain lower levels of calcium and phosphorus (Prince, 1997; Bayer et al., 1989), increased proportions of moisture, protein and lipid in the affected lesions were expected.

The lower content of protein in the hemolymph of affected lobsters (dry weight basis), coupled with the lack of significant differences in their moisture content, indicate that lower amounts of protein are found in the intact hemolymph of affected lobsters. Prince (1997) noted lower levels of serum protein and lower numbers of circulating hemocytes in the hemolymph of affected lobsters and postulated that blood cells are destroyed through phagocytosis and lysis to ward off the infection. The lower protein content of the hemolymph of affected lobsters further implies lower levels of hemocyanin, coagulogen, enzymes, hormones, transport proteins, free amino acids, etc. and therefore, a lower ability to fight off infection, to repair the damaged exoskeleton, and to transport nutrients and wastes.

The significantly lower moisture and lipid contents of the hepatopancreas of affected lobsters contributed to their lower hepato-somatic indices (HSI). A low index is indicative of poor health, reduced feed intake and possibly a loss of lipid-storing capacity. The possibility of loss of some lipid-storing capacity is supported by the observation that lobsters affected with shell disease continue to feed and that the lobsters consumed lipid-containing fish frames while in the pound. The low HSI was inconsistent with this feeding behavior. The organ is a known reserve for nutrients (Factor, 1995;

Torreblanca et al., 1993), which are utilized during starvation (Leung et al., 1990), or when the animal fails to properly feed (e.g., during disease conditions). In a study of American lobster larvae, loss of the lipid-storing capacity of the R-cells of the hepatopancreas was induced by starvation and the larvae eventually died even if feeding was successfully resumed (Anger et al., 1985). Direct or indirect consequences of shell disease may have caused similar damage to the hepatopancreas of affected lobsters. The significantly lower levels (dry weight basis) of lipid (nearly half of that in healthy lobsters) and  $n - 3$  HUFAs (a third of those in healthy lobsters) in the hepatopancreas of affected lobsters are too large to be attributed to reduced feed intake and does not coincide with visual observations of shell diseased lobsters feeding upon fish and fish frames. Also, if reduction in the hepatosomatic index were due to reduced feed intake, then there would have been a corresponding reduction in the protein content of the hepatopancreas. This was not observed. In fact, crude protein values of the hepatopancreas of shell-diseased lobsters were slightly but significantly higher, an observation that will be inconsistent with reduced feed intake.

The HUFAs,  $20:5n - 3$  and  $22:6n - 3$ , are dietary essentials for marine crustaceans (D'Abramo, 1997) and are usually incorporated into the phospholipids that make up the cellular membranes. Their lower content (dry weight basis) in the hepatopancreas (the main digestive organ) of affected lobsters indicates nutritional deficiency in these fatty acids possibly brought about by a loss of the lipid-storing capacity of the hepatopancreas and not by dietary unavailability.

Crustaceans severely affected by shell disease usually fail to molt a new, clean exoskeleton, since the old exoskeleton does not completely separate from the underlying tissue. Molt-death syndrome in juvenile American lobsters, a condition characterized by incomplete molting and death, had been linked to dietary deficiencies in phospholipid (D'Abramo et al., 1981), cholesterol and amino acid balance (Kean et al., 1985). Phospholipids, which are rich in the  $n - 3$  PUFAs, play important roles in nutrient transport and are themselves structural components of cellular membranes. However, this study cannot ascertain whether the lower lipid content and lower total  $n - 3$  PUFA content of the hepatopancreas of affected lobsters are underlying causes or merely complications from the disease. The significantly lower amounts of fatty acids in the hemolymph of affected lobsters but the lack of any significant differences in the amount of total hemolymph lipid between the two groups of lobsters imply that the content of non-fatty acid lipids (e.g. sterols) in the hemolymph is not affected by shell disease.

The significantly lower level of total carotenoids in the exoskeleton of affected lobsters, compared to that of healthy lobsters, was expected. The lesions do not allow for normal pigmentation and mineralization to occur, and are aggravated by a possibly hampered transport capability of the hemolymph. The significantly lower levels of total carotenoids in the exoskeleton of shell disease affected lobsters (in both lesion-free and ulcerated parts) also explains the slightly lighter color when these lobsters are cooked, and contributes to the lack of visual appeal in comparison to the deeper, red-orange coloration of cooked healthy lobsters.

Severely affected parts of the exoskeleton of affected lobsters contained significantly lower ratios of the amino acids, tyrosine and tryptophan. Tyrosine is involved in melanization and tryptophan is involved in crustacyanin–astaxanthin binding and the

binding of vitamin A in proteins (Zagalsky et al., 1991). The significantly lower levels of arginine in the muscle of affected lobsters imply catabolism of this amino acid. Arginine is highly important as the main reserve of ATP in crustaceans and is conserved even on starvation (Hird, 1986). However, lobsters affected with shell disease do not necessarily cease to feed and the significantly higher levels of arginine found in the hepatopancreas of affected lobsters may indicate reduced transport of this dietary essential amino acid to the muscles. Due to the lack of knowledge of the roles of the other amino acids in crustacean metabolism, it is difficult to speculate on the possible reasons why the proportions of some of the amino acids were highly elevated or highly reduced in the protein of the various tissues of affected lobsters. Noteworthy in affected lobsters are the elevated levels of arginine, and the lowered levels of histidine and aspartic acid in the ulcerated exoskeleton; the elevated levels of histidine in the hemolymph; the elevated levels of glycine in the muscle; the consistent reduction in the levels of phenylalanine and threonine in the hepatopancreas and hemolymph (Table 2). Aside from being building blocks of protein, amino acids are fuel sources and precursors for other types of biological molecules (Goldberg, 1991). Aspartic acid is part of the urea cycle and contributes to the formation of purines and pyrimidines. Histidine is the precursor of histamine, which is involved in tissue trauma and antigen-antibody allergic reactions. In vertebrates, glycine is a major component of collagen. Phenylalanine can convert to tyrosine, which participates in melanization.

Pounded lobsters affected with shell disease exhibit lower weight gain (Prince, 1997). Though reduced feed intake or starvation has not been established as the cause, the high density conditions of the pound may create hierarchical social interactions that inhibit affected lobsters from properly feeding, further aggravating their diseased condition. The eroded exoskeleton of affected lobsters also provides less protection and the breaks in the cuticle may provide the pathway for the release of attractant amino acids that promote their cannibalism by healthy lobsters. Glycine, highly elevated in the muscle and hepatopancreas of affected lobsters, is an effective attractant for the American lobster (Carter and Steele, 1982) and has been found to be one of two major amino acids (the other is taurine) involved in olfactory sensing in the aesthetascs of spiny lobsters (Trapido-Rosenthal et al., 1990).

The protein content of the carapace samples (<4% of dry tissue) reported in this study is considerably lower than the levels reported by other authors on other crustacean species (22% in the shrimp *Penaeus monodon*, Sarac et al., 1994; 22–48% in shrimp waste, Gagne and Simpson, 1993). Sarac et al. (1994) acknowledged the possibility of overestimating protein, as the Kjeldahl total nitrogen method used for analysis would also measure non-protein nitrogen such as chitin/chitosan. These amino-carbohydrates make up about 20% of crustacean exuvia (Welinder, 1997). Gagne and Simpson (1993), on the other hand, studied demineralized shrimp waste, which may have contained other tissues like meat and hepatopancreas, thus elevating protein levels. The protein content in this study was estimated using a modified Lowry procedure, which relies on the content of the aromatic amino acids in the sample.

As far as the biomedically important essential fatty acids are concerned (e.g., 20:5 $n$  – 3, 22:6 $n$  – 3), there is hardly any difference in the nutritional value of the meat, the most edible part, between affected and healthy lobsters. Though the proportions of

total  $n - 3$  PUFAs in the meat of affected lobsters are slightly but significantly higher, total  $n - 3$  content (dry weight basis) was not significantly different between the two sets of lobsters. However, consumption of hepatopancreas or 'tamale' of affected lobsters will have less benefit in terms of  $n - 3$  HUFA nutrition as their hepatopancreas contains approximately only  $1/3$  the  $n - 3$  HUFA content (dry weight basis) of healthy lobster hepatopancreas. The same can be said of the hemolymph but overall human nutrition derived from it is insignificant. In humans, these essential fatty acids have been associated with intelligence, decreased incidence of cardiovascular disease (Carroll and Woodward, 1989), improved brain function and a general sense of well-being (Robinson, 1998), and have also been suggested as a therapy for arthritis, autoimmune diseases such as lupus, and multiple sclerosis (Challem, 1998). The proportions of total  $n - 3$  and total  $n - 6$  PUFAs in the meat of affected lobsters was significantly elevated partly at the expense of monounsaturated fatty acids, which may have been catabolized for energy.

Various therapeutic regimes (mostly chemical) have been reported for the treatment of shell-disease, but none has been consistently effective (Prince, 1997). Slightly affected animals could probably molt into a new lesion-free exoskeleton and be free of the affliction, but severely affected ones will most likely not be able to molt nor survive the stress of molting. Induction of molting, frequently through water exchange, is a common strategy in the treatment of shell disease in tropical shrimp farming. The improved water quality thereby allows or induces the shrimps to molt and cast off their diseased exoskeleton. However, this strategy may have little significance in lobster pounds where the animals molt infrequently because of their relatively advanced age and large size. Furthermore, they are stocked in the pounds mostly as newly molted (soft-shell) lobsters and are also held for only short periods (3–6 months) when molting may not coincide with their residency in the pound. The cold water temperatures in the winter, when pounding is prevalent, are also not conducive for molting, which largely occurs in the summer and fall when water temperatures are high. However, if an effective technique that artificially induces molting en masse (by manipulating holding temperature, salinity, water quality or nutrition) of isolated, affected lobsters could be developed, the economic loss (up to a third of stock in some Nova Scotian farms) due to shell disease could be seriously addressed.

The exact etiology of lobster shell disease remains unknown. It has been theorized to be due to a 'metabolic disturbance' that prevents normal chitin deposition from keeping up with the chitinoclastic activity of microorganisms (Sindermann, 1991). Challenging healthy lobsters in the laboratory with chitinoclastic bacteria associated with the lesions caused the disease only when the epicuticle was damaged (Malloy, 1978). All the lobsters in this study were free of shell disease at the time of pounding and how and why the affected lobsters developed the disease in a short span of time are not known. Rough handling during fishing or their prior biochemical composition or nutritional history in the wild may have predisposed them to the disease. The appearance of the lesions of shell disease in pounded lobsters is also different from that observed in the wild. Lesions in wild-caught lobsters are darker (M. Loughlin, personal communication). In the light of the results of this study on the hemolymph, hepatopancreas and exoskeleton of affected lobsters, novel directions for therapy against shell-disease could

be geared towards improving the hemolymph's capacity to mount an immuno-defense and effectively restore nutrient transport capabilities by encouraging hematopoiesis, and increasing the lipid and overall PUFA and  $n-3$  content of the hepatopancreas. Approaches to accomplishing this (therapeutic nutrition, drugs, immunostimulants), as well as more investigation into the etiology of shell disease and mineral transport/deposition in crustaceans, are topics for future research.

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