New Zealand Journal of Marine and Freshwater Research, 2009, Vol. 43: 173–183
0028–8330/09/4301–0173

A long-term assessment of the physiological effects of herring (*Clupea harengus*) as a dietary component of the American lobster (*Homarus americanus*)

ANNA MYERS
MICHAEL F. TLUSTY
Edgerton Research Laboratory
New England Aquarium
Central Wharf
Boston, MA 02110, United States
email: mtlusty@neaq.org

Abstract Given the economic and ecological importance of American lobster (*Homarus americanus*) to the western North Atlantic, particular attention has been on factors that may increase susceptibility to disease. However, little focus has been on the possible role of dietary stress. There is strong evidence that wild lobsters feed on bait in traps, typically herring (*Clupea harengus*), yet evade capture. If the bait is nutritionally insufficient for lobsters, then the pervasive use of bait (up to 3 kg for each kg of lobster harvested) may compromise the health of lobsters, thus making them more susceptible to diseases. It has been shown previously that lobsters fed a diet solely of herring had higher incidences of shell disease and were less likely to survive than lobsters fed other diets. Here, physiological status including haemolymph protein, calcium, sodium, potassium and magnesium, hepatopancreas moisture and ash content, and the mineral constituents, thickness and hardness of the cuticle of lobsters fed 100% herring was compared to that of lobsters fed a diet consisting of rock crab (*Cancer irroratus)*, blue mussel (*Mytilus edulis*) and *Spirulina* algae. An artificial diet, as well as paired combinations of the three diets were also included for comparison. Juvenile lobsters (approximately 1.25 years of age) were fed one of these six diets for c. 330 days. Diet most affected the cuticle minerals, and lobsters with increased incidence of shell disease had a lower Ca/P ratio. Although few physiological parameters correlated to the previously observed disease and survival results, all physiological parameters tended to change with diet, and those lobsters fed herring overall increased haemolymph and cuticle mineral values and had lower shell physiological values than lobsters fed the other diets. These results suggest that rather than affecting lobsters via a single mechanism, nutritional stress is likely to impact a number of physiological variables, and thus make it difficult to assess health status through a single parameter.

Keywords bait; cuticle; diet; fishery; haemolymph; health; nutrition; shell disease; stress

INTRODUCTION

In the past two decades, stocks of most Gulf of Maine groundfish species and some invertebrates (such as the northern shrimp *Pandalus borealis*) have been severely reduced by overfishing, yet the American lobster (*Homarus americanus* Milne-Edwards 1837) fishery has continued to thrive despite increased numbers of fishers and traps (Grabowski et al. 2005). It is likely that the significant increase in landings and abundance of American lobster in recent years is associated with the use of bait in the lobster industry (Saila et al. 2002; Grabowski et al. 2005). In Maine, United States, fishers use about 99.8 kilo-tonnes of herring (*Clupea harengus*) as bait per year (NEFMC 1999) to land approximately 40.8 kilo-tonnes of lobster (Thunberg 2007), a bait-to-catch ratio that is also observed in the Nova Scotian (Canada) fisheries (Harnish & Wilson 2008). The addition of bait to coastal Gulf of Maine is an organic energy subsidy equivalent to about 1.8 times the natural primary production (Saila et al. 2002). Many fishers claim that through food supplementation, wild lobsters have essentially been "cultured" (Saila et al. 2002) with bait contributing to approximately 80% of the lobster's diet (Steneck 1987). There is concern from both fishers and researchers that bait may not be an appropriate diet for lobsters, as lobsters naturally ingest a wide variety of prey items

M07070; Online publication date 24 February 2009
Received 30 November 2007; accepted 19 July 2008

(Ojeda & Dearborn 1991), and may be contributing to recent disease outbreaks (Tlusty et al. 2008). The lobster fishery is an example where through fishing practices, the natural diet of a wild species may have been impacted, and the full implications of this diet change have not been assessed.

Recently, the survival for 352 days and disease status of 1-year-old lobsters fed: (1) 100% herring; (2) a diet of rock crab (*Cancer irroratus)*, blue mussel (*Mytilus edulis*) and *Spirulina* algae; (3) a commercially manufactured artificial shrimp diet (Progression 3™, Salt Creek, Inc., Salt Lake City, United States); or (4) paired combinations of three diets were assessed (Tlusty et al. 2008). The lobsters fed the 100% herring diet demonstrated higher initial moulting rates but, within the time of the experiment, all either contracted shell disease or died. These lobsters, and those fed the artificial diet also had the highest number of shell disease lesions. Mixing diets resulted in a higher survival and lower incidence of disease. The significant onset of disease and mortality occurred around the time of the third moult, indicating the lobsters required time in which to fully integrate the new diets into their shell and thus become susceptible to the bacterial agents responsible for the disease (Tlusty et al. 2008).

There is much interest in developing physiological measures that correlate to health status in different species of lobster to better forecast diseases in wild populations (Balcom & Pearce 2005; Tlusty et al. 2007), as well as determine quality of harvested animals (Ozbay & Riley 2002; Gardner & Musgrove 2006). Stressors such as pollution and water temperature can suppress lobster's ability to respond to pathogens, making it more likely that they will succumb to diseases such as gaffkemia (Sindermann 1990) or paramoebiosis (Balcom & Howell 2005). These stressors along with diet may also influence the onset of shell disease (Fisher et al. 1978; Smolowitz et al. 1992; Prince et al. 1995; Tlusty et al. 2007, 2008). In the interest of more comprehensively assessing the overall health of American lobster as it develops shell disease, here we related physiological parameters of lobsters to disease and survival data previously recorded in a controlled laboratory study assessing impacts of diet on lobster health (Tlusty et al. 2008). This study aimed to better understand physiological factors that may accompany changes in disease and survival. Chemical constituents of the haemolymph, hepatopancreas and the exoskeleton were analysed from the perspective of both the diet treatment and the resultant intensity of shell disease. We tested the alternative hypothesis that disease and mortality are negatively associated with physiological measures, compared with the null hypothesis that disease and mortality are not related to health status.

MATERIALS AND METHODS

Lobsters used in this experiment were part of a previous study that determinded survival and disease rates in relation to different diets (Tlusty et al. 2008, see Table 1). Lobsters were hatched and reared at New England Aquarium in Boston, MA, United States. Sea water was collected from Boston Harbor, and entered the hatchery at a rate of 10% replacement daily. It was filtered to 5 μm, UV-sterilised before entering the system and tested weekly to maintain 30.5–34.0 PSU salinity, pH

Table 1 Survival, disease status and severity, and growth of juvenile American lobster (*Homarus americanus*) fed one of six diets, as reported by Tlusty et al. (2008). Disease severity was categorised as an increasing index indicating increasing number and size of shell disease lesions. Diets were: (A) artificial—Progression 3™*; (H) herring; (W) wild—mix of rock crab (*Cancer irroratus*), blue mussel (*Mytilus edulis*) and *Spirulina* algae; or paired combinations of the three single diets. Results are for the terminal sample of a 352 day feeding trial.

Diet	*n*	Survival (%)	Healthy (%)	Diseased (%)	Disease index	% Growth day^{-1}
A	40	71.0	19.4	51.6	4.02 ± 0.47	0.58 ± 0.07
H	40	28.1	0.0	28.1	3.68 ± 0.37	0.43 ± 0.05
H/A	40	83.3	16.7	66.7	1.31 ± 0.47	0.66 ± 0.07
H/W	40	97.4	18.4	78.9	1.79 ± 0.44	0.47 ± 0.03
W	40	69.4	36.1	33.3	0.44 ± 0.18	0.29 ± 0.04
W/A	40	87.5	9.4	78.1	2.03 ± 0.38	0.60 ± 0.11

*Containing squid meal, soy protein, protein powder, fish protein, torula yeast, purified fish oils, lecithin, algae meal, plankton meal, binder, minerals, vitamin C (phosphate), betaine (an attractant), vegetable oil, cholesterol, vitamins, astaxanthin and/or canthaxanthin, and ethoxyquin.

7.84–7.97, and <70 ppm ammonium. All containers and trays containing animals were regularly cleaned. Lobsters were provided 11–13 h of wide spectrum fluorescent light per day. During the study period, water temperatures ranged from 14 to 18°C.

Lobsters were hatched in May and June 2004 from a wild-caught ovigerous female. The larvae and early benthic juvenile stages were reared according to standard hatchery techniques (Tlusty et al. 2005; Fiore & Tlusty 2005). In July 2005, 240 sibling lobsters of approximately 420–450 days old were randomly selected and equally divided into 6 experimental diet treatments. All animals were initially measured for carapace length (mm) and weight (g) respectively using a Mitutoyo Digimatic IP67, Series 500 caliper and an Ohaus Galaxy™ 160 scale. Lobsters were then placed individually into 9 cm diameter mesh cups in one of two flowing seawater trays, with three experimental groups per tray. Each tray was 193 cm × 18 cm and filled to a depth of 2.5 cm that was part of a larger 1705 litre recirculation system.

Table 2 Proximate analyses of the diets fed to American lobsters (*Homarus americanus*). Diets were: (A) artificial—Progression 3™; (H) herring; (W) wild—mix of rock crab (*Cancer irroratus*), blue mussel (*Mytilus edulis*) and *Spirulina* algae; or paired combinations of the three single diets. Fatty acid analyses were conducted on only the 100% diets (H, W, and A, $n = 3$ per treatment). Paired diets (H/A, H/W, W/A) were assumed to be an average of the two dietary constituents.

	Diet					
Proximate analysis	A	H	H/A	H/W	W	W/A
% Protein	37.85	14.01	29.62	13.54	15.91	27.49
% Fat	6.92	3.72	6.68	3.45	0.22	4.14
% Ash	6.41	2.61	5.35	1.89	1.59	4.99
% Fiber	0.65	0.00	0.55	0.16	0.04	0.55
Amino acid (% of protein)						
Alanine	5.91	8.86	6.04	4.59	10.25	6.34
Arginine	6.36	6.21	6.07	4.59	5.76	5.99
Aspartic acid	9.74	9.78	10.01	9.04	9.69	9.79
Cystine	1.69	1.12	1.74	1.38	2.39	2.23
Glutamic acid	19.99	14.77	19.32	22.66	14.19	18.91
Glycine	7.24	8.86	7.10	8.27	4.92	6.79
Histidine	2.23	1.83	2.02	1.38	1.97	2.14
Hydroxyproline	1.37	2.44	1.24	2.76	0.56	0.98
Isoleucine	3.89	3.87	3.98	5.05	4.78	4.23
Leucine	7.19	7.13	7.39	9.34	7.58	7.19
Lysine	6.28	8.04	6.78	11.33	6.74	6.34
Methionine	3.06	3.46	3.27	2.31	4.07	3.49
Phenylalanine	3.68	3.36	3.98	2.14	3.93	3.93
Proline	5.07	4.38	4.94	4.29	2.81	4.74
Serine	4.78	4.38	4.62	3.22	3.93	4.56
Threonine	4.02	4.28	4.12	3.06	4.49	4.24
Tyrosine	3.03	3.05	3.09	1.99	3.79	3.22
Valine	4.51	4.48	4.40	2.62	8.29	5.05
Fatty acid (% of lipid)						
Linoleic acid (C18:2ω6)	16.34	2.4			14.88	
ALA (C18:3ω3)	8.43	1.01			1.13	
ETA (C20:3ω3)	0.13	0.25			0.00	
ARA (C20:4ω6)	0.98	1.06			3.82	
EPA (C20:5ω3)	7.72	11.72			19.79	
DHA (C22:6ω3)	10.03	24.23			12.24	
Σ ω-3	30.10	38.73			34.58	
Σ ω-6	17.59	3.46			18.71	
Polyunsaturated	unknown	42.35			53.73	
Saturated	unknown	30.0			32.81	
Unsaturated	unknown	27.65			13.46	

Experimental design and feed formulation

Individual lobsters received one of six diets consisting of artificial feed (A), a "natural" diet of blue mussel, rock crab and *Spirulina* algae (W), herring (H), or 50:50 paired combinations of the diets (H/W, A/W, A/H, see Table 2 for proximate composition, amino acid and basic fatty acid profiles). The artificial feed was Progression 3™, a shrimp aquaculture feed (Salt Creek, Utah, United States) that was previously identified as being of suitable quality for lobsters (Tlusty et al. 2005).

For the W diet, the crabs and mussels were purchased live and the tissue from each animal was removed raw, whereas the *Spirulina* (Florida Aqua Farms Inc, Florida, United States) was in a dry, powdered form. The H diet consisted of herring, which was received frozen but whole. To remove any effect of particle size on food consumption (Fiore & Tlusty 2005), all diets were bound by gelatin after ingredients were placed in a Cusinart food processor and blended 150 g at a time to a slurry. Ninety ml of gelatin dissolved in distilled water was mixed with the resulting slurry. The gelatin allowed diets to retain a solid state in the water, preventing food from drifting to adjacent groups. Lobsters were fed once daily, with the amount of food equal to c. ½ the size of the last abdominal segment. In the rare instances in which food was left over in a lobster's cup, it was removed after a minimum of 4 h.

Laboratory analysis

Beginning at 330 days, 60 lobsters, ranging from 9 to 12 from each diet treatment group, were selected and tested for haemolymph content, shell thickness and hardness, while five additional lobsters from each diet treatment were selected for analysis of shell mineral constituents and hepatopancreas ash content. Because haemolymph and hepatopancreas volume and constituents can change during the moult cycle (Musgrove 2001), the moult stage was standardised by selecting lobsters 2 weeks after moulting. However, lobsters from the H group experienced particularly high degrees of mortality at the end of the experiment and testing of lobsters before mortality occurred became more important than the exact timeframes between last moult and sacrifice. This sampling necessity increased the range in days after moult for the H lobsters, but did not affect their respective moult stage. Lobsters were assessed for moult stage using the methods outlined by Waddy et al. (1995). Setae were removed from the pleoplods with dissecting scissors and observed under an MBC-10 dissecting microscope at a power of 56× magnification.

Haemolymph was extracted using 1 cc syringes, with the needle inserted at the basal joint of the 4th walking leg to extract haemolymph from the sternal cavity. Protein concentration was assessed as refractive index (RI, Leavitt & Bayer 1977), and was measured using a Leica TS Meter™ refractometer. The concentrations of sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{++}) and magnesium (Mg^{++}), were analysed on a Nova Biomedical Stat Profile Critical Care Xpress™ blood analyser. Haemolymph was first placed in microcentrifuge tubes and centrifuged in a Clay Adams Triac™ centrifuge for 3 min. Liquid was separated from the resulting pellet and diluted at a 1:5 ratio with de-ionised water. If any parameter exceeded the upper detection limit of the blood analyser, the sample was secondarily diluted and reanalysed. For all haemolymph parameters, there were never less than eight samples per diet treatment group.

Pieces of cuticle approximately 40 mm × 40 mm were cut with dissecting scissors from the carapace of sacrificed lobsters. Shell that was cut from above the dorsal midline was considered "dorsal;" shell cut from the carapace just above the walking legs was considered "ventral". Any tissue lining the inside of the shell pieces was removed. Each piece was dried with Kimwipes™ absorbant towels. Thickness was recorded using a Kaufer 0.001 mm analog thickness gauge.

Cuticle samples were assessed for hardness. They were broken into fragments approximately 5 mm in length, and fastened to a circular metal plate. The plate was placed in a Wilson Instruments Tukon® microhardness tester. Using a microscope, the shell was assessed to be of uniform height and free of cracks or colour abnormalities. A 4-sided 136° diamond with a 0.2 kg load was then gradually placed against the shell fragment for approximately 20 s. The length and width of the resulting impression was measured with a micrometer attached to a computer which calculated the surface area of the indentation. Vicker's hardness was calculated by dividing 0.2 kg by the mm^2 surface area of the impression (Smith & Sandland 1922).

To assess shell mineral consitutents (n = 5 per diet treatment), samples of dorsal cuticle were cut out, and weighed with an Ohaus Galaxy™ 160 scale. They were then dried in a VWR 1300U drying oven at 40°C for 24 h to determine dry weight. Samples were assessed for Ca, copper (Cu), iron (Fe), K, manganese (Mn), Mg, Na, P and zinc (Zn), (Cornell University College of Veterinary Medicine diagnostic laboratory, Ithaca, New York). The ash

content of the hepatopancreas was assessed by excising tissue from sacrificed lobsters and storing at –80°C for subsequent dry weight assessment analysis as described previously. The samples were then ignited at 500°C for 2 h in a Thermolyne 62700 furnace, and the loss of material (ashed weight) was recorded.

Proximate analysis and amino acid composition of all diets (n = 3 per diet) were carried out at New Jersey Feed Lab, Inc. (Trenton, New Jersey, see Table 2).

Statistical analyses

Comparisons between diet treatment groups were conducted for the three distinct data sets—haemolymph parameters, shell physical parameters (hardness and thickness), and shell mineral content. For each data set, a nested MANOVA (JMP 7.0, Carey NC) with the Hotelling-Lawley comparison was used to examine tray (the nested factor) and diet treatment effects (Quinn & Keough 2002). For those data sets with significant diet effects, each parameter was separately tested with a one-way nested ANOVA and Tukey's HSD (Quinn & Keough 2002). Pearson product-moment correlation and partial correlations were also calculated within each data set (Quinn & Keough 2002), and to the disease index of each lobster (Cohen 1988). Because large numbers of correlations were calculated, P values were protected by reducing significance proportionately by the number of correlations conducted ($0.05/n$, where n is the number of correlations tested) (Quinn & Keough 2002). The protected P value was 0.0024 for the haemolymph parameters, 0.005 for the physical cuticle parameters, and 0.0014 for the mineral properties of the cuticle. For each data set, a backward-stepwise regression (SigmaStat 3.0, Systat, Richmond, CA) with $F > 4.0$ to enter, was calculated to determine how the disease rating could be predicted from the specific parameters.

Finally, to determine the overall trend in data across diet treatments, a high-low (HL) index was calculated for each of the three data sets. These meta-analyses rank the average of each parameter across the diet treatments, and analyse the ranks (one-way nested ANOVA, Johnson et al. 2002). Thus, for the haemolymph data set, there were six parameters (RI, Ca^{++}, Mg^{++}, K^{+}, Na^{+}, and CL^{-}) over the six diet treatments for a total of 36 observations. The physical properties of the cuticle (dorsal and ventral hardness and thickness) had 24 total observations, whereas the mineral properties of the cuticle had 48 total observations.

RESULTS

The period between moulting and haemolymph sample day ranged from 10 to 110 days, and did not differ between trays (nested ANOVA, $F_{1,54} = 0.51$, $P > 0.4$), but did differ between diet treatment ($F_{4,54} = 6.23$, $P < 0.001$). This interval was largest for lobsters fed diet H (Tukey's HSD, $F_{1,54} = 26.26$, $P < 0.001$, power $\alpha_{0.05} = 0.98$) with all other diets statistically similar to one another. All lobsters were at similar points in the moult cycle, being either stage C_4 or D_0.

The diet treatment had a statistically significant effect on haemolymph parameters (nested MANOVA, $F_{24,134} = 1.89$, $P < 0.001$), whereas the tray did not have a significant effect ($F_{6,35} = 1.23$, $P > 0.30$). However, none of the single parameter nested ANOVAs for RI, Na^{+}, K^{+}, Cl^{-}, Ca^{++}, or Mg^{++}, were statistically significant across diet treatments (nested ANOVAs, all $F_{5,40} < 1.75$, $P > 0.10$). Ranking the average values for each treatment-parameter demonstrated the lobsters fed H had generally elevated haemolymph values compared with those fed A, all other diets were intermediate (nested one-way ANOVA, $F_{4,30} = 3.1$, $P < 0.03$, Table 3). Correlating disease rating to haemolymph parameters, the most significant Pearson product-moment correlation coefficient was 0.20 for the association between the disease rating and Ca^{++} (Table 4). A partial correlation improved this value to 0.31. The lack of fit between disease rating and haemolymph parameters precluded a backward stepwise regression model.

For the hardness and thickness of the dorsal and ventral cuticles, neither the diet treatment (nested MANOVA, $F_{16,198} = 0.54$, $P > 0.90$) nor the tray were statistically significant ($F_{4,51} = 1.78$, $P > 0.10$). The HL index indicated that diet treatments were statistically different (nested one-way ANOVA, $F_{4,18} = 6.27$, $P < 0.005$). Lobsters fed A and A/W diets had the thickest and hardest shells, whereas lobsters fed the H diet had the thinnest and softest shells (Table 5). Disease rating also had a small correlation to the physical shell parameters, with the most significant being a value of 0.11 (simple correlation) for dorsal thickness, which increased to 0.14 for a partial assessment (Table 6). The small correlations also precluded a stepwise regression model for the physical shell parameters on disease rating.

For cuticle minerals, the Ca/P ratio had a higher correlation to the disease rating than Ca or P individually (–0.66 compared with –0.22 or 0.54, respectively), and thus the ratio was used in subsequent analyses. Diet treatment had a statistically significant

effect on cuticle minerals (nested MANOVA, $F_{32,62}$ = 3.26, $P < 0.001$), whereas the tray did not have a significant effect ($F_{8,17} = 1.31$, $P > 0.30$). Multiple mineral components of the shell, including Ca/P, Na, K, Cu, and Zn were statistically significantly different when assessed independently (Table 7, nested one-way ANOVAs, all $F_{4,24} > 3.21$, $P < 0.05$). Overall, these components had the highest concentrations in the lobsters fed the H diet, and were equally low in lobsters fed the mixed diets H/A, W/A, and H/W (HL ranking, Table 7, nested one-way ANOVA, all $F_{4,42} = 5.61$, $P < 0.001$). Of the cuticle mineral parameters, the disease rating was most correlated to the Ca/P ratio (simple –0.66, partial –0.73, Table 8). A backward stepwise regression indicated that the disease rating could be determined by only shell mineral constituents Ca/P and Fe (ANOVA, $F_{2,27} = 17.99$, $P < 0.001$, $r^2 = 0.57$) in the form of the equation:

$$D = 6.97 - (0.22 \times Ca/P) - (0.0011 \times Fe).$$

Table 3 Haemolymph parameters (mean ± SE, $n = 60$) (refractive index RI, and ions measured in mmol/litre) for American lobster (*Homarus americanus*) fed one of six experimental diets (artificial, A; herring, H; wild, W, and paired combinations). Statistically similar treatments (nested one-way ANOVA, Tukey's HSD, $P < 0.05$) are indicated with like superscripts. HL ranking is a nested one-way ANOVA of the ranks (low to high) of the six haemolymph parameters.

Diet	RI	Ca^{++}	MG^{++}	Na^{+}	K^{+}	Cl^{-}	HL ranking
A	87.80 ± 8.16	8.61 ± 0.42	11.64 ± 2.13	405.00 ± 45.81	9.34 ± 0.49	479.63 ± 13.58	2.50 ± 0.67 [b]
H	85.63 ± 7.74	10.08 ± 0.47	15.95 ± 3.71	480.00 ± 22.39	10.09 ± 0.51	535.67 ± 22.29	5.52 ± 0.50 [a]
H/A	94.44 ± 6.31	8.42 ± 0.47	12.05 ± 1.70	474.38 ± 13.97	9.40 ± 0.37	504.78 ± 10.23	3.67 ± 0.72 [a,b]
H/W	67.91 ± 2.45	8.50 ± 0.20	12.63 ± 1.53	467.92 ± 7.52	9.30 ± 0.30	504.92 ± 5.87	2.83 ± 0.60 [a,b]
W	70.00 ± 3.54	8.46 ± 0.18	15.01 ± 1.74	459.29 ± 4.68	9.38 ± 0.30	493.17 ± 7.49	2.83 ± 0.48 [a,b]
W/A	86.00 ± **6.93**	8.93 ± **0.53**	**10.29 ± 1.41**	**411.11 ± 52.31**	**10.03 ± 0.64**	**518.59 ± 41.05**	**3.67 ± 0.72** [a,b]

Table 4 Partial (above diagonal) and simple Pearson product moment correlations (below diagonal) between haemolymph parameters and disease rating of individual American lobster (*Homarus americanus*). Significant values at a protected $P = 0.00024$ in bold. (RI, refractive index.)

	RI	Na^{+}	K^{+}	Cl^{-}	Ca^{++}	Mg^{++}	Disease rating
RI	–	0.38	0.15	**–0.49**	**0.58**	–0.03	–0.15
Na^{+}	–0.04	–	–0.12	**0.95**	–0.28	**–0.66**	–0.12
K^{+}	–0.22	0.48	–	0.27	–0.11	0.13	0.16
Cl^{-}	–0.26	**0.93**	**0.61**	–	0.43	**0.62**	0.01
Ca^{++}	0.40	0.47	0.21	**0.43**	–	–0.22	0.31
Mg^{++}	–0.55	–0.04	**0.37**	**0.24**	–0.20	–	–0.08
Disease rating	–0.01	–0.13	0.07	–0.07	0.20	0.01	–

Table 5 Physical properties (mean ± SE, $n = 60$) (thickness, mm; hardness, Vicker's hardness) of the cuticle of American lobster (*Homarus americanus*) fed six different experimental diets (artificial, A; herring, H; wild, W, and paired combinations). Statistically similar treatments (nested one-way ANOVA, Tukey's HSD, $P < 0.05$) are indicated with like superscripts. HL ranking is a nested one-way ANOVA of the ranks (low to high) of the four cuticle parameters.

	Thickness		Hardness		
	Ventral	Dorsal	Ventral	Dorsal	HL ranking
A	0.25 ± 0.03	0.42 ± 0.06	57.38 ± 4.36	53.05 ± 9.82	5.00 ± 0.71[a]
H	0.23 ± 0.03	0.31 ± 0.04	38.30 ± 6.56	49.58 ± 5.39	1.50 ± 0.50[b]
H/A	0.24 ± 0.04	0.37 ± 0.06	48.34 ± 10.03	47.31 ± 7.27	2.50 ± 0.65[a,b]
H/W	0.30 ± 0.03	0.36 ± 0.05	46.59 ± 6.36	52.42 ± 6.82	3.75 ± 0.85[a,b]
W	0.27 ± 0.03	0.31 ± 0.03	55.03 ± 7.04	47.34 ± 5.07	3.00 ± 0.58[a,b]
W/A	0.27 ± 0.03	0.38 ± 0.04	57.54 ± 8.80	53.02 ± 6.18	5.25 ± 0.25[a]

Lobsters fed the H diet had significantly greater hepatopancreatic % dry weight than all other groups (nested one-way ANOVA, $F_{4,24} = 16.13$, $P < 0.001$, with a significant tray effect, $F_{1,24} = 6.57$, $P < 0.05$), but ash weight did not differ between diet treatments (nested one-way ANOVA, $F_{4,24} = 1.85$, $P > 0.10$). Of the dietary constituents analysed, no singular element or combination of elements was identified as a definitive cause for the lack of shell disease among lobsters that consumed diet W. The concentration

Table 6 Partial (above diagonal) and simple Pearson product moment correlations (below diagonal) between physical cuticle parameters and disease rating of individual American lobster (*Homarus americanus*). Significant values at a protected $P = 0.005$ in bold.

	Ventral thickness	Ventral hardness	Dorsal thickness	Dorsal hardness	Disease rating
Ventral thickness	–	0.20	**0.46**	0.19	–0.09
Ventral hardness	**0.50**	–	0.24	0.27	–0.06
Dorsal thickness	**0.62**	**0.51**	–	0.10	0.13
Dorsal hardness	**0.44**	0.36	0.42	–	0.08
Disease rating	–0.01	–0.01	0.11	0.08	–

Table 7 Mineral properties (mean ±SE, $n = 5$; Ca, P, Mg, Na and K, % composition; Cu, Fe, Mn and Zn, mg/kg) of the cuticle of American lobster (*Homarus americanus*) fed six different experimental diets (artificial, A; herring, H; wild, W; and paired combinations). Statistically similar treatments (nested one-way ANOVA,Tukey's HSD, $P < 0.05$) are indicated with like superscripts. HL ranking is a nested one-way ANOVA of the ranks (low to high) of the mineral parameters.

Diet	Ca/P	Mg	Fe	K
A	$14.38 \pm 4.11^{b,c}$	2.47 ± 0.16	682.00 ± 403.08	$0.22 \pm 0.10^{a,b}$
H	12.54 ± 2.57^{c}	2.83 ± 0.15	990.60 ± 289.77	0.38 ± 0.10^{a}
H/A	$18.65 \pm 2.65^{b,c}$	2.38 ± 0.18	744.00 ± 466.57	0.16 ± 0.00^{b}
H/W	$19.51 \pm 2.99^{b,c}$	3.00 ± 0.31	299.60 ± 84.77	$0.20 \pm 0.04^{a,b}$
W	27.99 ± 5.85^{a}	2.72 ± 0.21	573.40 ± 78.96	$0.29 \pm 0.10^{a,b}$
W/A	$20.95 \pm 5.38^{a,b}$	2.69 ± 0.18	280.40 ± 74.86	0.16 ± 0.00^{b}

	Cu	Na	Mn	Zn	HL ranking
A	76.80 ± 18.29^{b}	$1.516 \pm 0.144^{a,b}$	92.40 ± 3.52	$93.60 \pm 15.25^{a,b}$	$3.12 \pm 0.44^{a,b}$
H	199.40 ± 47.96^{a}	2.168 ± 0.249^{a}	93.60 ± 11.51	246.80 ± 56.97^{a}	5.25 ± 0.11^{a}
H/A	53.80 ± 12.16^{b}	1.174 ± 0.031^{b}	85.80 ± 5.04	$96.60 \pm 36.44^{a,b}$	2.87 ± 0.50^{b}
H/W	44.00 ± 9.80^{b}	$1.736 \pm 0.218^{a,b}$	66.20 ± 10.98	79.60 ± 19.80^{b}	2.62 ± 0.57^{b}
W	$113.60 \pm 22.59^{a,b}$	1.994 ± 0.137^{a}	80.00 ± 14.22	$155.20 \pm 55.86^{a,b}$	$4.50 \pm 0.47^{a,b}$
W/A	62.60 ± 17.60^{b}	1.938 ± 0.154^{a}	67.00 ± 3.54	$95.20 \pm 17.31^{a,b}$	2.62 ± 0.43^{b}

Table 8 Partial (above diagonal) and simple Pearson product moment correlations (below diagonal) between chemical constituents of the cuticle and disease rating of individual American lobster (*Homarus americanus*). Significant values at a protected $P = 0.0014$ in bold.

	Ca/P	Mg	Na	K	Cu	Fe	Mn	Zn	Disease rating
Ca/P	–	0.03	0.08	–0.25	0.23	–0.37	–0.24	–0.08	**–0.73**
Mg	0.05	–	**0.64**	–0.33	–0.22	0.14	0.16	0.07	0.11
Na	0.00	0.51	–	**0.51**	0.09	–0.08	–0.27	0.22	0.01
K	–0.20	0.05	**0.68**	–	0.23	0.11	0.04	0.14	–0.22
Cu	–0.29	0.00	0.53	**0.75**	–	0.38	0.43	**0.60**	**0.47**
Fe	–0.21	0.02	0.28	0.51	0.52	–	–0.04	–0.21	**–0.48**
Mn	–0.35	–0.07	0.10	0.38	**0.58**	0.37	–	–0.06	–0.18
Zn	–0.08	0.16	**0.68**	**0.76**	**0.80**	0.41	0.38	–	–0.31
Disease rating	**–0.66**	–0.01	–0.14	–0.13	0.10	–0.21	0.11	–0.16	–

of protein in the diets did not appear to explain the differences in survivability between groups (see Table 1). The treatment in which lobsters were most likely to survive (H/W) involved a diet that had a protein content similar to that fed lobsters in treatments that exhibited the lowest survival (Table 2). Patterns of disease also did not correspond to protein content. The H lobsters were most likely to get diseased, whereas lobsters consuming diet W were least susceptible, with protein concentrations being similar in these diets.

Like protein, overall lipid content did not adequately explain trends in mortality and disease. Lipid content was highest in the A group (6.92%), in which lobsters had an intermediate likelihood of becoming shell diseased or dying during the experimental period (Table 1), whereas the H diet had an intermediate lipid content (3.72%). The H diet had a significantly lower level of omega-6 fatty acids compared with omega-3s than any of the other diets, and the omega-3:omega-6 ratio was high in this diet, nearly 13:1, but only 2:1 in the A and W diets (Table 2).

DISCUSSION

This study indicates that lobsters consuming a diet that leads to increased disease and lower survival had elevated chemical constituents of the haemolymph and the cuticle, and decreased thickness and hardness of the cuticle. Of the three assessments, haemolymph-chemical, cuticular-chemical and cuticular-physical, the shell chemical constituents were the most influenced by the diet treatment, as indicated by a statistically significant HL index and MANOVA. In addition, there were significant single parameters effects, and ultimately, these constituents could be associated with disease rating in a stepwise regression. The cuticular-physical parameters were least influenced, as significant diet effects were observed only through the HL index.

From a single constituent perspective, the results from this experiment appear contradictory to previous research. For example, the cuticular Ca/P levels varied with diet treatment. Gallagher et al. (1983) demonstrated that increased calcium can inhibit use of phosphorous and lead to shell abnormalities. Yet lobsters fed the H diet had low Ca/P ratios with elevated shell disease levels. Protein concentration varied as a function of diet (Table 2) but was not correlated with the disease rating. In contrast, haemolymph protein of shell diseased lobster was 40% lower than that of healthy lobsters (Floreto et al. 2000). However, others studies indicate that protein increases with stress (Lavallée et al. 2000; Dove et al. 2005). Therefore, the relationship between haemolymph protein content and disease remains unclear and needs further investigation. Protein levels are likely a function of the status of lobsters, and in Lavallée et al. (2000), the increase in protein was likely a result of dehydration. Within this study, responses may have been a function of the high protein concentrations of all lobsters tested, with no measurement being less than 55 mg/ml. Stewart et al. (1967) found that haemolymph protein concentration could predict growth only up to 55 mg/ml. Floreto et al. (2000) found that shell-diseased lobsters had significantly lower haemolymph protein concentrations than healthy lobsters, but in their study, concentrations averaged approximately 32 mg/ml. It is possible that juvenile lobsters generally have higher haemolymph protein concentrations than adult lobsters, perhaps owing to their shorter intermoult period and subsequently greater growth rate. Haemolymph protein may therefore be less useful as a health indicator when analysing juveniles compared with adults. Finally, the results of the hepatopancreas quality within this study were contradictory to those of previous studies. It has been shown that lobsters experiencing nutritional stress can suffer from inadequate hepatopancreas function, and under such conditions, lipid metabolism may be compromised (Rosemark et al. 1980). Both starvation and shell disease induce the loss of lipid-storage capacity, leading to increased ash content (Anger et al. 1985; Floreto et al. 2000). However, lobsters in this study had equivalent proportions of hepatopancreatic ash indicating that they were not starving, and thus differences in disease and survival were a result of dietary incompatibility as opposed to nutritional insufficiency. Because changes in diet did not result in a large change in single physiological components, but rather had a lesser but widespread effect on a number of physiological components, diet appears to affect lobster health from a whole animal perspective, instead of through a single mechanism. The lack of a single definitive causative agent indicates that it will be difficult to determine a single parameter which confers health status in American lobster.

Arginine, methione and lysine are important in lobster diets because they may contribute to better health (Gendron et al. 2001). In this study, arginine was approximately 6% of protein in all diets. Methione was only slightly greater in the W diet

(4%) versus other groups (2% H/W; 3% in all other groups). Lysine was also highest in the H/W (11%) and H (8%) groups as opposed to approximately 6% in other groups. Histidine has been considered an important amino acid in past research in lobster nutrition (Boghen & Castell 1981), but comprised only 2% of protein in all groups with the exception of the H group, in which it was 1%. Tyrosine, important in shell mineralisation (Floreto et al. 2000), followed a similar pattern, with a content of approximately 3% of protein in all diets, except the H diet, which contained 2%.

Little research has been conducted on the use of shell hardness as a measure of health in lobsters. Donahue et al. (1997, 1998) found that shells of lobsters fed cod (*Gadus morhua*) racks, which are high in essential fatty acids, were thicker and more resistant to compressive force when compared with those of lobsters fed a diet of herring or an artificial diet. In this study, lobsters fed herring had overall thinner and weaker cuticles compared with lobsters on the other diets. Ventral and dorsal hardness and thickness were highly correlated with each other. In the exocuticle, the α-chitin-protein stacks are arranged in much finer layers, and are thus denser than the endocuticle (Raabe et al. 2005), so that the exocuticle is harder than the endocuticle (Raabe et al. 2006). It is possible that the diamond in the microhardness tester penetrated the endocuticle in thinner shells, thereby producing a lower hardness measurement in them.

Previously, Fisher et al. (1978) found that lobsters fed a synthetic diet were more likely to become shell diseased. It was suggested that the diet lacked nutrients necessary for building the lipid-based epicuticular layer, thus allowing chitinolytic bacteria to parasitise the exo- and endocuticle (Fisher et al. 1978). In this study, those lobsters fed the artificial diet survived similarly to lobsters fed a more natural diet, and also had less disease than those fed only herring. However, a potential limitation of essential nutrients is likely as diets become more narrow in their composition. Research on American lobster indicates that diets with the highest diversity of components promoted the highest growth and survivability (Tlusty et al. 2008). The W/A diet was a combination of at least 8 food sources (rock crab, mussel, algae, squid meal, soy protein, fish protein and oils, torula yeast, and plankton meal). Although this study did not test every nutrient that may confer benefits to lobsters, a greater diversity of ingredients in the W/A diet may have provided unidentified nutrients or interactions of nutrients necessary for growth and survival. Although lobsters consume a significant amount of herring from traps (Grabowski et al. 2005), it is most likely not their only source of nutrition. Lobsters in a fished area of Maine grew more than those in unfished areas (Grabowski et al. 2005). In the wild, lobsters have more available food sources than only mussels, crab and algae, and Ojeda & Dearborn (1991) observed approximately 12 taxa in a lobster's diet. However, there are likely growth benefits of consuming herring as lobsters fed the W/H diet grew more than those fed the W diet (Tlusty et al. 2008).

An additional factor to consider is local variation in the cuticle minerals. In this study, samples were not taken from diseased areas of the cuticle, and correlative samples between diseased and healthy areas were not assessed. Thus it is unclear to what degree the mineral analysis of the healthy samples reflected status of diseased shell.

These results may have important implications for future management of the lobster industry, both in holding and feeding animals before shipment to market, and in use of bait within the lobster fishery. Studies have shown that multiple stressors can lead to disease events (Tlusty et al. 2000). Although the Maine lobster fishery has not experienced significant losses owing to disease, seawater temperature is consistently rising (Drinkwater et al. 2003). The impact of diet may become more significant when compounded with environmental stress. To fully address this issue, a better understanding of how bait impacts health and physiological functioning is necessary, as is determining the actual bait consumption by wild lobsters. It is crucial to understand how lobsters naturally forage for bait compared with other food items. If lobsters preferentially consume bait, then as environmental conditions become more stressful it may become prudent to consider bait when managing this species.

ACKNOWLEDGMENTS

This work was supported by the Darden Environmental Trust, and we thank G. Williams for his assistance with funding. Immense thanks go to A. Metzler, J. Ayers, B. Rosengaus and the large number of interns and volunteers who assisted in all phases of this work. Lobsters were maintained in accordance with NEAq ACUC protocol no. 06-01.

REFERENCES

Anger K, Storch V, Anger V, Capuzzo JM 1985. Effects of starvation on moult cycle and hepatopancreas of stage 1 lobster (*Homarus americanus*) larvae. Helgoländer Meeresuntersuchungen 38: 481–482.

Balcom N, Pearce J 2005. The 1999 Long Island Sound lobster mortality event: findings of the comprehensive research initiative. Journal of Shellfish Research 24(3): 691–697.

Balcom N, Howell P 2005. Responding to a resource disaster: American lobsters in Long Island Sound, 1999–2004. Storrs, Connecticut, Connecticut Sea Grant. 24 p.

Boghen AD, Castell JD 1981. Nutritional value of different dietary proteins to juvenile lobsters, *Homarus americanus*. Aquaculture 22: 343–351.

Cohen J 1988. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale, NJ, Lawrence Earlbaum Associates. 567 p.

Donahue DW, Bayer RC, Work TM, Riley JG 1997. The effect of diet on weight gain, shell hardness, and flavor of new-shell American lobster, *Homarus americanus*. Journal of Applied Aquaculture 7(4): 71–77.

Donahue DW, Bayer RC, Riley JG 1998. Effects of diet on weight gain and shell hardness of new-shell American lobster, *Homarus americanus*. Journal of Applied Aquaculture 8(2): 79–85.

Dove ADM, Allam B, Powers JJ, Sokolowski MS 2005. A prolonged thermal stress experiment on the American lobster, *Homarus americanus*. Journal of Shellfish Research 24 (3): 761–765.

Drinkwater KF, Petrie B, Pettipas RG, Petrie WM, Soukhotsev V 2003. Physical oceanographic conditions on the Scotian Shelf and in the Gulf of Maine during 2002. Science Council Research Document NAFO no. 03/31. 45 p.

Fiore DR, Tlusty MF 2005. Use of commercial *Artemia* replacement diets in culturing larval American lobsters *(Homarus americanus*). Aquaculture 243: 291–303.

Fisher WS, Nilson EH, Steenbergen JF, Lightner DV 1978. Microbial diseases of cultured lobsters: a review. Aquaculture 14: 115–140.

Floreto EAT, Prince DL, Brown PB, Bayer RC 2000. The biochemical profiles of shell-diseased American lobsters, *Homarus americanus* Milne Edwards. Aquaculture 188: 247–262.

Gallagher ML, Bayer RC, Rittenburg JH, Leavitt DF 1983. Studies on the mineral requirements of the adult American lobster. Progressive Fish Culturist 44: 210–212.

Gardner C, Musgrove R 2006. Quantifying shell hardness in the southern rock lobster (*Jasus edwardsii*). Journal of Aquatic Food Product Technology 15: 17–35.

Gendron L, Fradette P, Godbout G 2001. The importance of rock crab (*Cancer irroratus*) for growth, condition and ovary development of adult American lobster (*Homarus americanus*). Journal of Experimental Marine Biology and Ecology 262: 221–241.

Grabowski JH, Clesceri E, Baukus A, Yund PO 2005. Are we using herring to farm lobsters? The effects of herring bait on lobster growth and diet composition. www.fishresearch.og/project_reports/ProjectReport_Home.asp?id=27 [accessed 15 July 2008].

Harnish L, Wilson JHM 2008. Efficiency of bait usage in the Nova Scotia lobster fishery: a first look. Journal of Cleaner Production 17: 354–347.

Johnson VE, Deaner RO, Van Schaik CP 2002. Bayesian analysis of rank data with application to primate intelligence experiments. Journal of the American Statistical Association 97: 8–17.

Lavallée J, Hammell KL, Spangler ES, Cawthorn RJ, Dohoo IR 2000. Descriptive statistics of fishing practices, postharvest health status and transport conditions in the Prince Edward Island lobster (*Homarus americanus*) industry. Journal of Shellfish Research 19(1): 265–274.

Leavitt DF, Bayer RC 1977. A refractometric method of determining serum protein concentration in the American lobster. Aquaculture 12: 169–171.

Musgrove RJB 2001. Interactions between haemolymph chemistry and condition in the southern rock lobster, *Jasus edwardsii*. Marine Biology 139: 891–899.

New England Fisheries Management Council (NEFMC) 1999. Atlantic herring fishery management, incorporating the environmental impact statement and regulatory impact review (including the regulatory flexibility analysis). Saugus, MA, United States. www.nefmc.org/herring [accessed 15 July 2008].

Ojeda FP, Dearborn JH 1991. Feeding ecology of benthic mobile predators: experimental analyses of their influence in rocky subtidal communities of the Gulf of Maine. Journal of Experimental Marine Biology and Ecology 149: 13–44.

Ozbay G, Riley JG 2002. An analysis of refractometry as a method of determining blood total protein concentration in the American lobster *Homarus americanus* (Milne Edwards). Aquaculture Research 33: 557–562.

Prince DL, Bayer RC, Galagher ML, Subramanyam M 1995. Reduction of shell disease with an experimental diet in a Nova Scotia lobster pound. Journal of Shellfish Research 14: 205–207.

Quinn GP, Keough MJ 2002. Experimental design and data analysis for biologists. Cambridge, Cambridge University Press. 537 p.

Raabe D, Romano P, Sachs C, Al-Sawalmih A, Brokmeier HG, Yi SB, Servos G, Hartwig HG 2005. Discovery of a honeycomb structure in the twisted plywood patterns of fibrous biological nanocomposite tissue. Journal of Crystal Growth 283: 1–7.

Raabe D, Romano P, Sachs C, Fabritius H, Al-Sawalmih A, Yi SB, Servos G, Hartwig HG 2006. Microstructure and crystallographic texture of the chitin-protein network in the biological composite material of the exoskeleton of the lobster *Homarus americanus*. Materials Science and Engineering A421: 143–153.

Rosemark R, Bower PR, Baum N 1980. Histological observations of the hepatopancreas in juvenile lobsters subjected to dietary stress. Proceedings of the World Mariculture Society 11: 471–478.

Saila SB, Nixon SW, Oviatt CA 2002. Does lobster trap bait influence the Maine inshore trap fishery? North American Journal of Fishery Management 22: 602–605.

Sindermann CJ 1990. Principal diseases of marine fish and shellfish, vol 2. San Diego, Academic Press, Inc. 516 p.

Smith RL, Sandland GE 1922. An accurate method of determining the hardness of metals, with particular reference to those of a high degree of hardness. Proceedings of the Institution of Mechanical Engineers 1: 623–641.

Smolowitz RM, Bullis RA, Abt DA 1992. Pathologic cuticular changes of winter impoundment shell disease preceding and during intermoult in the American lobster, *Homarus americanus*. Biological Bulletin 183: 99–112.

Steneck R 1987. Lobstermen pull traps for research. Gulf of Maine Foundation Newsletter, Making Waves 2(4): 1–2.

Stewart JE, Cornick JW, Foley DM, Li MF, Bishop CM 1967. Muscle weight relationship to serum proteins, hemocytes and hepatopancreas in the lobster *Homarus americanus*. Journal of the Fisheries Research Board of Canada 24: 2339–2354.

Thunberg EM 2007. Demographic and economic trends in the northeastern United States lobster (*Homarus americanus*) fishery, 1970–2005. US Department of Commerce, Northeast Fishery Science Center Reference Document 07-17. 64 p.

Tlusty MF, Lightner D, Goldstein J, White B 2000. Potential synergistic stressors trigger a mortal infection in juvenile *Homarus americanus*. The Lobster Newsletter January 13: 6–8.

Tlusty MF, Fiore D, Goldstein J 2005. Use of formulated diets as replacements for Artemia in the rearing of juvenile lobsters (*Homarus americanus*). Aquaculture 250(3-4): 781–795.

Tlusty MF, Smolowitz RM, Halvorson HO, DeVito SE 2007. Host susceptibility hypothesis for shell disease in American lobsters. Journal of Aquatic Animal Health 19(4): 215–225.

Tlusty MF, Myers A, Metzler A 2008. Short and long-term dietary effects on disease and mortality in American lobster, *Homarus americanus*. Diseases of Aquatic Organisms 78: 249–253.

Waddy SL, Aiken DE, De Kleijn DPV 1995. Control of growth and reproduction. In: Factor RJ ed. Biology of the lobster, *Homarus americanus*. San Diego, Academic Press. Pp. 217–265.