

Factors affecting post-capture survivability of lobster *Homarus americanus*

David Basti^{1,*}, Ian Bricknell², Ken Hoyt¹, Ernest S. Chang³, William Halteman⁴, Deborah Bouchard¹

¹University of Maine Animal Health Laboratory, ²School of Marine Sciences, and ⁴Department of Mathematics and Statistics, University of Maine, Orono, Maine 04469, USA

³Bodega Marine Laboratory, University of California–Davis, PO Box 247, Bodega Bay, California 94923, USA

ABSTRACT: Technological advances in gear and fishing practices have driven the global expansion of the American lobster live seafood market. These changes have had a positive effect on the lobster industry by increasing capture efficiency. However, it is unknown what effect these improved methods will have on the post-capture fitness and survival of lobsters. This project utilized a repeated measures design to compare the physiological changes that occur in lobsters over time as the result of differences in depth, hauling rate, and storage methodology. The results indicate that lobsters destined for long distance transport or temporary storage in pounds undergo physiological disturbance as part of the capture process. These changes are significant over time for total hemocyte counts, crustacean hyperglycemic hormone, L-lactate, ammonia, and glucose. Repeated measures multivariate analysis of variance (MANOVA) for glucose indicates a significant interaction between depth and storage methodology over time for non-survivors. A Gram-negative bacterium, *Photobacterium indicum*, was identified in pure culture from hemolymph samples of 100% of weak lobsters. Histopathology revealed the presence of Gram-negative bacteria throughout the tissues with evidence of antemortem edema and necrosis suggestive of septicemia. On the basis of these findings, we recommend to the lobster industry that if a reduction in depth and hauling rate is not economically feasible, fishermen should take particular care in handling lobsters and provide them with a recovery period in recirculating seawater prior to land transport. The ecological role of *P. indicum* is not fully defined at this time. However, it may be an emerging opportunistic pathogen of stressed lobsters. Judicious preemptive antibiotic therapy may be necessary to reduce mortality in susceptible lobsters destined for high-density holding facilities.

KEY WORDS: Lobster · *Homarus americanus* · Stress · Clinical biochemistry · Depth · Hauling rate · *Photobacterium indicum* · *Aerococcus viridans*

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INTRODUCTION

Since the mid-1800s the American lobster industry in the Northwest Atlantic has represented a source of revenue and a way of life for generations of families. The heritage and tradition of the lobster industry, as well as the economic benefits, continue to be vital to the survival of the coastal communities in the Northeast, many in rural, economically challenged areas. While other industries such as cod, shrimp, scallops, and sea urchins have plummeted, the lobster fishery has enjoyed an upswing in catches and demand since

the late 1980s. Today, the Maine lobster fishery is considered to be well regulated and sustainable, and supports more than \$500 million in industry-related income. In 2008, 63.4×10^6 pounds ($\sim 28.8 \times 10^6$ kg) of live lobster, worth over \$222.6 million, were landed in Maine (Pritchard 2009).

Briefly, in the chain of live marketing, lobsters are captured in baited traps set on the seafloor at depths of 10 to 200 m, depending upon season, local abundance, and prevailing weather patterns. 'Strings' of traps (2 or more) are identified with buoys and recovered to the surface with the aid of hydraulic power haulers. Lob-

*Email: david.basti@umit.maine.edu

sters are immediately removed from the traps, graded, and placed into totes and stored either on deck or below deck in recirculating seawater. 'Shorts' and oversized lobsters are discarded overboard. Marketable lobsters are brought to dealers who truck them to large cities such as Boston, where they are distributed nationwide, to Canada, or overseas within 3 to 4 d. In the late summer and fall, as the weather begins to deteriorate and the market price begins to drop, US pound owners also purchase lobsters from fishermen, store them over the winter in pounds, and release them during the holidays and early spring as the market demand increases.

A lobster pound is a 3-sided embayment that is typically a part of the natural shoreline, with the fourth side being a gate to the open ocean, allowing for semi-diurnal flushing (tidal water exchange). There are approximately 49 active pounds in Maine. They average 2 to 6 m in depth, cover 4000 to 40 000 m², and are capable of storing from 9000 to 135 000 kg of lobsters at densities as high as 11 ind. m⁻² of bottom area (Floreto et al. 2000).

Anecdotal information and data from a Maine Lobster Pound Association survey conducted in 2006 suggest that there has been an increase in lobster mortality, termed 'shrinkage' (a decrease in outgoing poundage), during the fall-winter impoundment cycle. Although detailed records are not always available, estimates among survey respondents now place the loss at 14 to 27 % in 2006. These losses have occurred despite the lack of clear evidence of a disease epizootic or reported changes in handling and shipping practices, and in the face of widespread prophylactic antibiotic use.

A number of studies have examined and compared the physiological changes that occur in various crustacean species, from trawling and handling practices at sea and in overseas transport, to survival in a controlled laboratory setting (Taylor & Whiteley 1989, Taylor et al. 1997, Gomez-Jimenez et al. 2000, Dove et al. 2005, Ridgway et al. 2006a, 2007, Lorenzon et al. 2007). Harris & Andrews (2005b) monitored the post-capture effects of trawling and emersion on the physiology and survival of 'discarded' and 'escaped' *Nephrops norvegicus* by placing them within cages on the seafloor, and then recovering them with divers. Ridgway et al. (2007) describes idiopathic muscle necrosis in post-capture *N. norvegicus*, and Bernasconi & Uglow (2008) compared the effects of emersion and reimmersion on trawled and creel-caught *N. norvegicus*.

Although many fishermen believe that handling practices within the American lobster fishery have not changed, a number of pound owners blame increased mortality (shrinkage) on rough handling and advances in gear technology that now allow fishermen to maintain more traps by hauling faster and from greater

depths. Lavallée et al. (2000) examined risk factors associated with the loss of vigor in lobsters arriving at processing plants in Prince Edward Island, Canada. However, to our knowledge, there are no references that compare the effects of depth at capture, hauling rate, and storage methodology on the post-capture survival of *Homarus americanus*. Subjective measures of stress based upon physical characteristics may be imprecise (Taylor et al. 1997, Basti 2008). The purpose of this experiment was to combine the techniques of hematology, bacteriology, endocrinology, clinical biochemistry, and histopathology to quantitatively compare physiological changes over time in long-term survivors. This information will help us to identify points of vulnerability in the movement of lobsters so that corrective strategies and best management practices can be developed that will benefit the lobster industry and ultimately the consumer.

MATERIALS AND METHODS

Care and management of lobsters. One hundred and forty-eight market size lobsters (mean weight 400 to 700 g) were purchased from a pound owner. The lobsters were captured and held overnight within the pound in plastic totes. Lobsters were then packed with damp seaweed into Styrofoam™ insulated boxes and transferred to a seawater holding facility (within an approximate emersion transport time of 2 h). The lobsters were individually compartmentalized, to reduce agonistic behavior and cannibalism, within a 5400 l artificial seawater (Crystal Sea® Marinemix) recirculating system and allowed to acclimate for 3 wk prior to the field experiment. During this time, water quality parameters were monitored by spectrophotometry and digital titration (Hach Company) and kept at the following recommended levels with partial water changes and foam fractionation. The total ammonia nitrogen and un-ionized ammonia levels were maintained at <1.0 mg l⁻¹ and <0.3 mg l⁻¹, respectively, at pH 7.8 to 8.0 (Young-Lai et al. 1991), nitrite <0.1 mg l⁻¹, salinity 33 to 35, specific gravity 1.023 to 1.025, oxygen >7.0 mg l⁻¹. System water passed through four 65 W ultraviolet sterilizers and was kept at 9 to 11°C to match real-time data obtained from the Gulf of Maine Ocean Observing System (GoMoos 2008) buoy closest to the approximate origin of the experimental lobsters. Light intensity was reduced by covering normal output fluorescent tubes with black plastic mesh. Light:dark duration was maintained at Eastern Standard Time. All lobsters were individually fed every 48 h on an alternating schedule of cooked Maine shrimp, predominantly *Pandalus borealis*, locally caught frozen smelt, predominantly *Osmerus mordax*, and fresh soft shell

clams *Mya arenaria*. The tanks were flushed daily to remove uneaten food and fecal waste.

Experimental design. At the end of a 20 d acclimation period, 121 surviving lobsters were examined for overall appearance, sex, molt stage by carapace and exoskeletal characteristics (Aiken 1973), the presence and severity of active lesions (open wounds), shell disease, and other abnormalities. They were then tagged and sampled for baseline physiology and bacteriology. Following an additional 8 d resting period, 94 lobsters were taken into the field, and 22 lobsters were left behind as the control group. The experimental lobsters were quickly transferred into an insulated carrier filled with approximately 400 l of seawater. Ambient air temperature within the transport vehicle was 3.9°C. Because of the time of year, it was easier to control the confounding effects of abrupt temperature changes during transport. Immersion time to dockside and placement into the vessel's recirculating seawater storage was approximately 4.5 h.

Experimental sites were bounded by approximately 67° 50' to 68° 00' N and 44° 10' to 44° 25' W, off Petit Manan, Maine. At the shallow water site, 47 lobsters were randomly allocated (random numbers were generated by thrown dice) into 4 standard size (122 × 55 × 35 cm) plastic-coated wire traps that were individually compartmentalized. Trap and tag numbers as well as weather conditions were recorded. A temperature data logger (Omega Engineering) was attached, and the traps were lowered to a depth of 10.7 m. Each string of 2 traps was weighted down with 23-kg anchors at each end to secure its position on the seafloor in anticipation of inclement weather. It took an additional 1 h to reach the deep water site, and the remaining 47 lobsters were placed into the 4 traps and lowered to the seafloor at 131.8 m. These traps were also prevented from drifting with the aid of 23-kg anchors.

The lobsters were recovered after 8 d at sea. Lobsters were pre-randomized into their designated treatment groups based upon trap identification, prior to the retrieval day, to minimize confounding variables at sea in potentially adverse weather. Lobsters were retrieved first at the deep water site. At the deep water site, the first string of 2 traps was hauled at the normal commercial rate. Elapsed retrieval time was standardized as the instant the rope was captured by the rotating disc of the hydraulic hauler, until the time the traps broke the water surface. The calculated retrieval rate was 75 m min⁻¹. Surviving lobsters were then allocated into air transport storage above deck in totes or below deck in totes in recirculating seawater on the basis of pre-randomization. The last 2 traps at the deep water site were hauled at a slow rate calculated to be 21.3 m min⁻¹ and the surviving lobsters allocated into their designated treatment groups for transport storage. The

vessel arrived at the shallow water site approximately 55 min later. The first 2 traps were hauled to the surface at a rate calculated to be 64 m min⁻¹ and the last 2 traps hauled at 21 m min⁻¹. All surviving lobsters were again allocated into their predetermined transport groups as the vessel returned to port. At dockside, lobsters from all treatment groups were loaded into 4 plastic totes to replicate the standard commercial land transport method. Care was taken to minimize injury by orienting lobsters parallel to each other in the same direction, with tails curled under the body to protect the vulnerable ventral abdomen from puncture wounds sustained from the protruding rostrum of the lobster immediately beneath. They were also packed in sufficient density to minimize excessive movement and subsequent impact injuries during transport (S. Sargent pers. comm. 2008). Transit time to the seawater holding facility was 2.5 h. The total air exposure time for the above-deck transport groups was approximately 4.5 h.

Upon arrival at the holding facilities, all surviving lobsters, including the resident control group, were sampled for baseline physiological parameters and bacteriology. A total of 48 lobsters survived from the shallow water site, and 28 survived from the deep water site. This discrepancy in numbers of lobsters recovered from the shallow water site compared to the numbers originally placed at that site is attributed to inaccurate counting and adverse weather conditions at sea. During the reacclimation period, lobsters were observed 3 times d⁻¹ for evidence of distress or morbidity (weakness). For the purpose of this experiment morbidity was judged by the following criteria: (1) flaccid paresis, described as the diminished or absent 'claw display,' 'arched back' posture, and vigorous abdominal muscle contraction ('tail flip') when held out of water; (2) ataxia, described as the inability to maintain or return to a level position in the water column; and (3) poor response to tactile stimuli. In the experience of the authors, weak lobsters never spontaneously recover, and are considered mortalities. Weak lobsters were sampled for physiological data and bacteriology prior to euthanasia with an intrasinus injection of potassium chloride solution (300 mg ml⁻¹) (Battison et al. 2000). Their tissues were then preserved for histopathology. Lobsters that died between observation times were sampled only for bacteriology. Three weeks post-retrieval, all surviving lobsters, including resident controls, were sampled for bacteriology and physiological data and then euthanized. In addition, a random sub-sample of 5 ind. from each treatment group, including the control group, had their tissues preserved for histopathology.

Bacteriology. The dorsal arthrochial membrane was disinfected with 70% ethanol. Two milliliters of

hemolymph were collected from the dorsal abdominal artery into a sterile 3.0 cc syringe with a 23 gauge needle. A 200 μ l aliquot of hemolymph was inoculated into thioglycollate broth with 1.5% Instant Ocean[®] to increase sensitivity and screen for possible anaerobic bacteria. The media were placed on ice for transport to the laboratory and were incubated at 16°C for 5 d. Hemolymph samples exhibiting microbial growth were streaked for isolation on Trypticase Soy Agar with additional NaCl. Preliminary biochemical profiling included Gram stain, oxidase reaction, 0129 vibriostatic susceptibility, and carbohydrate utilization prior to final identification using the Biolog[®] Microbial Identification System.

Total hemocyte counts. A 200 μ l aliquot of hemolymph was transferred into 800 μ l of chilled 10% neutral buffered formalin in a 5 ml glass vial. Care was taken to prevent the hemolymph from coming into contact with the sides of the vial to avoid hemocyte agglutination or lysis. Vials were stored at 4°C prior to cell counting. Cell counting was performed using a Kova[®] Glasstic[®] slide 10 with quantitative grids (Hycor Biomedical).

Crustacean hyperglycemic hormone (CHH). A 500 μ l aliquot of hemolymph was transferred into 500 μ l of chilled glycine ethyl ester hemolymph dilution buffer prepared fresh before each use (Chang et al. 1998). Samples were immediately frozen at –80°C and then shipped later on dry ice to Bodega Marine Laboratory, Bodega Bay, California, USA, to be evaluated by the enzyme-linked immunosorbent assay (ELISA) method of Chang et al. (1998, 1999).

Analytes. Approximately 1.0 ml of hemolymph was centrifuged at 10 000 $\times g$ for 4 min at 4°C to obtain cell-free hemolymph. Cell-free hemolymph was used in this experiment to reduce the possibility of unknown constituents from lysed hyalinocytes interfering with testing procedures (Battison 2006). All samples were kept frozen at –80°C. Thawed plasma samples were analyzed for glucose, L-lactate (Hammen 1980), and ammonia on a Beckman-Coulter (Synchron clinical system) CD4 Pro Biochemistry analyzer.

pH. *In vivo* hemolymph pH (Thermo Electron portable pH meter) was determined by disinfecting

the dorsal abdomino-cephalothoracic arthrothoracic membrane with 70% ethanol and placing a 16 gauge beveled tip, pH microprobe (Microelectrodes) within the pericardial sinus (Dove et al. 2005). The pH electrode was calibrated against pH 7.00 and pH 10.00 standard solutions every hour at air temperature, and then recalibrated to read pH at the experimental water temperature.

Total hemolymph protein. A 50 μ l aliquot of hemolymph was placed on a Schuco clinical refractometer (model 5711-2020, Schuco International) to measure total hemolymph protein.

Histology. Samples of heart, hepatopancreas, gonad, antennal gland, eye, intestine, ventral nerve chord, gills, deep abdominal flexor muscle, and exoskeleton were placed into 10% neutral buffered formalin and processed routinely for hematoxylin and eosin staining of paraffin-embedded sections.

Data analysis. SYSTAT[®] version 12 (2007) (Systat Software), Microsoft[®] Excel 2007 and R v.2.10.1 were utilized in the analysis. Since the data were collected on most lobsters at 3 time points, the data were analyzed as a repeated measures design. The measured analytes were treated as the response variables, and the categorical variables mortality, depth, hauling rate, and storage were used as the independent explanatory variables. The measures recorded as ‘weak prior to death’ were entered at the third time point regardless of the actual time of death. All cases with data at <3 time points were removed for this analysis. Because of missing data, a full analysis with all possible interaction terms could not be performed. Thus, 3 hierarchical models were analyzed and compared (Table 1).

There are 2 approaches to a repeated measures analysis: univariate (used when ANOVA tables are subsequently presented), which requires a strong assumption about the composition of the variance, called sphericity; and multivariate (used when MANOVA tables are subsequently presented), which does not require assumptions about the variance structure, but is less powerful and is not as likely to detect small differences. For all analytes the null hypothesis is that the least complex model is an adequate substitute for the

Table 1. *Homarus americanus*. Models for stressor analysis. Mort: mortalities

Model	Terms	Equation
3	Only single-factor terms	$y = \text{Mort} + \text{Storage} + \text{Depth} + \text{Haulrate}$
2	Single-factor and 2-factor terms	$y = \text{Mort} + \text{Storage} + \text{Mort} \times \text{Storage} + \text{Depth} + \text{Mort} \times \text{Depth} + \text{Haulrate} + \text{Mort} \times \text{Haulrate} + \text{Storage} \times \text{Depth} + \text{Storage} \times \text{Haulrate} + \text{Depth} \times \text{Haulrate}$
1	Single-factor, 2-factor, and the few estimable 3-factor terms	$y = \text{Mort} + \text{Storage} + \text{Depth} + \text{Haulrate} + \text{Mort} \times \text{Storage} + \text{Mort} \times \text{Depth} + \text{Mort} \times \text{Haulrate} + \text{Storage} \times \text{Depth} + \text{Storage} \times \text{Haulrate} + \text{Depth} \times \text{Haulrate} + \text{Mort} \times \text{Storage} \times \text{Depth} + \text{Mort} \times \text{Depth} \times \text{Haulrate} + \text{Storage} \times \text{Depth} \times \text{Haulrate}$

more complex model. For brevity, only significant terms are presented in the ANOVA and MANOVA tables, although the actual fitted models were those presented in Table 1.

RESULTS

Mortality

Between retrieval day (29 November 2008) and the termination of the experiment (19 December 2008) there were 19 mortalities. Thirteen lobsters were sampled as weak prior to death. Six lobsters died between observation times and were sampled only for bacteriology. All 19 lobsters cultured positive for *Photobacterium indicum* within the hemolymph (Table 2). *P. indicum* is a Gram-negative, 0129 vibriostat susceptible, oxidase-positive, alkaline/acid triple-sugar iron-agar-reacting bacterium; it was further identified by the Biolog[®] using extensive computer algorithms of positive-, negative-, and borderline color reaction patterns produced during substrate utilization. Using this system we obtained repeatable (100% probability) patterns establishing the isolate as *P. indicum*. Two of these lobsters had suffered exoskeletal injuries (cheliped fracture and pereopod avulsion), one noted prior to the field portion of the experiment and the other on retrieval day. Two other lobsters were sampled as weak: one in the control group, and the other, unidentified because of a lost tag, in the field portion of the experiment. All lobsters were in post-molt stage C₃–C₄ by carapace and exoskeletal characteristics (Aiken 1973). Morbid lobsters were clinically characterized as profoundly weak with mild generalized edema evident at the arthrodistal joints.

Total hemocyte counts

Non-transformed data for total hemocyte counts (THC) are shown in Fig. 1a for survivors, and in Fig. 1b

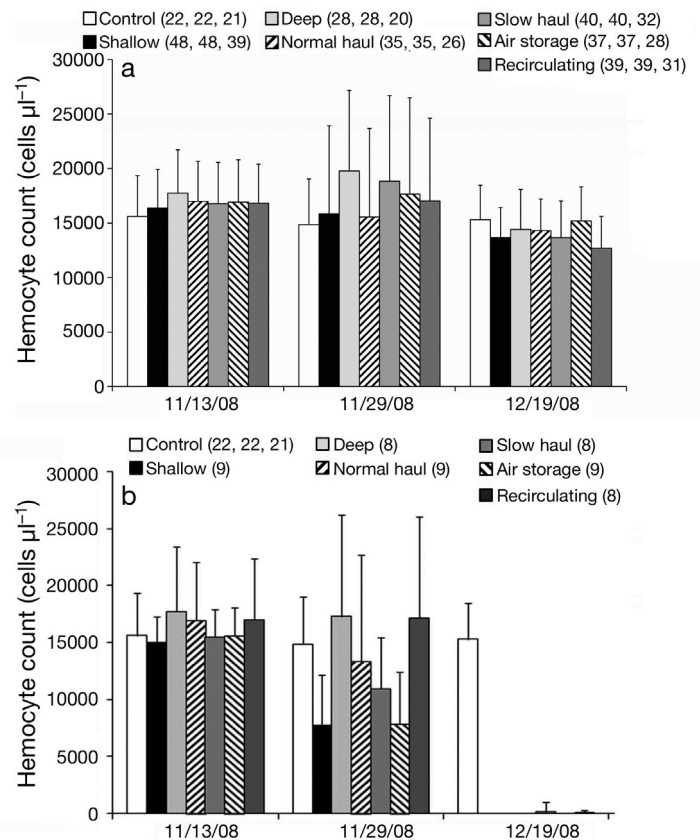


Fig. 1. *Homarus americanus*. Non-transformed mean (+SE) total hemocyte counts (cells μl^{-1}). (a) Survivors. Numbers within parentheses: n for each treatment group by date of sampling (mo/d/yr). Differences in n between 29 November and 19 December 2008 represent mortalities. (b) Mortalities. Numbers within parentheses: n for each treatment group

for mortalities. The ANOVA table comparing the levels of complexity of the 3 models (ANOVA table not shown) indicates that the least complex model (no significant interaction) is sufficient to explain the data. The MANOVA (Table 3) shows that the mean THC differs by mortality status and that this difference changes over time. Fig. 2 depicts changes in THC means and standard errors (SE) over time.

Table 2. *Homarus americanus*. Summary of bacteremia

	13 Nov 2008 (Initial sampling)	29 Nov 2008 (Second sampling)	Mortalities ^a (All treatments + 1 control + 1 no ID)	19 Dec 2008 (Terminal sampling of survivors)
Total lobsters	122	92 ^b	19	80
Bacteria cultured	1 <i>Flavobacterium</i> spp. 1 mixed growth	All negative	All <i>Photobacterium indicum</i>	1 <i>Vibrio splendidus</i> 1 <i>Vibrio tubiashii</i>

^aWeak and dead sampled after 29 November 2008 and before 19 December 2008
^bResults only for samples with known tags and treatment

Table 3. *Homarus americanus*. MANOVA for total hemocyte counts (Type II repeated measures MANOVA tests: Pillai test statistic). Mort: mortalities

	Approx. F	df Numerator	df Denominator	p
Mort	9.10	1	62	0.004
Time	22.83	2	61	<0.001
Mort × Time	17.994	2	61	<0.001

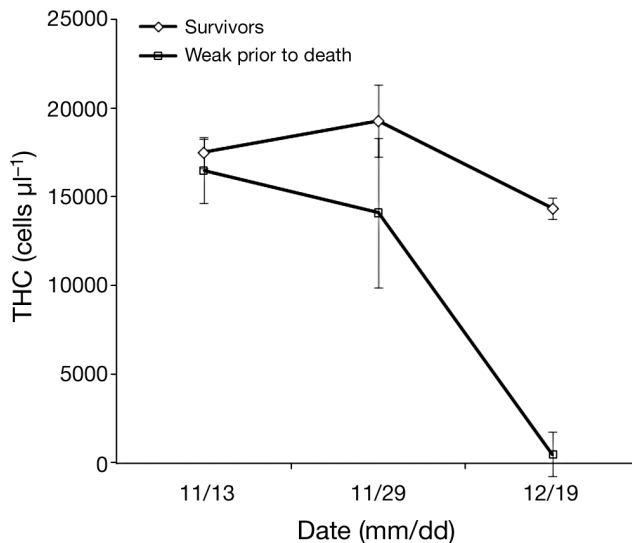


Fig. 2. *Homarus americanus*. Total hemocyte counts (THC; cells μl⁻¹) means ± SE by date of sampling

Analytes

L-lactate. Non-transformed data for L-lactate are shown in Fig. 3a for survivors and in Fig. 3b for mortalities. The ANOVA table comparing the 3 models (data not shown) indicates that the least complex model (no significant interaction) is sufficient to explain the data, which is not analyzable as repeated measures due to an excess of '0' data in the first and third time periods. A simple analysis of variance was performed on the lactate data from 29 November. Residual analysis revealed 1 extreme outlier (studentized residual = 5.97) that substantially reduced the model R² (38% from 52%) and was therefore deleted from the final model. The remaining data were square root transformed to achieve constant variance. Table 4 shows that there are significant differences in mean lactate levels for the effects of 'depth', 'hauling rate', and 'storage' on 29 November, but they are not differentially associated with survival.

Ammonia (NH₃). Non-transformed data for ammonia are shown in Fig. 4a for survivors and in Fig. 4b for mortalities. The data, particularly on 19 December, were skewed because of the presence of several atypically high, but likely true values within the data set, which tend to pull the mean upward. Therefore, the natural log of NH₃ was analyzed to reduce the influence of these atypical values. The ANOVA table comparing the 3 models (ANOVA table not shown) indicates that the least complex model (no significant interaction) is an adequate explanation of the data. The MANOVA (Table 5) shows that the mean levels of NH₃ differ by mortality status and that this difference changes over time. Fig. 5 depicts the changes in hemolymph ammonia means (± SE) over time.

Crustacean hyperglycemic hormone. Non-transformed data for CHH are shown in Fig. 6a for survivors and in Fig. 6b for mortalities. The data were skewed because of the presence of several atypically high, but likely true values within the data set, which tend to pull the mean upward. Therefore, the log of CHH + 0.1 was analyzed to reduce the influence of these atypical values. The ANOVA table comparing levels of complexity between the 3 models (data not shown) indicates that the less complex model (no significant inter-

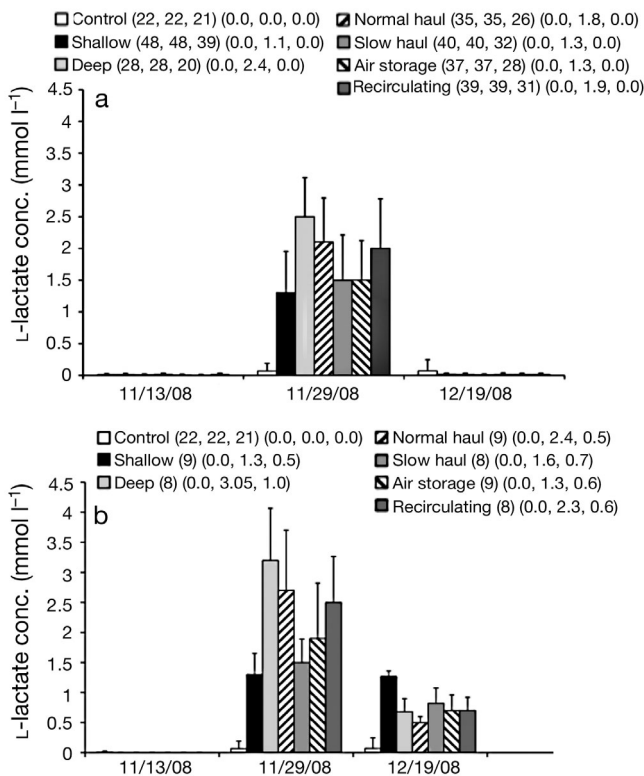


Fig. 3. *Homarus americanus*. Non-transformed mean (+SE) L-lactate (mmol l⁻¹). (a) Survivors. Numbers within the first parentheses: n for each treatment group by date of sampling. Differences in n between 29 November and 19 December 2008 represent mortalities. Numbers in the second parentheses: sample median for each treatment group. (b) Mortalities. Numbers within the first parentheses: n by date of sampling. Numbers within the second parentheses: sample median for each treatment group

Table 4. *Homarus americanus*. ANOVA for L-lactate. Mort: mortalities

	df	SS	MS	F value	p
Mort	1	1.9704	1.9704	5.8883	0.0179
Storage	1	1.8354	1.8354	5.4851	0.022
Depth	1	15.1903	15.1903	45.3954	<0.001
Haulrate	1	6.1437	6.1437	18.3603	<0.001
Residual	69	23.0889	0.3346		

Table 5. *Homarus americanus*. MANOVA for ammonia (Type II repeated measures MANOVA tests: Pillai test statistic). Mort: mortalities

	Approx. F	df Numerator	df Denominator	p
Mort	12.121	1	60	0.0009
Time	179.105	2	59	0
Mort × Time	52.705	2	59	<0.001

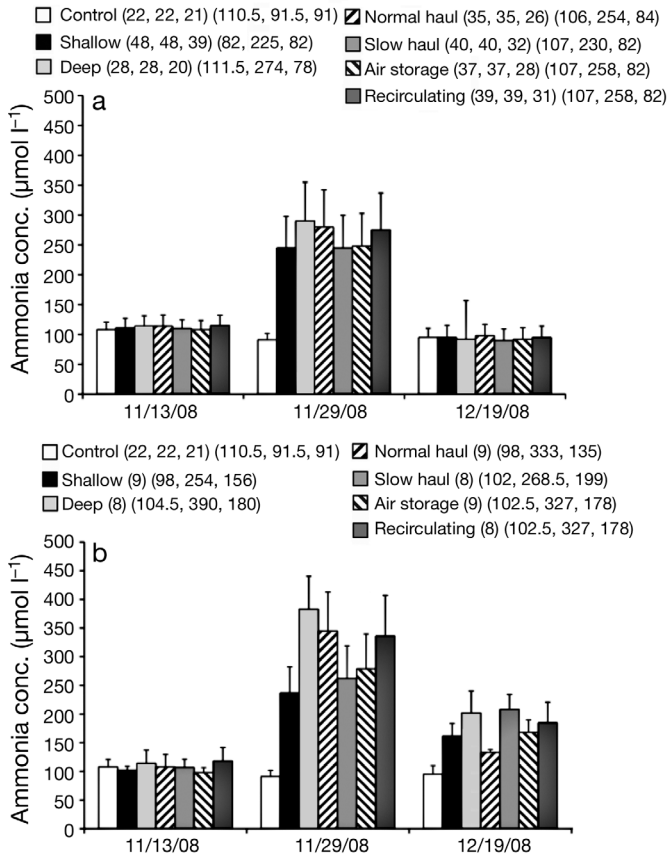


Fig. 4. *Homarus americanus*. Non-transformed mean (+SE) ammonia data ($\mu\text{mol l}^{-1}$). (a) Survivors. Numbers within the first parentheses: n for each treatment group by date of sampling. Differences in n between 29 November 2008 and 19 December 2008 represent mortalities. Numbers within the second parentheses are the sample median for each treatment group. (b) Mortalities. Numbers within first parentheses: n by date of sampling. Numbers within second parentheses: sample median for each treatment group

action) is the simplest model that explains the data. The MANOVA (Table 6) shows that the mean levels of CHH differ by mortality status and that this difference changes over time. Fig. 7 depicts changes in hemolymph CHH means ($\pm\text{SE}$) over time.

Glucose. Non-transformed data for glucose are shown in Fig. 8a for survivors and in Fig. 8b for mortalities. The data were skewed because of the presence of

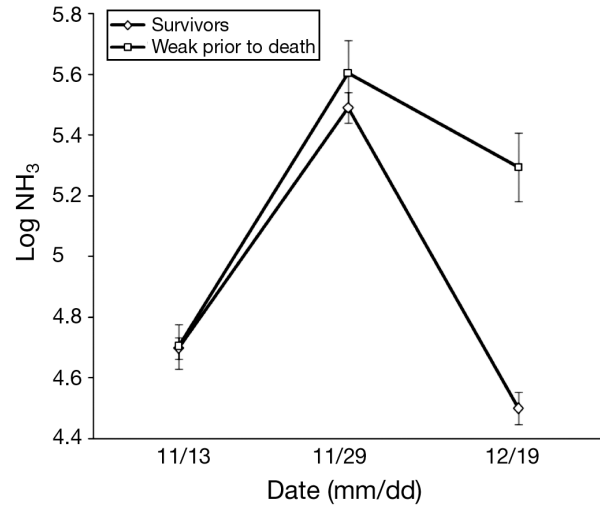


Fig. 5. *Homarus americanus*. Log hemolymph ammonia (NH_3 , $\mu\text{mol l}^{-1}$) mean ($\pm\text{SE}$) by date of sampling

several atypically high, but likely true values within the data set, which tend to pull the mean upward. Therefore, the natural log of glucose was analyzed to reduce the influence of these atypical values. Table 7 shows that Model 3, the least complex, is not sufficient to adequately explain the data. The p-value for comparing Model 1 to Model 2 (0.0629) is close to significant, and a conservative approach would be to continue with the more complex model. Tests for sphericity were rejected so the multivariate approach is presented in Table 8. Fig. 9 illustrates how storage and depth affect alive versus dead lobsters differently. For the lobster stored in air, the changes in glucose level are similar over time, with a severe drop for both alive and dead lobsters on 19 December, whereas for those stored in recirculation, the glucose levels for the dead lobsters stayed constant through time while the levels for the live lobsters dropped at a constant rate. Fig. 10 presents the mean ($\pm\text{SE}$) log glucose levels for the combinations of depth and mortality over time. For shallow water, the alive and dead group means start together and end up apart, whereas for deep water, the mean log glucose levels for alive and dead start apart and end up together.

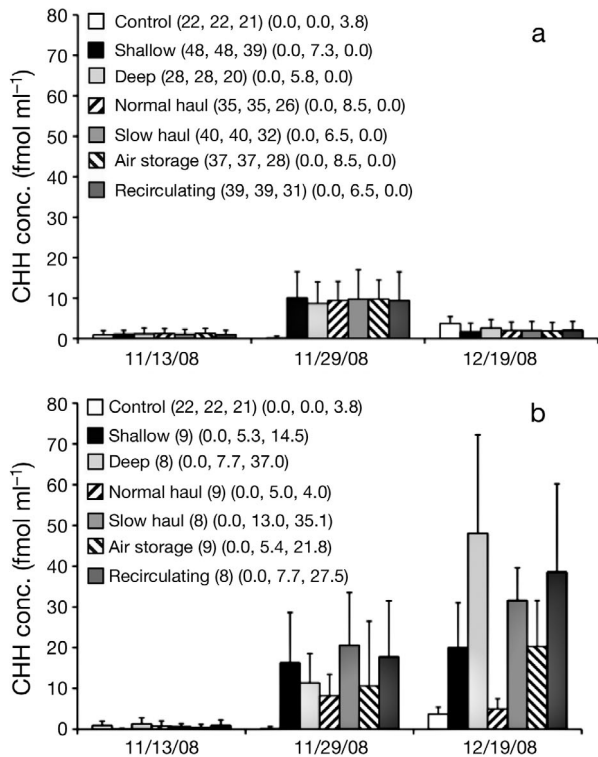


Fig. 6. *Homarus americanus*. Non-transformed mean (+SE) crustacean hyperglycemic hormone (CCH, fmol ml⁻¹) data with means +1 SE. (a) Survivors. Numbers within the first parentheses: n for each treatment group by date of sampling. Differences in n between 29 November 2008 and 19 December 2008 represent mortalities. Numbers within the second parentheses are the sample median for each treatment group. (b) Mortalities. Numbers within the first parentheses: n by date of sampling. Numbers within the second parentheses are the sample median for each treatment group

Table 9 shows mortalities by treatment group and Chi-square analysis. The results suggest a trend in higher mortalities in this experiment for lobsters originating in deep water, hauled to the surface at the commercial rate, and stored above deck in air.

Total hemolymph protein and *in vivo* pH. Both parameters decreased in non-survivors sampled on retrieval day, although the decrease was not significant (data not shown).

Table 6. *Homarus americanus*. MANOVA for crustacean hyperglycemic hormone (Type II repeated measures MANOVA tests: Pillai test statistic). Mort: mortalities

	Approx. F	df Numerator	df Denominator	p
Mort	8.873	1	64	0.004
Time	82.784	2	63	0
Mort × time	17.032	2	63	<0.001

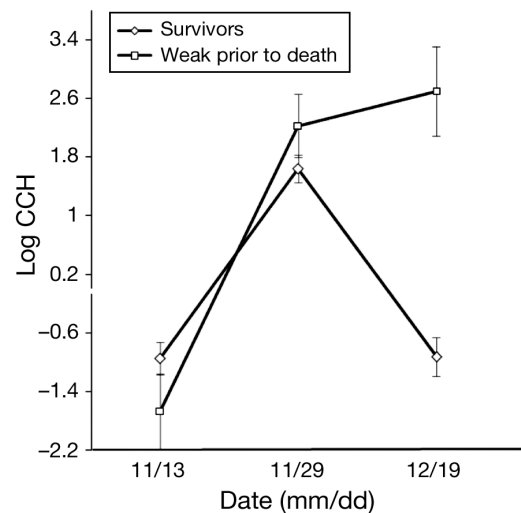


Fig. 7. *Homarus americanus*. Log crustacean hyperglycemic hormone (CCH, fmol ml⁻¹) mean (±SE) by date of sampling

Histology

Large (up to 5 μm in length), Gram-negative (Gram stain not shown), rod-shaped pleomorphic bacteria were found throughout most tissue sections of all weak lobsters (Figs. 11 to 14). There is evidence of antemortem tissue edema and necrosis. The overall pattern of tissue change is suggestive of bacterial septicemia. Unidentified apicomplexans, ciliates, and helminths were observed in low numbers within the intestinal tract and gills of the subsampled survivors.

DISCUSSION

The introduction of high tensile strength synthetic rope in the late 1950s, the widespread use of lightweight plastic-coated wire traps during the 1970s, and the evolution of high-speed fiberglass boat hulls and hydraulic power blocks through the 1980s (Miller 1995) have had a profound effect on the lobster fishery. Today, it is not uncommon for an experienced fisherman and a sternman to haul, bait, and set several hundred traps per day in waters as deep as 150 m, and to bring to market over 400 kg of lobster. The average 1 horsepower hauler utilized on most nearshore lobster boats is capable of raising 90 kg off the seabed at a rate of 98 m min⁻¹. A large offshore hauler can displace 1100 kg of deadweight at a rate of 152 m min⁻¹ (Prybot 2006). Although fishing practices vary, the net result is the same—an increase in fishing efficiency as more traps can be set over a wider geographic area per unit of time.

Stress has been well characterized in terrestrial animals and fish. Stress is recognized as a potential con-

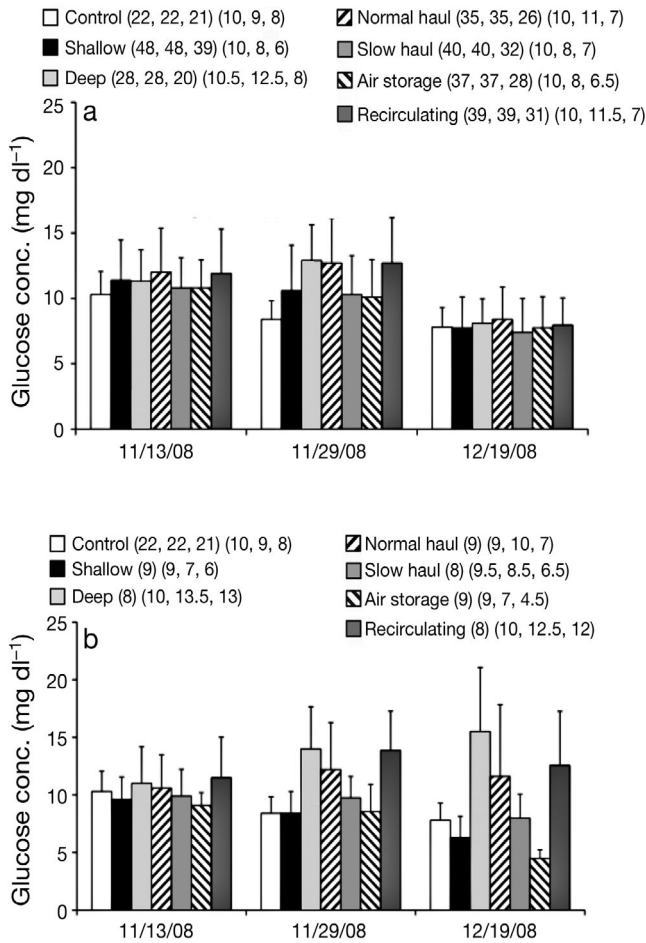


Fig. 8. *Homarus americanus*. Non-transformed mean (\pm SE) glucose data (mg dl^{-1}). (a) Survivors. Numbers within the first parentheses: n for each treatment group by date of sampling. Differences in n between 29 November 2008 and 19 December 2008 represent mortalities. Numbers within the second parentheses are the sample median for each treatment group. (b) Mortalities. Numbers within the first parentheses: n for each treatment group by date of sampling. Numbers within the second parentheses are the sample median for each treatment group

tributor to disease and mortality of lobsters held in temporary storage and to their decreased survivorship during transport (Balcom 2003, Lorenzon et al. 2007).

The stress response in lobsters is a normal physiological reaction to disturbances in homeostatic mechanisms resulting from changes in the environment, injuries, behavioral interactions, or pathogens (Evans 1999). Healthy lobsters can compensate for minor stress of short duration. However, acute or chronic stress can result in irreversible tissue injury and death, or the impairment of defense mechanisms, reproduction, and growth, and predispose lobsters to infection (Lorenzon et al. 1999, Ridgway et al. 2006b, 2007).

The immune system of lobsters consists of a nonspecific but effective first line of defense to control the proliferation and spread of microorganisms within hours of exposure. Circulating hemocytes release prophenoloxidasases that, when activated, participate in the encapsulation and melanization of foreign microbes (Terwilliger 2007). THC is an indicator of stress but tends to vary non-specifically owing to changes in environmental conditions such as temperature, salinity, and other natural rhythms (Gomez-Jimenez et al. 2000, Le Moullac & Haffner 2000). In addition, there is great intra-specific variation in total numbers of cells within a range of 11.0×10^3 to 28.7×10^3 cells μl^{-1} (Cornick & Stewart 1978). Bacterial challenge or injury tends to result in a decreased circulating hemocyte count, as cells are mobilized to the site of attack or exoskeletal breach. As hemocytes encapsulate bacteria at the site of invasion, small aggregates are moved to the gills where they are cleared by fixed phagocytes (Martin et al. 1998, Ridgway et al. 2006a). Although transient bacteremia is not an uncommon finding in *Homarus americanus* (Cornick & Stewart 1966, Bartlett et al. 2008), all lobsters cultured negative for bacteremia at the sampling on 29 November 2008. It is possible that the relative hemocytopenia noted in non-survivors on 29 November (Fig. 2) is more of a response to lipopolysaccharide (endo-toxin) and subpatent infection with *Photobacterium indicum* than a direct result of

Table 7. *Homarus americanus*. Models for stressor analysis with respect to log glucose levels. Mort: mortality

a)				
Model 1: Log glucose ~ Mort \times Storage \times Depth + Mort \times Depth \times Haulrate + Storage \times Depth \times Haulrate				
Model 2: Log glucose ~ Mort \times Storage + Mort \times Depth + Mort \times Haulrate + Storage \times Depth + Storage \times Haulrate + Depth \times Haulrate				
Model 3: Log glucose ~ Mort + Storage + Depth + Haulrate				
b)				
	Approx. F	df		p
		Numerator	Denominator	
Model 1 vs. Model 2	1.85424	9	153	0.0623
Model 2 vs. Model 3	2.98332	18	153	<0.001

Table 8. *Homarus americanus*. MANOVA for glucose (Type II repeated measures MANOVA tests: Pillai test statistic). Mort: mortality

	Approx. F	df Numerator	df Denominator	P
Time	306.118	2	50	0
Mort × Time	1.139	2	50	0.328
Storage × Time	3.795	2	50	0.029
Depth × Time	10.482	2	50	<0.001
Haulrate × Time	2.009	2	50	0.145
Mort × Storage × Time	13.179	2	50	0
Mort × Depth × Time	11.915	2	50	<0.001
Storage × Depth × Time	0.495	2	50	0.612
Mort × Haulrate × Time	1.108	2	50	0.338
Depth × Haulrate × Time	4.89	2	50	0.012
Storage × Haulrate × Time	4.514	2	50	0.016
Mort × Storage × Depth × Time	1.276	2	50	0.288
Mort × Depth × Haulrate × Time	0.458	2	50	0.635
Storage × Depth × Haulrate × Time	4.471	2	50	0.016

stress (Lorenzon et al. 1999). *P. indicum* (Xie & Yokota 2004), a Gram-negative, pleomorphic rod previously identified as a *Vibrio fluvialis*-like bacterium, was implicated as the cause of 'limp lobster disease' that resulted in severe economic loss in Maine during the autumn of 1997 to 1998 (Tall et al. 2003). It is of particular interest that *Aerococcus viridans*, the causative agent of gaffkemia ('red tail' disease), historically reported as a major cause of mortality in impounded lobsters, was not detected in this study or in active disease surveillance in Maine for the last 4 yr (Basti 2008).

Lobsters that are held out of water become hypoxic owing to gill collapse and their inability to maintain ventilation despite much higher levels of atmospheric oxygen. The resulting ventilation-perfusion mismatch results in low tissue oxygen levels, carbon dioxide re-

tention, and a mixed respiratory and metabolic acidosis due to a switch to anaerobic metabolism and L-lactate production (Hammen 1980, Taylor et al. 1997). L-lactate levels rise from low resting levels in the hemolymph (Lorenzon et al. 2007, Basti 2008) and were found to be highly correlated to tissue levels in *Nephrops* (Harris & Andrews 2005b), suggesting that they may also be a good indicator of exertional or hypoxic stress in homarid species. When lobsters are resubmerged and left undisturbed, full recovery from hypoxia should occur by increased oxygen consumption, carbon dioxide off-gassing, and the reoxidation of lactate brought about by an increase in ventilation

and heart rate (Taylor et al. 1997). Hemolymph lactate levels usually return to resting levels within 48 h of reimmersion (Whiteley & Taylor 1992, Lorenzon et al. 2007).

CHH is a diabetogenic neuropeptide synthesized in the medulla terminalis x-organ of the eyestalk, is stored in the sinus gland, and is released via a negative feedback loop with glucose in response to stress and increased energy demand (Chang 2005). It triggers glycogenolysis of glycogen stores in the hepatopancreas and muscle and may regulate lipid metabolism (Santos et al. 1997). The presence of Gram-negative bacteria and lipopolysaccharide may also stimulate CHH release directly or through the action of acute phase cytokines (Lorenzon et al. 1997). During extreme exertion, such as repeated 'tail flipping' escape

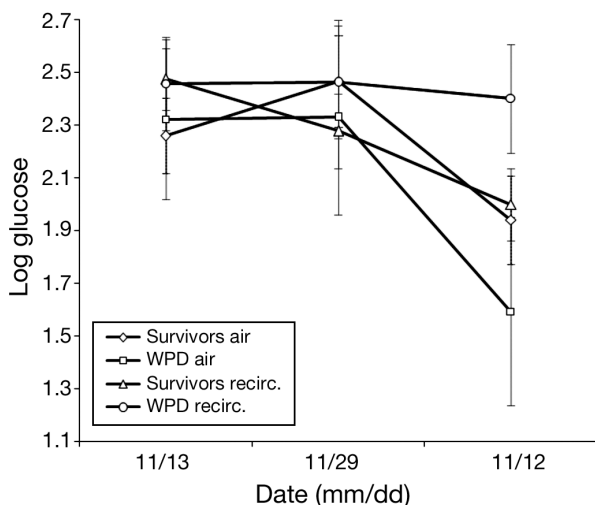


Fig. 9. *Homarus americanus*. Log glucose (mg dl^{-1}) means (\pm SE) for storage and mortality by date of sampling. WPD: weak prior to death; recirc.: recirculating water

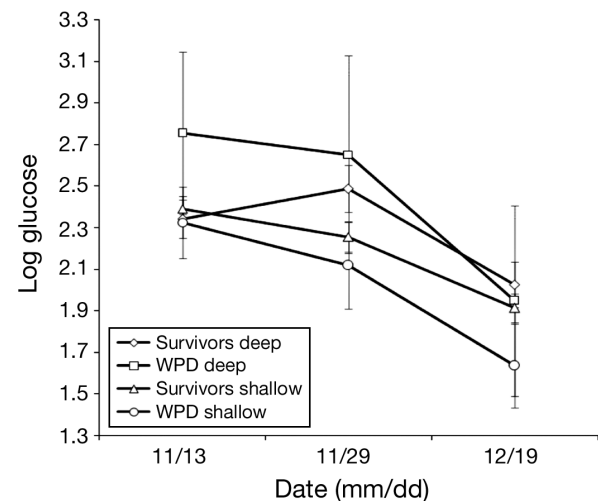


Fig. 10. *Homarus americanus*. Log glucose (mg dl^{-1}) means (\pm SE) for depth and mortality by date of sampling. WPD: weak prior to death

Table 9. Mortalities of lobsters *Homarus americanus* by treatment group and χ^2 analysis

Treatment	Total	Mortalities
S = Shallow site (10.7 m)	48	9
D = Deep site (131.8 m)	28	8
F = Commercial hauling rate (64 to 75 m min ⁻¹)	35	9
SL = Slow hauling rate (~21 m min ⁻¹)	40	8
W = Transport in recirculating sea water	39	8
A = Air transport in totes	37	9
S + F	23	5
S + SL	25	4
S + W	22	4
S + A	26	5
D + F	12	4
D + SL	16	4
D + W	17	4
D + A	11	4
F + W	18	3
F + A	17	6
SL + W	21	5
SL + A	20	3
S + F + W	10	1
S + F + A	13	4
S + SL + W	12	3
S + SL + A	13	1
D + F + W	8	2
D + F + A	4	2
D + SL + A	7	2
D + SL + W	9	2
χ^2 analysis	p	
D × S	0.046	
F × S	0.049	
A × W	0.050	

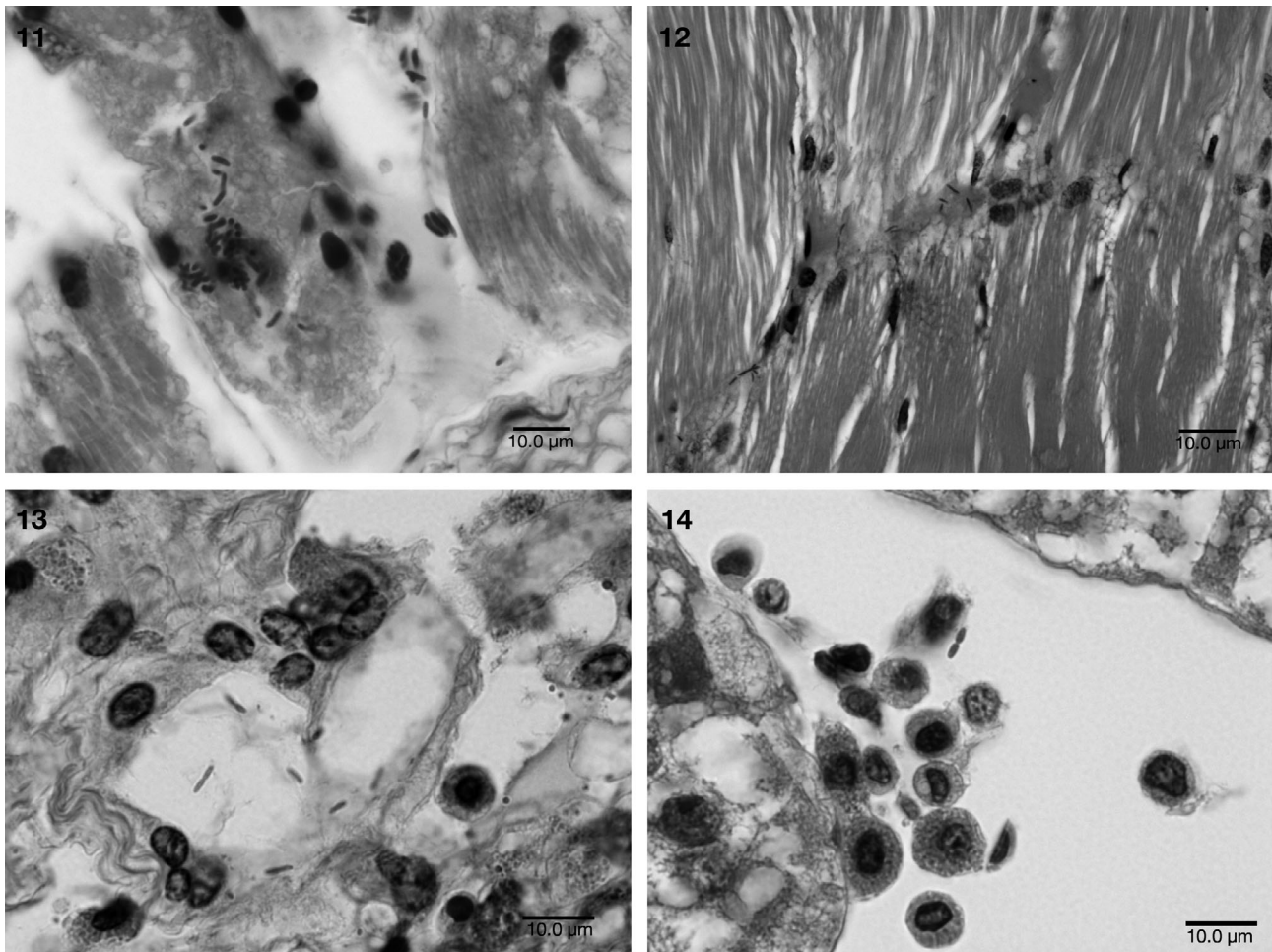
behavior during hauling and handling or emersion hypoxia, lactate accumulation drives the positive feedback of CHH, resulting in exhaustion of glycogen stores as glucose is converted into pyruvate and then lactate, the end product of anaerobic metabolism (Spicer et al. 1990, Ridgway et al. 2006b). The overall effect of handling and short term emersion hypoxia on retrieval day (29 November 2008) is reflected in elevated CHH, lactate, and glucose above control levels. This is a normal physiological response. The effect is significant for lactate in non-survivors that originate from deep water and are hauled to the surface at the commercial rate.

Ammonia is the excretory end product of protein and amino acid metabolism in lobsters and is thought to passively diffuse from the hemolymph across the gill membranes. It is generally held that an increase in hemolymph ammonia above resting levels is indicative of gill dysfunction. Work by Young-Lai et al. (1991)

implies that ammonia may be actively transported across a concentration gradient in an energy-mediated step in exchange for other ions such as sodium. Harris & Andrews (2005a), working with escapee and discarded *Nephrops norvegicus*, proposed that the catabolic breakdown of protein during extreme exertion is an additional energy source reflected in elevated hemolymph ammonia. Although the exact etiology of hyperammonemia on retrieval day is unknown, it tends to be higher in non-survivors and may partially explain the profound neurological deficits noted in weak lobsters prior to death.

A major advantage of this experimental design is that it provides the observer with a retrospective review of physical and physiological parameters that could represent risk factors that may contribute to the prediction of disease or mortality. On the basis of this work, it is apparent that clinically normal lobsters are at equal risk of mortality prior to the onset of stressful conditions, and that there are trends in higher levels of lactate and ammonia in non-survivors originating in deep water and hauled to the surface at the commercial rate. The relatively short-term emersion hypoxia and the intense agitation of below-deck storage in rough seas are the likely cause of the confounding effects noted between the different modes of transport in all analytes except glucose. The significant interaction noted in the MANOVA table for glucose (Table 7) implies that glucose levels in survivors are more likely to return to pre-stress levels in lobsters taken from shallow water and held temporarily in recirculating water. The magnitude of change in glucose levels may distinguish capture-related stress from other forms of stress such as bacterial septicemia and requires further research. It is interesting that the weak lobsters sampled after the experimental retrieval date (29 November 2008) still had significantly higher levels of measured analytes, in particular lactate and ammonia, compared to survivors sampled on the termination date (19 December 2008) of the experiment, despite a return to oxygenated water. This implies a failure in homeostatic mechanisms and/or distress from bacterial septicemia.

Although open wounds and compression fractures resulting in fluid and electrolyte loss must increase the risk of opportunistic infection, this observation alone does not explain similar wounds in long-term survivors. It is possible that internal injuries may be undetected or underestimated. Lobsters are routinely caught in traps set in over 300 m of water in the offshore canyons of the continental shelf (Miller 1995). During the course of this experiment, lobsters retrieved from the deep water site (132 m) at the normal commercial hauling rate (75 m min⁻¹) underwent a 14 atmosphere change in pressure in less than 2 min. Lob-



Figs. 11 to 14. *Homarus americanus*. Photomicrographs of lobster tissue (weak prior to death), showing large, rod-shaped pleomorphic bacteria (H&E stain). Fig. 11. Cardiac muscle. Fig. 12. Deep abdominal flexor muscle. Fig. 13. Connective tissue of intestine. Fig. 14. Hepatopancreatic sinus, with granular hemocytes.

sters lack air-filled sinuses and lungs and so would not be expected to suffer from gas expansion injuries and arterial gas emboli. However, it is not clear if rapid decompression can result in microbubble formation and ischemic necrosis as observed in fish and terrestrial animals. Further research should examine whether nitrogen accumulates or moves between different tissue compartments and the efficiency of nitrogen elimination across the gill membranes (Joiner 2001). There are also differences in the oxygen-carrying capacity of hemocyanin and hemoglobin that may affect susceptibility to rapid decompression injury (McMahon 1995).

In conclusion, the results of this experiment show that depth at capture, hauling speed, and emersion transport increase the relative risk of post-capture mortality in lobsters. However, it is less clear if hypoxia, over-exertion, rapid decompression, or a combination of these factors is responsible for the observed

physiological changes. These findings also raise concerns for the survivability of non-marketable discarded lobsters. Although the pathogenesis of death in this experiment is unknown, there is evidence that concomitant infection with *Photobacterium indicum* may initiate a fatal systemic inflammatory response in the more severely stressed lobsters. This may occur secondary to immunosuppression, intestinal mucosal injury, or through the release of virulence factors (Ridgway et al. 2008). Ongoing work will examine the ecological role of this bacterium and its contribution to decreased survivorship of lobsters during transport and long-term storage. To improve post-capture survival we recommend that the following changes to the current practices be adopted:

- Although lobsters will continue to be hauled at high speed from great depths, they should be afforded a quiescent period in recirculating seawater at ambient temperature to allow for partial recovery from the

effects of high-speed hauling and rapid decompression. Lobsters judged as 'weak' or 'fair' in vigor could be temporarily exhausted from vigorous escape activity during hauling but may recover if reimmersed.

- Care should be taken during handling to avoid injury and over-exertion from repeated 'tail flipping'. Lobsters should be packed within totes in sufficient density to minimize aggressive behavior and shifting. If lobsters are stored above deck in totes, they should be placed parallel to each other in the same direction with the tails curled under to protect the vulnerable ventral abdomen from punctures, and packed in sufficient density to minimize shifting. This should also apply to transport on land.

- Although it may be weight prohibitive, lobster injuries and desiccation may be reduced by packing in moist seaweed. During land-based storage, workers should maintain high-quality water conditions, good circulation, and temperatures at or below 10°C, which may be outside the thermal preference of *Photobacterium indicum*. It is also worth considering that judicious preemptive antibiotic therapy may be necessary to reduce mortality in lobsters susceptible to secondary bacterial infection caused by *P. indicum*.

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