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in female-biased conditions, competition for mates is as strong between females as between males and thus female reproduction may be more limited than suggested by the relative parental contributions of the sexes.

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Potential synergistic stressors trigger a mortal infection in juvenile *Homarus americanus*

From: Michael Tlusty, Don Lightner, Jason Goldstein and Brenda White

The American lobster (*Homarus americanus*) has recently been subject of several severe disease outbreaks. The 1999 Long Island Sound fishery was decimated by paramoebiasis and shell disease to the point that federal monies were necessary to assist in the relief effort (NY CT Sea Grant 2000). It is likely that there is one or more stressors in the environment, be it an environmental change, a toxin or a shift in food resources, which is a major factor in making these animals more susceptible to pathogens (Prince et al. 1995, Sindermann 1989). However, the role of stress in triggering or producing disease in lobsters is poorly elucidated.

Of the 1,669 citations for "stress and disease" found in the NISC Aquatic Biology, Aquaculture & Fisheries Resources database from 1971 to the present (NISC 2000), only 15 were concerned with "lobster". The one published example of multiple stressors influencing the subsequent health of lobsters was discussed by Lavallee et al. (1998). They observed that adults caught using mackerel (Scombridae) as bait were more likely to be graded as "weak" at the processing plant compared to animals caught with other bait types. Their rationale was that unrefrigerated mackerel had high bacterial loadings, and this stressor, coupled to post capture handling stress resulted in significant decreases in health indices. As a compliment to this observation, we present an observation of mortality in captively cultured juvenile lobsters that appear to be a result of the synergistic effect of multiple stressors.

The New England Aquarium (NEAq) has operated a lobster hatchery and rearing facility (LRF) since 1987 that has historically provided quality animals of known larval stage or juvenile age for use by neurobiology and molecular biology researchers (Goldstein 1998, Tlusty unpub. data). Hatchery disease incidents tend to be extremely low and for the most part nonexistent. We attribute this primarily to our efficient system design and life support, consistent, disciplined, and well documented husbandry protocols and cultured animals, which are raised individually as opposed to communally. Because embryos are obtained from the wild, gravid females are put through a strict quarantine process before being integrated into

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the rest of the system. Some of the major life support components that contribute to maintaining low disease incidence includes mechanical seawater filtration to 5 µm, UV sterilization, venturi foam fractionation, fluidized filter beds, and gravity sock filtration (Goldstein unpub. data). The worst we have suffered in the past is a minor outbreak of *Leucothrix mucor*, but the excellent attention to water quality and animal husbandry (Goldstein unpub. data) has made the occurrence of disease a rare event.

In March of 1999, the NEAq-LRF shipped 115 fourth stage (0.5 g average weight) early benthic phase American lobsters to the University of Arizona (UAZ) to test if they were susceptible to White Spot Syndrome Virus (WSSV). These animals were all from a single female. While at the aquarium, these animals were fed live enriched *Artemia* nauplii during stage I and II, then weaned to frozen enriched adult *Artemia* at stage III. At stage IV, the animals were placed in "condo trays", a system used to rear animals individually. Each condo tray measured 12 cm x 24 cm, and was divided into 36 – 4 cm x 2.5 cm compartments, with each compartment holding one animal. These animals all appeared healthy, and the cohort that remained at NEAq-LRF all survived, and grew well.

Pathology

The lobsters were packed for shipping to UAZ in three condo trays, with seven being placed individually in plastic film canisters. They were shipped Federal Express overnight, which is NEAq-LRF's usual methodology. When the animals arrived at UAZ, they had a high prevalence and severity of bacterial infections. These infections were typically presented in lobsters with an atrophied hepatopancreas (HP) with no (grade 0 or G0) stored lipids. Such HPs showed a generalized intratubular hemocytic congestion interspersed with melanized and unmelanized hemocytic nodules (HEN). Rod-shaped bacteria were apparent in the centers of many of these HEN. While the HP lipid content was not a good indicator of disease state, the other signs of these infections were near 100% prevalence in lobsters that died prior to, or early in the study, but absent in lobsters that survived to termination (day 14) of the study. Of the 16 animals histologically examined, three had HP lipid scores greater than G0, with two of these surviving to day 14. However, 10 of the 11 animals that died during the study had severe HP atrophy, while all five that were sampled on day 14 did not exhibit this condition. This result is identical for the presence of HEN where those dying early in the study were HEN positive, and those five sacrificed at the end of the study were HEN negative. Other signs of symptomatic disease, such as susceptibility to protozoan infection, did not demonstrate any significant trend. Three of the five animals sampled at day 14 had G2 or greater loricate protozoan fouling of the appendages, similar to those that died earlier in the study.

This opportunistic observation on disease mortality in American lobsters is instructive since it links multiple stressors to a mortal bacterial infection. However, the multiple stressors cannot be exactly pinpointed. Shipping stress likely involves a change in water quality or elevated temperature, but could also include physical jarring. As for the physical condition of the lobsters, either chronic infection or poor diet can result in HP atrophy. Thus, while a link between physiological status and stress is suggested, we cannot

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implicate a strict causal chain. However, in this case, the prevalence of HP atrophy and G3 HEN in animals that died upon arrival at UAZ suggests that this state was present prior to shipping. The time between arrival and mortality was to brief to allow for HP atrophy and HEN development. The animals that remained at NEAq demonstrated the importance of the shipping stress in advancing the disease state. When the shipping stress was absent, the animals exhibited virtually no mortality

What this observation suggests is that the NEAq-LRF animals had the ability to survive a single stressor, but it was the synergistic combination of stressors that resulted in a disease state. The rod shaped bacteria and disease state most likely implicate vibriosis. *Virbrio* is ubiquitous in the water at NEAq (Dr. B. Turnbull, NEAq veterinarian, pers. comm.). The *Artemia* are cultured at room temperature for 24 to 48H at 22ºC, conditions that are prone to increased bacterial loadings (Dehasque et al. 1991). Thus, even with UV sterilization, the animals are subject to increased bacterial contact. This background loading makes the lobsters subject to severe disease difficulties when appropriate conditions prevail. Any factor to increase stress in an animal, such as shipping, can decrease the animal's ability to defend against this bacterial load leading to a severe disease state.

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