EFFECTS OF AIR EXPOSURE ON DESICCATION RATE, HEMOLYMPH CHEMISTRY, AND ESCAPE BEHAVIOR OF THE SPINY LOBSTER, PANULIRUS ARGUS

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ABSTRACT

Desiccation rates and hemolymph pH, lactic acid and ammonia concentrations of spiny lobsters, Panulirus argus, exposed in air for up to 2 hours were measured. Desiccation rates were faster in smaller lobsters. During a 2-hour exposure, hemolymph lactic acid levels increased more than 11 times, pH decreased more than one-half unit, and ammonia concentrations nearly doubled. Exposure-induced changes in hemolymph parameters occurred most rapidly in the first 30 minutes and began to level off by 2 hours. Lobsters exposed for 2 hours, then reimmersed for 24 hours, survived and had normal hemolymph chemical values. However, 75% of the reimmersed spiny lobsters had a delayed or absent tail-flip escape response; most individuals also exhibited diminished antennal defensive motions. Results suggest that desiccation and hemolymph chemical changes, caused by exposure, do not directly cause mortality, but rather induce secondary physiological damage, manifested as aberrant defensive and escape behavior.

The South Florida fishery for spiny lobster, Panulirus argus (Latreille, 1804), uses sublegal (<76 mm carapace length, CL) lobsters, locally called shorts, as living attractants in traps for legal-sized lobsters. Shorts used in this manner are customarily held in wooden boxes on deck until replaced in traps. Aerial exposure ranges from a few minutes to several hours but is typically about 1 h (Bill Moore). Hunt et al. (1986) reported an average 26.3% mortality rate after 4 wk for lobsters that had been exposed between ½ and 4 h and estimated that 600,000 to 3.7 million shorts die annually as a result of handling and exposure. Because this mortality is incurred by sublegal lobsters which otherwise would soon contribute to legal harvest, economic loss to the fishery is considerable, perhaps as high as $9.0 million annually.

This study examines desiccation rate, hemolymph chemistry, and escape behavior of spiny lobster to document physiological and behavioral changes induced by air exposure. The relationship between these changes and mortality is discussed.

MATERIALS AND METHODS

One hundred seventy intermolt spiny lobsters, averaging 80.2 mm CL (range, 56.7-120.7 mm), were collected from traps at the Atlantic reefs south of Marathon, FL, in the Florida Keys. Approximately 26 lobsters at one time were allowed to acclimate for a minimum of 2 d in a 800 L (179 x 76 x 60 cm) outdoor fiberglass tank. The tank was fully shaded by three plywood sections which could be removed individually, allowing easy access while minimizing disturbance. Flow-through water circulation was maintained by a pump drawing approximately 3,600 L/hour from a clean, well-oxygenated canal. Complete water exchange occurred every 15 min. Periodic canal water samples had oxygen concentrations of 5-7 ppm and no detectable ammonia or lactic acid. There were resident spiny lobsters in the canal.

Shelter inside the tank was provided by a double layer of two-hole cinder blocks (39.5 x 19.5 x 19.5 cm) centered and aligned parallel to the long axis of the tank. This arrangement of blocks allowed for easy removal of spiny lobsters by the antenna-tug technique, described later. In rare instances when a spiny lobster evaded capture on the first attempt, sampling of that animal was postponed for at least 24 h. This was necessary because repeated tail-flips depressed hemolymph pH (unpubl. data). Spiny lobsters were not fed during confinement or held longer than 10 d. Both sexes were used equally.

Desiccation Rate

Spiny lobsters were randomly selected from the acclimation tank at 10 min intervals, marked for in-
individually identified, and alternately assigned to either an exposure or control group.

After marking, control spiny lobsters were weighed to the nearest 0.1 g and promptly placed inside a shaded, wood-slat fish box two-thirds submerged inside the acclimation tank. Weights were also recorded at 1 and 2 h. Excess water clinging to the exoskeleton and inside the branchial chambers was removed prior to each weighing by holding the spiny lobster around the carapace in a head down position and gently moving it through a short downward arc six times. Exposed spiny lobsters were marked and weighed as above, but were held in a fish box located in a fully shaded outdoor area. Evaporative water loss was indicated by weight decrease over time.

During the period when desiccation experiments were performed (late March to early May 1984), relative humidity was 61-72%, air temperature 22-30°C, wind speed 10 km/h or less, and cloud cover ranged from clear to lightly scattered or hazy. Experiments were not performed on very wet or windy days to avoid excessive variation in desiccation rates between experiments.

**Hemolymph Chemistry**

To assess effects of exposure on hemolymph chemistry, spiny lobsters were air-exposed in fish boxes for ½, 1, or 2 h as previously described. Control spiny lobsters were removed directly from the acclimation tank.

Hemolymph sampling was via cardiac puncture. A 1.6 mm (¼-in) hole drilled through the dorsal carapace directly over the heart allowed easy hydrodermic removal of 8-10 mL of hemolymph. There is no suitable chemical method to prevent hemolymph clotting (Young 1972). At ambient temperature, spiny lobster hemolymph forms a tough rubbery clot within seconds. Prompt cooling of the hemolymph by immersion of the syringe in an ice water bath (4°C, 60 s) inhibited clotting long enough to prepare subsamples for pH, ammonia, and lactic acid analysis. All hemolymph samples were collected between the hours of 10:00 and 16:00 and analyzed the same day.

Intervals between netting and completion of hemolymph removal were 70 s or less, thus minimizing trauma associated with handling and cardiac puncture. Since net confinement reduced struggling, spiny lobsters were not removed from the net for hemolymph sampling unless access to the dorsal carapace was restricted. In preliminary experiments, repetitive handling and sampling of controls depressed hemolymph pH values. Consequently, each spiny lobster was sampled only once in experiments reported here. Hemolymph pH was determined by a digital pH meter with a calomel microelectrode. Hemolymph subsamples (2 mL) and a 7.0 buffer solution were chilled to 4°C in a second ice water bath before recording pH. Blood pH at 4°C probably varies from in vivo pH at ambient temperature, but this was an essential concession to retard clot formation. Anaerobic, radiometer-type pH measurements were also impossible due to clotting. However, care was taken to minimize hemolymph air contact since changes in CO2 equilibrium can alter pH values. Truchot (1975) reported the pH of crustacean blood exposed to air without mixing varies little from anaerobically obtained samples.

Serum was prepared by injecting the remaining 6-8 mL of chilled hemolymph into a 15 mL tissue grinder, then gently grinding for 1-2 min until the clotting hemolymph was liquefied. The still cool serum was then refrigerated in capped test tubes for subsequent ammonia analysis.

Ammonia was measured using the Conway microdiffusion method (Conway and Byrne 1938) with modifications suggested by Seligson and Seligson (1961). With this method, ammonia from a 0.5 mL blood sample was diffused onto an acidified glass rod inserted inside a microdiffusion cell. Microdiffusion cells were rotated for 50 min to facilitate diffusion, then the rods were washed off with 5 mL of Nessler’s reagent. Intensity of color developed in Nessler’s reagent, corresponding to ammonia concentration, was measured in a colorimeter at 420 nm. All samples were done in duplicate as were blanks and two concentration standards (10 μg/mL and 20 μg/mL) accompanying each group of unknowns.

Blood serum lactic acid concentrations were determined with a Sigma Chemical Company lactic acid analysis kit (826UV). With this kit, blood lactic acid is converted to pyruvic acid by lactate dehydrogenase, resulting in reduction of an equivalent amount of NAD. Reduction of NAD causes an increase in sample absorbance at 340 nm proportional to the initial lactic acid concentration. The same 2 mL blood sample used to measure pH was subsequently deproteinized with 4 mL of 8% perchloric acid and used in the lactic acid analysis. After 90-min incubation of the reaction mixture at 25°C, absorbance readings were stable, indicating the end point of the reaction. A chemical modification of the

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*Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.*
Sigma kit hydrazine buffer, recommended by Graham et al. (1983) for cases of end point instability, was not used because stable end points were obtained after 90 min.

One group of spiny lobsters was exposed for 2 h and then returned to the acclimation tank for 24 h before sampling to observe whether the blood chemistry changes which I observed immediately after exposure persisted after reimmersion.

**Escape Behavior**

Controls were individually netted, marked, and returned to the tank in less than 1 min. Exposed spiny lobsters were netted, marked, and placed in a shaded fish box for 2 h before being returned to the tank. Twenty-four h later, spiny lobsters were netted from the tank again and escape responses were recorded. A delayed or absent tail-flip after an antenna touch or tug was considered an impaired escape response; an immediate tail-flip was considered normal.

Student’s t-test was used to analyze all desiccation and hemolymph chemical data. Values of \( P < 0.05 \) were considered significant. All data are expressed as means ± 1 SE. Since the control hemolymph chemical values from each exposure interval and from the reimmersed exposed spiny lobster experiment were not significantly different, they were pooled.

### RESULTS

#### Desiccation Rate

Control spiny lobsters, which remained submerged except during weighings, maintained constant weights (Table 1). Percentage of initial weight remaining at the end of 2-h air exposure was 95.30% for shorts and 96.37% for legals, or an average weight loss of 2.35%/hour and 1.82%/hour respectively.

### Hemolymph Chemistry

During a 2-h exposure, hemolymph lactic acid levels increased more than 11 times (from 4.4 mg/100 mL to 49.5 mg/100 mL), pH decreased more than one-half unit (from 7.91 to 7.40), and ammonia concentration nearly doubled (from 7.22 \( \mu \)g/mL to 13.77 \( \mu \)g/mL) (Fig. 1). Exposure-induced changes in hemolymph parameters occurred very rapidly then leveled off. Lactic acid and pH changed more in the first 30 min of exposure than in the subsequent 90 min. Ammonia accumulation was also at its maximum rate during the first 30 min.

All spiny lobsters exposed for 2 h, then returned to the acclimation tank for 24 h before sampling, survived and had normal hemolymph parameters (Fig. 1). Evidently, acute hemolymph effects of exposure (i.e., elevated lactic acid and ammonia, depressed pH) do not persist beyond 24 h.

### Escape Behavior

Nonexposed spiny lobsters defended their positions in the concrete block holes with vigorous antennal movements directed toward an approaching hand until contact was made. Then a tap or, more frequently, a light tug on the tip of one antenna elicited an immediate tail-flip, propelling the lobster backward into the net. This method of removing lobsters from the acclimation tank was 100% effective on nonexposed lobsters.

Although hemolymph parameters of exposed spiny lobsters returned to normal within 24 h after reimmersion, it was evident that defensive and escape behavior of these lobsters was abnormal. In lobsters exposed for 2 h and then reimmersed for 24 h, defensive antennal movements were feeble or absent, and an antennal tap or tug usually failed to elicit an immediate escape response. When it did occur, the tail-flip response required several strong antennal tugs over a 3-4 s period. In some cases, a tail-flip could not be induced by any form of anten-
nal manipulation. The degree of antennal defensive movements was difficult to quantify, so the observation of feeble or absent movements is given here as anecdotal information. None of the immersed (control) lobsters showed an impaired tail-flip, whereas 75% of previously exposed lobsters showed some tail-flip impairment, i.e., tail-flip delayed or absent (Table 2). Controls tested again after 24 h still showed no tail-flip impairment, indicating that observed behavioral aberrations were not caused by netting and were not learned net-avoidance behavior.

**Table 2.**—Escape response (E.A.) impairment 24 h after 2-h exposure. Lobsters with delayed or absent tail-flip response to an antenna tug were considered impaired.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Total no. netted</th>
<th>Normal E.R.</th>
<th>Impaired E.R.</th>
<th>Impaired (%)</th>
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<td>Controls</td>
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<td>0</td>
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<td>15</td>
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<tr>
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<tr>
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<td>20</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
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<tr>
<td>(Second run)</td>
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**DISCUSSION**

Aquatic organisms suffer water loss and other stresses during air exposure. Inability to ventilate gills may lead to hypoxia, anaerobic metabolism, and accumulation of toxic metabolites. Intertidal crustaceans, which are periodically exposed to air, have behavioral, anatomical, and physiological adaptations that moderate deleterious effects of exposure. The barnacle, *Pollicipes polymerus*, easily recovers after a 40-50% water loss (Fyhn et al. 1972). Barnacles can reduce their metabolic rate during emersion and tolerate extended periods of anaerobiosis (Barnes et al. 1963). The stone crab, *Menippe mercenaria*, can survive severe hypoxia for at least 12 h at 28°-30°C and can tolerate high levels of hemolymph lactate (Albert and Ellington 1985). Crustaceans that have made a permanent transition from water to land have even more elaborate anatomical modifications of the gills and branchial chambers, including a reduction in gill number, volume, and area, which presumably minimize desiccation effects, and strongly sclerotized and ridged gills which do not collapse in air (Pearse 1950; Gray 1957; Edney 1960). Enhanced exoskeletal resistance to water loss is also a common adaptation of semiterrestrial and terrestrial crustacean species. Aquatic decapods in air lose 3-5 times as much water as do terrestrial decapods (Herreid 1969).

The spiny lobster lives subtidally throughout its life cycle and is only exposed to air as a byproduct of present fishery practices. Because there has been no selective pressure to evolve behavioral, anatomical, or physiological adaptations to aerial exposure, tolerance by spiny lobster should be low. Present results support this contention. Exposing spiny lobsters for even relatively short periods results in metabolic acidosis, accumulation of the toxic excretory product, ammonia, and impairment of defensive and escape behavior after reimmersion.
Desiccation Rate

Desiccation rate results are only valid for the range of weather conditions previously specified. Higher temperature and wind speed increases desiccation rate whereas higher relative humidity decreases it.

Because rate of water loss is directly proportional to surface area, smaller spiny lobsters, with higher surface area to volume ratios, lose water at a faster rate. If desiccation is indeed a major stress factor, smaller (sublegal) spiny lobsters will be more affected.

This size-desiccation rate relationship has also been noted by other investigators. Lazo-Wasem (1984) reported that smaller terrestrial amphipods, Arcitalitrus sylvaticus, lost water faster than did larger amphipods; he suggested a higher surface area to volume ratio and higher respiratory rate of smaller amphipods as two possible explanations. Davies (1969) reported that rate of water loss in a limpet, Patella sp., varied inversely with body weight. Price (1980) reported similar results in an intertidal snail, Melampus bidentatus.

Spiny lobsters exposed for 2 h lost only 3.6-4.7% of their initial weight, so it is unlikely that simple dehydration is a major source of exposure stress. This conclusion is supported by experiments showing that periodic wetting of spiny lobster with seawater during exposure did not improve survival (Hunt et al. 1986). McLeese (1965) also reported that continuous sprays of seawater did not increase survival of air-exposed northern lobsters, Homarus americanus. It has been suggested that gill damage caused by dehydration may contribute to documented mortality in exposed western rock lobsters, Panulirus cygnus (Anonymous 1980), but this has not been demonstrated.

Hemolymph Chemistry

Subtidal crustaceans are unable to extract oxygen effectively from air. In Cancer productus, gas exchange rate is reduced fivefold in air (deFur and McMahon 1978). The European lobster, Homarus vulgaris, only extracts one-seventh as much oxygen from air as from water (Thomas 1954). It has not been reported how much aerial respiration P. argus can achieve, but the rapid transition to anaerobic metabolism during exposure indicates oxygen extraction from air is not adequate to support normal aerobic metabolism. Although gill baiers continue their paddle-like motions in air, loss of fluid support for gill filaments causes them to collapse (pers. obs.).

Loss of gill surface area for gaseous exchange, coupled with a probable reduction in gill baier efficiency in air, leads to the hemolymph chemistry changes observed. Lactic acid, the primary product of crustacean anaerobic glycolysis (Albert and Ellington 1985) accumulates in quantities sufficient to overwhelm protein and bicarbonate-carbonic acid hemolymph buffering. Taylor and Wheatly (1980) reported a 0.44 unit drop in arterial pH for the crayfish, Austropotamobius pallipes, after 3 h of air exposure. They attributed this acidosis to a tenfold increase in hemolymph lactate and to accumulation of CO₂. Organisms generally regulate pH precisely, because a high or low pH can disrupt enzymatic reactions, ionic/osmoregulatory control, and cell membrane stability (Prosser 1973).

Jonas et al. (1962) found a close link between blood pH and mortality in trout. Death resulted when blood pH was lowered with either dilute lactic acid or hydrochloric acid from a normal mean pH of 7.3 to 6.8-6.9, a decrease of 0.4-0.5 units. Fatalities did not result when injection of the same quantity of either acid did not lower blood pH into this 6.8-6.9 range. This indicates that acidosis was the cause of death rather than the acids themselves. Spiny lobsters exposed for 2 h experienced a similar 0.5 unit drop in pH (7.91-7.40). Lobsters evidently have a higher tolerance for acidosis than do trout, since a 2-h exposure was not immediately lethal. However, a pH change this large must be considered a large physiological perturbation. Acidosis may also compound oxygen extraction problems, since hemocyanin oxygen affinity decreases as pH falls. Alternatively, because lactate increases hemocyanin oxygen affinity (Truchot 1980; Mangum 1983), these effects may offset each other.

Crustaceans do not have efficient systems for metabolizing lactate, so its removal from hemolymph is relatively protracted (Ellington 1983). Bridges and Brand (1980) subjected six species of crustaceans to 5-8 h of hypoxia and observed that intertidal and burrowing species returned to near normal hemolymph lactate levels much faster (4-6 h) than subtidal, nonburrowing species (20-24 h). They suggested that species more likely to encounter hypoxia in their natural environments are better adapted for removing accumulated lactate when aerobic conditions return. The spiny lobster, as a subtidal, nonburrowing species, probably removes lactate slowly even though normal concentrations were restored within 24 h.

Spiny lobsters are ammonotelic and eliminate ammonia by diffusion from the gills into the respiratory stream and out into the water. Removing spiny
lobsters from the water eliminates the respiratory stream and the normal excretory route for ammonia. This toxic product of protein catabolism can then accumulate in the hemolymph.

Binsn (1969) reported 16 \( \mu g/mL \) of ammonia in blood of freshly captured shore crabs, *Carcinus maenas*. Spaargaren (1982) reported blood ammonia concentrations between 4 and 9 \( \mu g/mL \) for this same species and also provided evidence for a close connection between ammonia excretion and extracellular ion regulation. Florkin (1960) reported average blood ammonia concentrations for 12 aquatic decapods to be 18 \( \mu g/mL \), range 4-25 \( \mu g/mL \). Normal hemolymph ammonia concentrations (7.22 \( \mu g/mL \)) for spiny lobsters are toward the low end of this range. After 2-h exposure, hemolymph ammonia concentrations for spiny lobster increased to 13.77 \( \mu g/mL \). It is not known if this concentration is toxic; however, hypoxia has been reported to increase toxic effects of ammonia in minnows (Wührmann 1952), rainbow trout (Downing and Merkens 1955), and mice (Warren and Schenker 1960). Exposure-induced hypoxia in the spiny lobster may interact synergistically with ammonia, leading to toxic effects at concentrations that would not normally cause problems.

Ionized ammonia (\( NH_4^+ \)) is less toxic than unionized ammonia (\( NH_3 \)) because of its lower tissue permeability (Warren and Nathan 1958). A decrease in hemolymph pH, as occurs during exposure, would shift the chemical equilibrium toward the less toxic \( NH_4^+ \) (Warren and Schenker 1962). Exposure-induced acidosis may afford some protection against ammonia toxicity by this mechanism if ammonia does indeed reach concentrations toxic to spiny lobsters. Ammonia, which functions as a base, may decrease pH in hemolymph and also partially offset the pH decrease caused by lactic acid.

**Escape Behavior and Conclusions**

All 32 spiny lobsters exposed for 2 h and then reimmersed were alive after 24 h and had normal hemolymph chemical values. Apparently the acute effects of exposure (acidosis, ammonia, and lactic acid accumulation) do not directly cause the increased mortality reported in previous studies (Lyons and Kennedy 1981; Hunt et al. 1986). Rather, secondary physiological damage, persisting after acute effects have vanished, may be the ultimate cause of mortality. Persistent physiological damage was manifested as aberrant defensive and escape behavior.

Spiny lobsters with diminished antennal defensive movements and tail-flip escape responses would be at increased risk from predators. Brown and Caputi (1983) observed that western rock lobsters, *Panulirus cygnus*, exposed to air for \( \frac{1}{2}-2 \) h were generally less active, slower in seeking shelter, incapable of defense, and more subject to attack by finfish and octopus.

Exposure effects severe enough to disrupt a basic reflex such as the tail-flip may also affect integrated nervous system functions such as feeding, locomotion, and social and sexual behavior. Nervous tissue is particularly susceptible to damage from hypoxia (Prosser 1973) and from fluctuations in osmotic and/or ionic concentrations of body fluids (Treherne 1980). Nervous system damage induced by hypoxia, acidosis, and perhaps osmotic imbalances is likely the cause of behavioral aberrations in exposed spiny lobsters.

Because the transition to anaerobic metabolism and resulting hemolymph changes occur so rapidly after emersion, the threshold at which physiological effects appear may be no more than a few minutes exposure. Fishery practices which allow exposures of 1 h or more must therefore be producing large numbers of spiny lobsters that are physiologically and behaviorally impaired.

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