Righting Response and Escape Response in Opsanus tau Are Temperature Dependent

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We sought to establish baseline values for two basic reflex behaviors and for ventilation rate in the toadfish, Opsanus tau, over a range of temperatures. Righting response and escape response were chosen as measurable reflexes indicative of activity level in these animals.

Between September, 1996, and January, 1997, ten fish were held in an isolated tank at ambient temperature in the Marine Resources Center at the Marine Biological Laboratory in Woods Hole, Massachusetts. The animals were subjected to a series of behavioral tests as the ambient water decreased in temperature. Fish were acclimated a minimum of 24 hours at each temperature before testing.

Tests were conducted in an experimental tank, 65 cm wide \( \times \) 100 cm long \( \times \) 10 cm deep, and filled with water at the same temperature to which the animals had been acclimated. Trials were recorded with a video camera mounted on a tripod above the tank. During testing, a single animal was placed in the tank, and righting response, escape response, and the ventilation rate were quantified consecutively.

The righting response was measured as follows. A fish was held ventral side up for several seconds and then released. The animal could then attempt to right itself. These trials were scored on an incremental scale of five levels based on the number of attempts required by the animal to right itself and on the effort exerted (4, successful on first attempt with accessory tail movement; 3, successful on first attempt with minimal tail movement; 2, successful after multiple attempts; 1, unsuccessful attempts; and 0, no attempts).

The escape response was quantified by probing and pinching the animal posterior to the last dorsal spine with toothed forceps. Three levels of pressure (probe, hold, hold, and pull) were applied to all animals with a 30-s recovery period between stimuli. From these three trials, a single escape response score was generated. Escape responses were divided into high velocity (>20 cm/s) and low velocity (<20 cm/s) responses. The stimulus of least force to which the animal responded at high velocity was the basis for scoring. If the animal did not exhibit a high velocity response to the first two levels of stimulus, the score was determined using the strongest stimulus applied (animal held and pulled with forceps). In general, scoring, using an incremental scale, was based on response velocity relative to the type of stimulus eliciting that response: 4, escape velocity \( \geq \) 20 cm/s when touched with forceps; 3, same velocity when held with forceps; 2, same velocity when held and tugged with forceps; 1, velocity < 20 cm/s when held and tugged; and 0, no escape response).

At the conclusion of the test, following a one-minute recovery period, we determined the ventilation rate by counting opercular movements for one minute. Afterwards, the fish were placed in a holding barrel until tests had been completed for all animals. These procedures were repeated for the same ten animals at seven temperatures between 12 and 2°C.

Activity levels for all tests show decreases with temperature. Also, an activity threshold in the toadfish appears at 3°C. At this temperature, activity levels begin dropping rapidly to an

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Physiological Stress Elevates Hemolymph Levels of Methyl Farnesoate in the Green Crab Carcinus maenas

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Methyl farnesoate (MF), a sesquiterpene from crustaceans, is structurally similar to insect juvenile hormone. Thus it may have a role in regulating crustacean metamorphosis, reproduction, and behavior \((1, 2)\). In preliminary studies, we measured the levels of MF in the hemolymph of several crustaceans daily and at different times during the molting and reproductive cycles. We found that MF levels often were elevated in animals that had been stressed by handling or disease. In this study, we have applied several types of physiological stress to green crabs and have examined the effects of these stressors on MF levels.

Green crabs, Carcinus maenas, were collected from wild stocks around Woods Hole, Massachusetts, and were maintained in running seawater (approximately 32 ppt salinity, 22°C, and saturated with \(O_2\)). The effects of changes in salinity, temperature, and oxygen concentration were tested in these experiments, and the severity of these stressful conditions was limited to that typically encountered by the organism in its environment \((3)\). None of the conditions had any long-lasting adverse effect on the animals \((4)\); some animals were monitored several days after their return to running seawater; MF levels had returned to control levels). Only intermolt males were used. To test the effects of salinity and temperature, animals were acclimated for at least 10 days \((\text{acclimation times reviewed in } \text{(4)})\); both control and treatment animals then were transferred \((t = 0)\) directly into recirculating, temperature regulated tanks with a biological filter and containing water at the appropriate salinity and temperature. Between 25 and 100% of the tank was exchanged daily, depending upon the number of crabs in a tank. Both control and treatment animals were removed and analyzed after the same exposure times. For hypotonic stress, animals were exposed to seawater diluted to 5 ppt with deionized water. For temperature stress, the water in the tanks was warmed to 32°C or chilled to 14°C. To test the effects of anoxia, animals were placed in a covered 1 or 2 liter flask containing seawater saturated previously for 30 min with nitrogen gas; saturation was maintained by bubbling with nitrogen throughout the experiment. Control flasks were bubbled with air. Oxygen concentration in treatment flasks was approximately 0.25 ppm, while that in control flasks was approximately 7.0 ppm. Hemolymph levels of MF were determined by HPLC as previously described \((5)\).

Three physiological stresses \((\text{increased temperature, anoxia, and decreased salinity})\) were followed by a significant \((p < 0.05)\) increase in the level of hemolymph MF \((\text{Table I})\). In contrast, cooling had no effect on MF levels, and transfer of animals acclimated to dilute seawater \((10 \text{ ppt salinity})\) to full-strength seawater was followed by a drop in MF to basal levels. Significant increases in MF levels were observed in both control and treatment animals during the first hour of the experiment \((\text{data not shown})\); this might be due to stress associated with handling. In any event, MF levels in controls typically returned to background levels by 2 h after exposure. MF levels in treatment animals exposed to increased temperature or anoxia increased significantly over levels in control animals in less than 2 h, but data were not reported because of the effect on controls during this time. While anoxia and increased temperature elicited increases in MF levels within 2 h, a significant

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