# MELATONIN MODULATION OF TIDE-ASSOCIATED PROCESSES IN THE GREEN SHORE CRAB, *CARCINUS MAENUS*

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Intertidal crustaceans respond to a variety of environmental cues involved in the entrainment of their tide-based, solar day, and seasonal cycles. These cycles can be observed in locomotor activity, cardiac regulation, neural activity, metabolism, respiration, endocrine function, and numerous other processes. While much is known about entrainment factors (e.g. temperature, hydrostatic pressure, salinity, photoperiod), little is known about the biological clock mechanisms in these animals. The eyestalks appear to be the location of both the environmental sensors and the biological clocks driving the rhythms (Naylor, E. and Williams, B.G., J. Exp. Biol. 49:107-116, 1968); however, the neural and/or endocrine transmission of this information has not yet been determined.

We have previously found that melatonin levels oscillate in the eyestalks of the fiddler crab *Uca pugilator*, with two peaks approximately 12 hr apart occurring in a 24-hr period (Tilden, A.R. et al., J. Pineal Res. 23:142-147, 1997). This cycle persisted in constant conditions, indicating a possible endogenous tide-based rhythm. We have also shown that melatonin affects a variety of processes in *U. pugilator* such as limb regeneration (Tilden, A.R. et al., J. Pineal Res. 23:142-147, 1997), hemolymph metabolite levels (Tilden, A.R. et al., J. Exp. Zool. 290:379-383, 2001), and locomotor activity (Tilden, A.R. et al., J. Exp. Zool. *In press*). These findings suggest that melatonin may be involved in the transduction of tidal and/or photoperiodic information. *U. pugilator* is an intertidal crab that is active nocturnally and during low tides. We further explored the role of melatonin in crustacean physiology and behavior in a comparative study using *Carcinus maenus*, an intertidal crab that is active nocturnally and during high tides.

STUDY 1. We monitored locomotor activity in response to melatonin injection (400 ng/g) in intact vs. eyestalk-ablated crabs. Eyestalk-ablated crabs were allowed to recover from surgery for 4 days before use in the study. Crabs were entrained in the laboratory to the natural Maine coast tide cycles and then placed in individual containers with $22^0$C water (high tide) or no water (low tide), at $22^0 \pm 4^0$C air temperature. The use of $22^0$C water was necessary to prevent direct temperature effects of inundation. Animals were video-monitored for 1 hr at various times of day. We found that, as expected, activity increased during high tides (Fig. 1). Unexpectedly, activity was significantly higher during a photophase than a scotophase high tide in intact animals. Eyestalk-ablated animals displayed overall reduced activity but still responded moderately to tidal inundation.

Next we injected melatonin or saline into intact or eyestalk-ablated crabs at various times during a 24-hr period. Melatonin significantly increased activity during high tides but not during low tides in intact animals (Fig. 2); these results are similar to our findings in *U. pugilator*. Activity in eyestalk-ablated, melatonin-treated crabs was not different from that of eyestalk-ablated saline-treated crabs in initial studies.

STUDY 2. Baseline hemolymph lactate and glucose levels were measured in intact and eyestalk-ablated crabs in response to tides and photoperiods. Crabs were placed in wire cages in the shelter of rocks and seaweed at the mid-tide point, such that inundation and emersion cycles were of equal duration and centered on high and low tides. Hemolymph samples were collected from 10 different crabs every ~3.1 hr during a complete tide cycle. Two tide cycles were studied: one with high tides centered at mid-photo and mid-scotophase, and a reverse cycle with low tides centered at mid-photo and mid-scotophase. Lactate levels in intact crabs were elevated during times of high tides in both tide cycles (Figs. 3 and 4). These results presumably reflect anaerobic metabolic processes that occur during bouts of locomotor activity. Hemolymph glucose levels were elevated during high tides occurring in both mid-photophase and mid-scotophase (Fig. 3) but not during a late-photophase high tide (Fig. 4). Glucose levels were also increased during a scotophase low tide; in fact, these levels were greater than at high tides. We hypothesize that both high tides and scotophase induce a hyperglycemic response, and that the low tide response is greater because of decreased metabolism of glucose during inactive periods. Eyestalk-ablated crabs had overall reduced levels of both lactate and glucose, reflecting their behaviorally inactive state and removal of eyestalk crustacean hyperglycemic hormone (Fig. 5). Glucose levels did not change over time; however, lactate levels cycle out of phase with those of intact crabs. Preliminary analysis shows that melatonin has hyperglycemic effects in *C. maenus*, as in our earlier studies in *U. pugilator*.

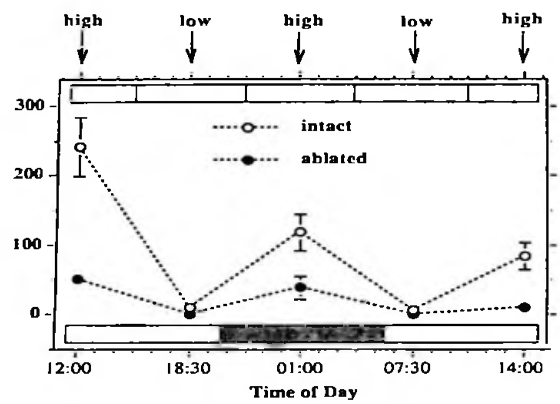



Figure 1. Locomotor activity of eyestalk-intact and –ablated *Carcinus maenus* in response to tide and photoperiod; each point represents the mean ± SEM of 8 crabs. Upper light and dark bars represent high and low tides, respectively; lower light and dark bars represent photo- and scotophase, respectively.

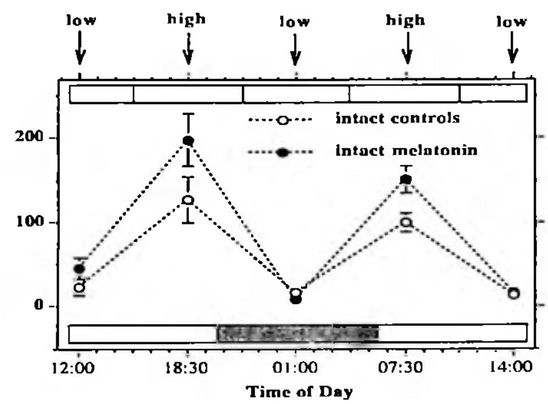
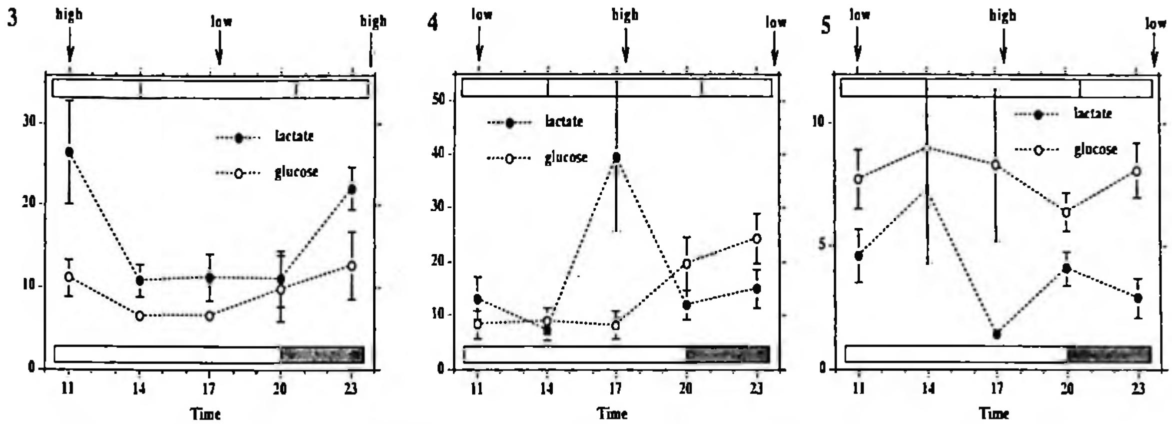



Figure 2. Locomotor activity of intact *C. maenus* in response to injection of melatonin or saline; each point represents the mean ± SEM of 8 crabs. Graphic representation as in Fig. 1.

STUDY 3. We monitored cardiac activity in intact and eyestalk-ablated *C. maenus* during photophase low and high tides and in response to melatonin. Crabs were acclimated to tide-based inundation cycles as in Study 1; they were then cold-anesthetized, and electrophysiological recordings were made through a small opening in the dorsal carapace. Intact crabs had elevated mean heart rates during high tides and decreased rates during low tides (Fig. 6). Eyestalk-ablated crabs had overall reduced heart rates, with no significant difference between high and low tides. Melatonin caused no significant change in heart rate in intact crabs but caused a significant increase in rate over 40 minutes in eyestalk-ablated crabs, whereas melatonin did not alter the heart rate in control ablated crabs (Fig. 7).




Figures 3-5. Hemolymph lactate and glucose levels in intact (3 & 4) and eyestalk-ablated (5) *C. maenus* in response to tide and photoperiod. Graphic representation as in Fig. 1.

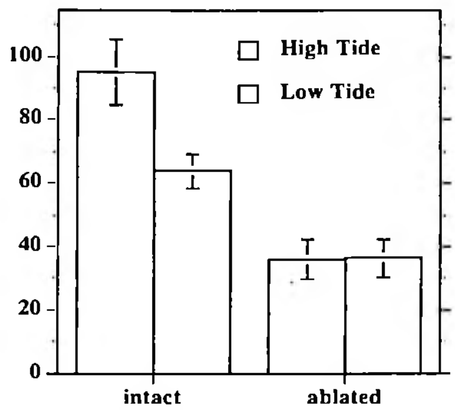



Figure 6. Heart rates of intact and eyestalk-ablated *C. maenus* at high and low tides. Each bar represents the mean ± SEM of at least 10 crabs.

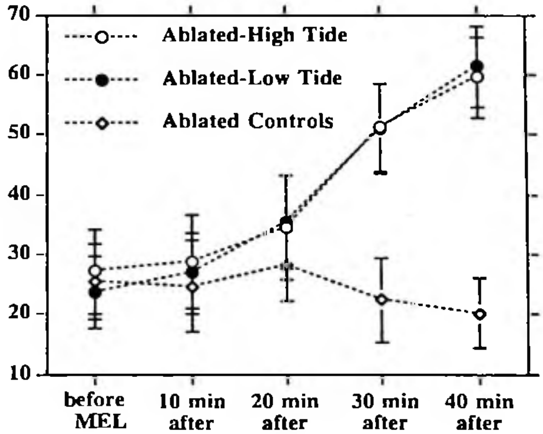



Figure 7. Heart rates of eyestalk-ablated *C. maenus* at low and high tides, in response to melatonin. Each point represents the mean ± SEM of at least 10 crabs.

Overall, these studies provide evidence for the role of melatonin in the modulation of tide- and photoperiod-variable processes. Melatonin appears to have high tide, activity-enhancing effects in *C. maenus*; it has similar effects in *U. pugilator*, except that these effects occur during low tide when this species is more active.

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