SALINITY EFFECTS ON AQUATIC SURFACE RESPIRATION IN *FUNDULUS HETEROCLITUS* DURING HYPOXIA

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Many fish utilize aquatic surface respiration (ASR) during hypoxia, since normally there is more oxygen at the surface. This respiration supports their metabolic demands, including the energy necessary for osmoregulation. Since killifish inhabit estuaries, they have access to a variety of salinities which range from fresh water (FW) to salt water (SW), extremes which require osmoregulatory work. These fish also have access to all mixtures of FW and SW, including isoosmotic water (ISO), in which osmoregulatory work should be minimum. In this water (salinity ≈ 10 ppt), oxygen consumption should also be minimum, assuming that osmoregulatory work stimulates respiration rather than diverting energy from other uses. Since most of the (passive) osmotic water flux is thought to occur through the gills, conditions which force increased ventilation should increase the osmoregulatory work load. In particular, decreasing the available oxygen from 21% (air) to 5% would require a 4-fold increase in ventilation to maintain a constant oxygen uptake rate. In fact, *F. heteroclitus* decreases its respiratory rate by 50% in response to this degree of hypoxia, so the ventilation increase is only 2-fold, implying prioritizing of energy utilization under oxygen stress. We have so far been unable to demonstrate a decrease in respiratory rate in isotonic water, which is also consistent with prioritization of energy utilization.

When experiments were performed in transparent chambers in a water bath at 10.5 °C with an underwater camera recording the fish movements, ASR was indeed seen in hypoxia. Unlike natural conditions, there is no more oxygen in surface water than in the bulk solution, so this behavior does not provide more oxygen under laboratory conditions. To investigate the possible effects of salinity on this response, three tall-form 1 liter beakers containing 500 ml of artificial sea water, fresh water, and a 10 ppt mixture (ISO) were placed in the water bath with the camera. They were gassed with air for 24 hours, with 5% O_2 in N_2 for 24 hours, and returned to air for the final day, with continuous time lapse VCR recording (1 frame/1.2 sec) with the underwater camera. A single frame is shown as Figure 1. On playback with a freeze-frame VCR, fish head positions were recorded as above or below the center of the beaker, for each frame during the central 5 minutes of each hour. These data are shown in Figure 2. Note particularly that while hypoxia immediately stimulates (futile) attempts at ASR in fresh water, and later in salt water, the isotonic fish seem not to feel hypoxic stress, and

Figure 1. Single frame from video recording, fish in 5% O_2 in (left to right) sea water, isotonic water, and fresh water. One fish in SW and two in FW are showing ASR at this moment.

do not respond. The time-lapse video recording was later analyzed by playing the recording into a small-screen monitor with three $CdSO_4$ photocells taped to the faceplate to record excursions of the fish into the upper part of the beakers by the decrease in light intensity. The result is shown in Figure 3, and shows excellent agreement with the manual method.

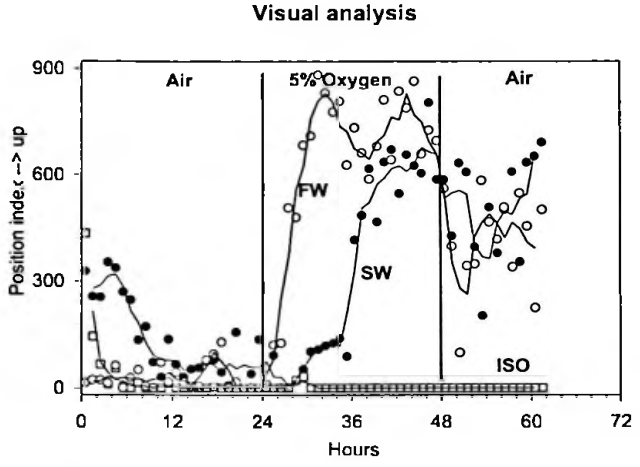

Figure 2. Fish head position vs time. Points are positions of the 3 fish in each solution, summed for 5 minutes (300 sec) of each hour; thus minimum value is 0 and maximum is 900 fish·seconds. (The recording was terminated when the illuminating lamp failed.)

Therefore, although we have not been able to demonstrate changes in oxygen consumption due to salinity changes, it appears that the fish do not respond to this degree of hypoxia when in isoosmotic water. This implies that hypoxia is not as stressful when osmoregulatory work is minimized.

The experiments reported above were conducted on November 18 – 20, 2002, using fish maintained in flowing sea water of gradually falling temperature, which had reached 9 °C by this time. Repeating this experiment in December and January, with fish acclimated to 1 – 2 °C gives rather different results. It is clear that these cold acclimated fish are responding differently from fish held at warmer temperatures. One might have expected this from the variation in plasma osmolality due to temperature, since warm-water (summer) fish in salt water have a plasma osmolality of 368.0 ± 2.7 mOsM (Kidder, G. W., Bull. MDIBL 37:79, 1998) while killifish in cold (3 °C) sea water measure 464.5 ± 3.8 by the same technique (David Petzel, personal communication). Likewise, osmoregulation measured by weight changes is sensitive to acclimation temperature (Kidder, G. W. Bull. MDIBL 40:76, 2001).

It is clear that for these and many other experiments, in this laboratory and others, more attention must be paid to the temperature and salinity conditions under which the fish are maintained prior to the experiment. (Supported by NSF C-RUI 0111860 to RLP, GWK and CWP.)

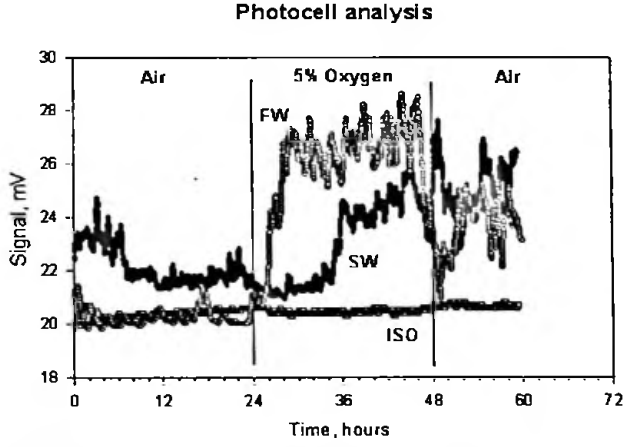

Figure 3. Analysis of this same record with photocells on the video faceplate. These data agree with the manual method, which will make routine use possible.