

**USE OF SURFACE RESPIRATION BY**  
***FUNDULUS HETEROCLITUS* AND *FUNDULUS MAJALIS***  
**FROM HYPOXIA-PRONE WESTERN LONG ISLAND SOUND**

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**Abstract**

Cyprinodonts such as the mummichog, *Fundulus heteroclitus*, and the striped killifish, *Fundulus majalis*, are abundant in stressed environments such as the bays and harbors of western Long Island Sound, which frequently become hypoxic during the summer months. Use of surface respiration during periods of environmental hypoxia could increase their tolerance to low dissolved oxygen (DO). We found that both species of killifish altered their respiratory behavior when exposed to progressive hypoxia in the laboratory and adopted aquatic surface respiration increasingly after oxygen concentrations declined below 5.0 mg O<sub>2</sub>/l. In addition, below 4.0 mg O<sub>2</sub>/l, both species were observed to break the water surface, apparently to take air into the buccal cavity. Striped killifish reached maximal opercular rate, and initiated the use of surface respiration, at higher dissolved oxygen concentrations (DO) than mummichogs. The frequency of surface respiration in striped killifish was at least twice that of mummichogs at dissolved oxygen concentrations from 5.0 to 2.0 mg O<sub>2</sub>/l. In severe hypoxia of 1.0 mg O<sub>2</sub>/l both species spent considerable time (>80%) at the surface using these alternate modes of respiration (ASR and air gulping).

**Introduction**

The Cyprinodonts are abundant in bays, harbors, and marshes along the East Coast of the North America (Bigelow & Schroeder, 1953; Diaber, 1982; Kneib, 1986; McEnroe et al., 1994) where they are import forage species for piscivorous fishes

and wading birds (Frederick and Loftus, 1993). Both mummichogs, *Fundulus heteroclitus*, and the striped killifish, *Fundulus majalis*, are estuarine species (Nixon & Oviatt, 1973; Kneib & Stiven, 1978). Although these congeners are sympatric, *F. heteroclitus* is common in both subtidal and intertidal areas of the marsh while *F. majalis* occurs primarily in subtidal areas of the marsh (Weisberg, 1986).

Long Island Sound is heavily impacted by anthropogenic inputs and the deeper waters of the western Sound usually become hypoxic during the summer (LISS, 1994). Near shore areas may also become hypoxic (Parker, 1991). We have found a diurnal variation in oxygen at our study site, with oxygen minima in the early morning and maxima in the afternoon (McEnroe, unpubl.). While both species of *Fundulus* are common in near shore areas of the Sound, field studies found *F. heteroclitus* occurs in high numbers, even at low dissolved oxygen levels while the abundance of striped killifish, *F. majalis*, is more variable (McEnroe et al., 1994).

Among fishes there is a wide array of physiological, morphological, and behavioral adaptations to hypoxia, including aquatic surface respiration (ASR), bimodal respiration and air breathing. Even in hypoxic water the air-water interface is relatively well oxygenated and the use of this water layer for respiration is termed aquatic surface respiration (Kramer, 1982). Such alternative modes of respiration are well documented for tropical freshwater fishes from hypoxia-prone habitats. However, fewer studies have been made on temperate estuarine species. In such habitats hypoxia is linked to anthropogenic inputs and presumably is of recent origin.

Surface respiration may permit fish to survive otherwise lethal oxygen levels (Kramer & McClure, 1981a) by increasing arterial oxygen content (Burggren, 1982). ASR is performed by fish moving to the surface of the water column and ventilating their gills with the top 1 cm of the water (Lewis, 1970; Gee et al, 1978; Kramer, 1982, 1983). Although energetically expensive (Kramer & McClure, 1981a) the cost of ASR may be reduced by use of a buccal bubble to enhance buoyancy (Gee, 1991; Gee & Gee, 1991). Killifishes (*Fundulus* spp.) have several morphological characteristics important in the use of surface respiration: a dorsally oriented mouth, neutral buoyancy, and small body size (Lewis, 1970). We investigated the respiratory responses, including use of surface respiration (ASR and air gulping), of *F. heteroclitus* and *F. majalis* to low dissolved oxygen.

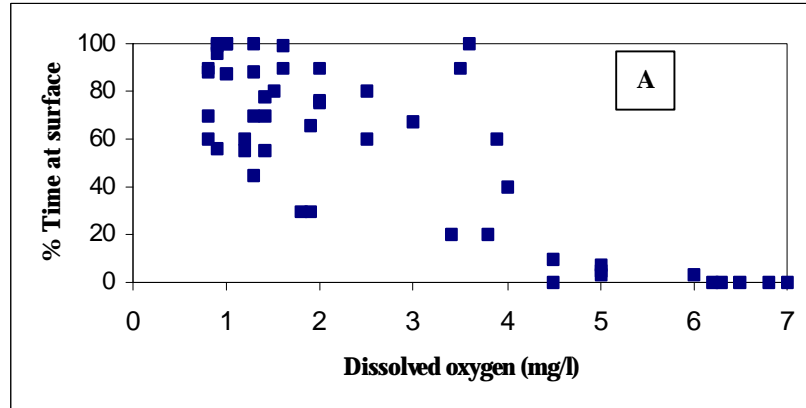
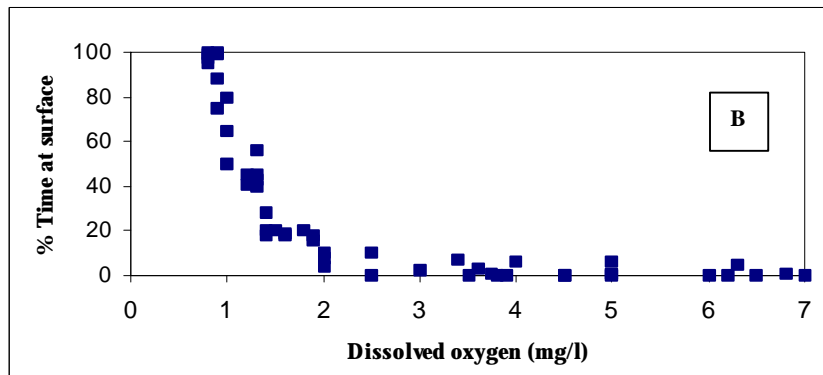


Figure 1. Time spent at the surface by *Fundulus majalis* (A) or *Fundulus heteroclitus* (B) exposed to progressive hypoxia. Each symbol represents the mean value for 5 fish at each oxygen level.



## Materials and Methods

Fish were collected by seine from in the western Sound in Rye NY. After transport to the laboratory they were held in aquaria in Long Island Sound water, at temperatures of 22-25 °C and salinity of about 26 ppt, typical of summer conditions in the Sound. Fish were fed Tetramin several times /d. The aquaria were fitted with undergravel filters and aerated to maintain dissolved oxygen concentrations at 6-7 mg O<sub>2</sub>/l. Water was renewed periodically.

Fifteen experiments were conducted, five on *F. majalis*, and ten on *F. heteroclitus*. For each, 5 fish were placed in a 25 liter glass experimental tank and acclimated for several days prior to experiments. Fish were fed several hours prior to the start of the experiment and left over food removed from the tank. Dissolved oxygen concentration (DO, mg O<sub>2</sub>/l) and temperature were measured using a YSI Model 58B Dissolved Oxygen meter fitted with a water stirrer, salinity was measured using a Riechert-Jung refractometer and control measurements obtained in normoxia. Then the dissolved oxygen (DO) concentration was lowered by stopping aeration and bubbling nitrogen (N<sub>2</sub>) into the tanks. In all experiments DO was continuously recorded and N<sub>2</sub> flow adjusted to decrease dissolved oxygen levels at a steady rate of about 1 mg O<sub>2</sub>/l/hr. Experiments lasted for 7 to 8 hrs, similar in length to the period of diel hypoxia in the marsh.

At each oxygen level, from normoxia (90-100% Saturation) to 1.0 mg O<sub>2</sub>/l, four measurements of respiratory behavior were made:

- a) opercular ventilation movements were counted for 15 sec on each fish.
- b) respiratory behavior was considered ASR when fish ventilated their gills at, or immediately below, the surface of the water. In each experiment, the total number of ASR events for 5 fish was counted during each 10 min. observation period.
- c) at low dissolved oxygen levels fish broke the surface with open mouths. This behavior was termed 'air gulping'; these events were quite distinct and easily differentiated from gill ventilation at the surface (ASR events). The total number of times the 5 fish in each experiment exhibited 'air-gulping' during each 10 min. observation period was counted.

d) percent time that all 5 fish in a tank spent at the surface was monitored over a 3 min period at each level of dissolved oxygen.

During each experiment the respiratory behaviors (a, b, c, d above) of the fish were measured at each of several levels of oxygen depletion, down to 1.0 mg O<sub>2</sub>/l.

## Results

### *Opercular Ventilation*

Measurement of opercular ventilation rate was made over a range of dissolved oxygen from 7.5 to 1.0 mg O<sub>2</sub>/l. Maximal opercular rates were similar, 170 bpm (*F. majalis*) and 175 bpm (*F. heteroclitus*), but *F. majalis* reached maximal opercular rate at 3.0 mg O<sub>2</sub>/l versus 1.0 mg O<sub>2</sub>/l in *F. heteroclitus*.

### *Percent Time at the Surface*

As the severity of hypoxia increased so did the use of ASR, thus fish spent more time near the surface of the water column (Figure 1a,b). In normoxia, 7.0 mg O<sub>2</sub>/l, both species spent less than 10% of the time at the surface of the water column. This increased to 93% and 87% respectively at 1.0 mg O<sub>2</sub>/l. However, striped killifish spent more time at the surface at intermediate oxygen concentrations of 6.0 to 3.0 mg O<sub>2</sub>/l.

### *Aquatic Surface Respiration*

Aquatic surface respiration was rarely observed at DO > 5 mg O<sub>2</sub>/l but as oxygen declined both species increased the frequency of ASR, exploiting the oxygen-rich surface layer of water to ventilate the gills. *F. majalis* initiated the use of ASR at a higher DO than did *F. heteroclitus*. The frequency of ASR increased in both species as oxygen levels fell but remained significantly greater in *F. majalis* than in *F. heteroclitus* until severe hypoxia of  $\leq 1.5$  mg O<sub>2</sub>/l (Figure 2). The percent of fish using ASR also increased with declining DO; ASR<sub>100</sub> was 2.0 mg O<sub>2</sub>/l in *F. majalis* and 1.0 mg O<sub>2</sub>/l in *F. heteroclitus*. Both species were observed to break the water surface, apparently to take air into the buccal cavity, as oxygen levels declined. This behavior was termed 'air gulping'; *F. majalis* initiated this behavior at a higher DO (3.0 mg O<sub>2</sub>/l) than did *F. heteroclitus* (1.2 mg O<sub>2</sub>/l).

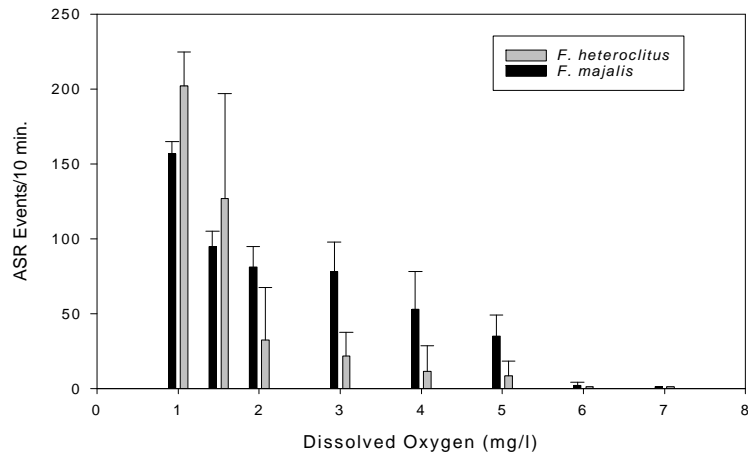


Figure 2. Use of aquatic surface respiration (ASR) by *Fundulus heteroclitus* and *F. majalis* exposed to progressive hypoxia. The mean  $\pm$  standard deviation is plotted for each of dissolved oxygen (DO) concentration. For both species the frequency of ASR was significantly higher (t-test,  $p < 0.005$ ) in hypoxia ( $DO \leq 3.0$  mg  $O_2/l$ ) than in normoxia ( $DO \geq 6.0$  mg  $O_2/l$ ). At intermediate oxygen levels of 3.0 to 5.0 mg  $O_2/l$ , *F. majalis* used ASR more frequently than did *Fundulus heteroclitus*.

### Survival

All fish survived exposures of several hours at 1.0 mg  $O_2/l$  were without apparent ill effects, the fish maintained their equilibrium and swam normally. At very low DO of 1.0 mg  $O_2/l$  both species appeared stressed: *F. heteroclitus* changed color, and decreased swimming activity; *F. majalis* swam in an agitated manner.

### Discussion

In western Long Island Sound hypoxia occurs every summer, and in the harbors and embayments dissolved oxygen concentrations fluctuate with the diel cycle, reaching

minima late in the night. Our measurements have confirmed that this cycle occurs nightly in Milton Harbor, Rye NY: the early morning measurements of oxygen approaching 3 mgO<sub>2</sub>/l (McEnroe et al., 1994). Mummichogs were abundant in this habitat, and remain there during hypoxic events (McEnroe et al., 1994). Such levels of hypoxia have been shown to produce physiological affects in marine fishes (Jensen et al., 1993). Hypoxia normally induces an increase in ventilation frequency (Burggren, 1982; McEnroe and Krozlowitz, 1997; Jensen et al., 1993) and stroke volume (Shelton, 1970; Jensen et al., 1993). Increasing gill ventilation passes more water over the gills per unit time, and can compensate for the decreased oxygen content of the water. However, there is a maximum ventilatory rate that can be achieved; as opercular rate increases, so does the amount of oxygen required to sustain it. Ventilatory costs in fish range from 0.5 to 43 % of the total oxygen consumption (Roberts, 1975; Holeyton, 1980), with an average cost in normoxia of about 10% (Hughes, 1973). With increasing severity of hypoxia the cost of ventilation increases, and may eventually exceed the amount of oxygen taken up the gills (Burggren, 1982; Hughes, 1973). When oxygen becomes limiting opercular rate may decline (Fritsche and Nilsson, 1993; McEnroe and Krozlowitz, 1998). As expected, both killifish species increased ventilation rate in direct response to declining oxygen concentrations, and maximal opercular rates were similar in both species: 175 bpm in *F. heteroclitus* and 170 bpm in *F. majalis*. In both species ASR increased as opercular rates reached maximal values. Although no decline in opercular rate occurred in these experiments where fish had surface access, subsequent experiments (McEnroe et al., in prep.) have found such a decline when fish are denied access to the surface, suggesting that ASR enhances oxygen uptake and hypoxia tolerance.

Both species spent significantly more time at the surface in hypoxia than in normoxia. Percent time at the surface increased as oxygen declined: above 5.0 mg O<sub>2</sub>/l both species spent less than 10% of the time at the surface, and at 1.0 mg O<sub>2</sub>/l, both species spent most of the time at the surface (>80%). *F. majalis* initiated the use of ASR at a higher oxygen level than did *F. heteroclitus*, and spent proportionately more time at the surface. (>50%) than did *F. heteroclitus* (<30%) at intermediate oxygen levels (5.0 to 2.0 mg O<sub>2</sub>/l). The percent time spent at the surface correlated with the number of surface respiration events. *Fundulus* are relatively small and both species of killifish studied possess dorsally oriented mouths and flattened heads that enable them to use the oxygen-rich surface layer of the water without altering swimming posture (Lewis, 1970). Furthermore, they are neutrally bouyant, an important feature for utilization of surface respiration. At oxygen levels below 5 mg O<sub>2</sub>/l the *F. heteroclitus* adopt ASR, swimming just beneath the air-water interface and utilizing the relatively oxygen-rich surface water to ventilate their gills. ASR was infrequent at dissolved

oxygen concentrations above 5 mg O<sub>2</sub>/l, and the frequency of ASR increased dramatically as oxygen levels fell with ASR<sub>20</sub> reached at 3.2, and ASR<sub>100</sub> at 1.0 mg O<sub>2</sub>/l. Striped killifish (*F. majalis*) initiated the use of ASR at higher oxygen levels and used ASR more frequently than mummichogs when DO ≥ 1.5 mg O<sub>2</sub>/l. Both species were observed to break the water surface, apparently to take in an air, as oxygen levels declined. Air bubbles held in the buccal cavity may be used to oxygenate water flowing past it, thereby enhancing oxygen uptake at the gills. Burggren (1982) found goldfish using such a technique, had significantly higher arterial oxygen saturation than did fish denied surface access. Increased buoyancy from such bubbles could reduce the metabolic cost of surface respiration and enhance the efficiency of ASR (Gee and Gee, 1991).

The absence of surface respiration in normoxia suggests that such behavior has a cost, either energetic or in terms of aerial predation (Kramer, 1983; 1987; Kramer and McClure, 1981). Frequent trips to the surface, even in oxygenated water, uses a large percentage of the daily energy budget (Kramer and McClure, 1981; Pandian and Vivekanandan, 1976). The energetic cost of surface respiration may differ between the two *Fundulus* species: *F. heteroclitus* intermittently swam to the surface to perform surface respiration, while *F. majalis* stayed near the surface and constantly swam along the top of the water column. Chapman et al. (1995) suggested that rapid, constant swimming at the surface might force more well-oxygenated water over the gill than could be achieved by opercular ventilation alone, thus reducing the energetic cost of ASR. A second potential cost of surface respiration is increased risk of aerial predation. Cyprinodonts are important forage species for wading birds (Frederick and Loftus, 1993) and use of surface respiration has been found to increase predation risk for other species of fish (Kramer, 1983; 1987; Kramer and McClure, 1981). Although they were frequently collected together at low tide (McEnroe et al., 1994), *F. heteroclitus* inhabits both intertidal and subtidal areas while *F. majalis* occurs primarily in the subtidal areas (Weisberg, 1986). In the laboratory *F. majalis* appeared more stressed by hypoxia than its congener: maximal opercular rate was reached at a higher dissolved oxygen than in *F. heteroclitus* (3.0 vs. 1.0 mg O<sub>2</sub>/l), and *F. majalis* initiated the use of ASR and air-gulping at higher DO than *F. heteroclitus*.

## Conclusions

Both species of killifish utilizes ASR and 'air-gulping' in hypoxia, as do many tropical fishes. In both, the frequency of surface respiration increased as O<sub>2</sub> declined. *F. majalis* initiated the use of surface respiration at higher O<sub>2</sub>

concentrations than *F. heteroclitus*. In both species the use of surface respiration significantly increased when maximal opercular rate was reached, suggesting that these alternative modes of respiration were used to supplement oxygen uptake once gill ventilation could no longer suffice. We have found that other common species of fish from the Sound may use adaptive behaviors when exposed to hypoxia (McEnroe and Krozlowitz, 1997), though not all respond in the same way. Conventional laboratory assays measuring LC<sub>50</sub>s, or oxygen consumption, in response to acute exposures do not usually take account of adaptive responses therefore they may provide an incomplete measure of tolerance to hypoxia.

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### **Acknowledgements**

This research was supported by Texaco Student Research Awards to H.M., Y. K, and J. A.

