

Morphological Adaptations of Cyprinodontoids for Inhabiting Oxygen Deficient Waters

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A previously unrecognized morphological adaptation of fishes to oxygen depletion is described. Natural oxygen depletion was simulated by use of sodium sulfite. It was demonstrated that some fishes are adapted to permit use of an oxygen rich layer of water at the atmosphere-water interface. Representative cyprinodontoids (*Fundulus notatus*, *Gambusia affinis*, and *Poecilia reticulata*) exhibited maximum adaptation and the greatest survival ability of the species tested. *Notemigonus crysoleucas*, *Brachydanio rerio*, *Lepomis macrochirus*, and *L. cyanellus*, representing other suborders, showed less adaptation to these conditions and correspondingly lower survival. *Micropterus salmoides* seemed unadapted for survival in oxygen depleted waters. *Carassius auratus* exhibited a physiological adaptation, but used the oxygen rich surface water as a supplement.

INTRODUCTION

SOME freshwater habitats, such as swamps, are characterized by periodic absence of dissolved oxygen in the water column, and some fishes are clearly adapted for living in these waters. Adaptations studied to date include morphological modifications for using atmospheric oxygen and physiological adjustments that increase tolerance to low oxygen levels. This paper reports the occurrence of anatomical adaptations to permit use of oxygen rich water at the atmosphere-water interface.

The importance of vertical oxygen gradients to aquatic fauna in general has long been recognized. Carter and Beadle (1931) commented on the significance of surface layers as a reservoir of oxygen for organisms in an oxygen depleted aquatic environment. Dusart (1963) and Carter (1931) mentioned the probable role of oxygenated surface layers in sustaining fishes during periods of oxygen depletion. Although it is often observed that fishes not adapted for aerial breathing respond to a reduction in dissolved oxygen by rising to the surface, it is not clear at present whether such behavior is accompanied by the use of atmospheric oxygen or dissolved oxygen, nor has the possible significance of surface layers as an oxygen source for fishes been quantitatively investigated.

Observations preliminary to the present investigation suggested that the surfacing response of a number of fishes is associated

with the use of an oxygenated film of water just below the air-water interface. It is postulated here that the small, dorsally oriented mouth and dorso-ventrally flattened head of such fishes as *Fundulus*, *Poecilia*, and *Gambusia* are adaptations for utilizing a well oxygenated surface film, and that these fishes are suited for occupying habitats characterized by periodic or continuous oxygen depletion.

METHODS

Sodium sulfite was used to remove dissolved oxygen from the water column. Oxygen is bound by the sulfite ion according to the equation: $2\text{SO}_3^{--} + \text{O}_2 \longrightarrow 2\text{SO}_4^{--}$. From the gram equivalent weight, 7.891 mg/l of sulfite are required to bind 1 ppm dissolved oxygen. Amounts in excess of the gram equivalent were used to increase the rate of reaction and to prevent reoxygenation of the water column during experiments. Experimental concentrations of sulfite did not constitute a significant osmotic challenge to the fish. There was no observable difference in behavioral response to water freed of oxygen with sulfite and water freed of oxygen by bubbling with nitrogen.

An oxygen analyzer of the galvanic cell type was used to measure dissolved oxygen, except very near the air-water interface. The dissolved oxygen concentration near the interface was approximated using Fick's Second Law employing the equation, $C_{x,t} = S(1-H(x/2D^{\frac{1}{2}} t^{\frac{1}{2}}))$ where: $C_{x,t}$ = oxygen concentration in water in ppm at a given depth

x (measured in cm) after a given time, t (sec after exposure to air. S = saturation concentration of oxygen in water in ppm at 25° C and standard pressure. H = the error function (U.S. Department of Commerce, 1954). D = the diffusion coefficient for oxygen in water at 25° C (Meites, 1955).

Experimental water temperatures ranged between 22 and 25° C.

Plastic pans with a depth of 12 cm and a surface area of 950 cm² served as experimental containers. A plastic tent over the tanks prevented air currents from disturbing the water surface. To prevent accumulation of metabolic carbon dioxide above the water, the containers were completely filled. A curb of screen wire around the outside of the container prevented fish from escaping.

Experimental fishes were kept in a common holding tank for four days before they were transferred to experimental tanks. The holding tank water always contained 3-4 ppm dissolved oxygen. All fish were thus acclimated to the same oxygen level prior to use.

Since handling or other excitation of fishes results in a significant physiological stress caused by high concentrations of lactic acid in the blood (von Buddenbrock, 1936; Auvergnat and Secondat, 1942; Black, 1955), fish were placed in the experimental tanks 12 hr before the beginning of each experiment.

The response of cyprinodontoids to oxygen depletion was compared to that of other fishes with different head and mouth morphologies. Experimental species considered to be morphologically adapted to habitats characterized by low levels of dissolved oxygen included: *Fundulus notatus* (Cyprinodontidae); *Gambusia affinis* and *Poecilia reticulata* (Poeciliidae). Experimental species not exhibiting such an adaptation included: *Notemigonus crysoleucas*, *Brachydanio rerio*, and *Carassius auratus* (Cyprinidae); *Micropterus salmoides*, *Lepomis cyanellus*, and *L. machrochirus* (Centrarchidae). *Gambusia affinis* was collected in the Piedmont area of North Carolina. *Poecilia reticulata* and *Brachydanio rerio* are both tropical forms and were obtained from commercial sources. *Notemigonus crysoleucas* was obtained from a commercial fish hatchery in southern Illinois. All other species were collected from the wild in southern Illinois. None of the experimental fish exceeded 90 mm SL.

Experiments were designed to yield four types of information: 1) the behavior of different species of fish in water depleted of dissolved oxygen, 2) the dependency of fishes upon surface breathing in oxygen depleted water, 3) the relationship between survival and the composition of the atmosphere above the water, and 4) the relationship between head morphology and ability to survive in oxygen depleted water.

Behavior of the fishes was observed after the addition of 300 ppm sodium sulfite to the water. This concentration of sulfite caused reduction of dissolved oxygen over a period of 1 hr or more, thus permitting observation of changes in behavior as the dissolved oxygen decreased.

To test the dependency of fishes on surface breathing when the water column was devoid of dissolved oxygen, sodium sulfite (750 ppm) was added to three tanks, each containing six fish. Three of the six fish in each tank were permitted free access to the surface and the remainder were caged below the surface. All species were tested in this manner.

To test the significance of gaseous exchange between the atmosphere and water at the interface, a covered aquarium containing *Poecilia* and *Brachydanio* was treated with 300 ppm sodium sulfite causing the subsurface oxygen to fall slowly to zero. The atmosphere above the tank was then rapidly replaced with pure nitrogen.

Extensive tests of ability to survive in oxygen depleted water were limited to *Gambusia*, *Notemigonus*, and *Carassius*, each representing one of three distinct types of response found in the experimental fishes. Excess sodium sulfite was added to the experimental tanks until a concentration was reached at which the test species remained at the surface continuously for 24 hr. Excess sodium sulfite eliminates oxygen from the water column and also limits the accumulation of oxygen entering by diffusion at the interface. Adding progressively more sulfite restricts the diffusion saturated surface water to a progressively thinner layer. The first appearance of continuous surface breathing is considered a uniform level of stress, and the concentration of sulfite needed to induce it is a measure of efficiency in use of surface water.

A more detailed comparison of the ability of the different species to effectively utilize

the surface film was made in terms of minimum surface area required for survival (weight of fish per unit water surface area, mg/cm²). Three tanks containing identical weights of a given species were treated with the amount of excess sodium sulfite which had been found in the preceding experiment to keep the fish at the surface. A fourth tank, the control, contained the same weight of fish but no sulfite was added. Dead fish were removed and weighed at 15 min intervals for the first 4 hr, and thereafter at 14 and 24 hr. The experiment was terminated 24 hr after the dissolved oxygen reached zero. This procedure was repeated for a range of densities to give the density-mortality relationship for each species. Experiments with the highest fish densities were terminated after 14 hr to avoid errors resulting from waste accumulation. The resulting data provide two types of information about each species: 1) mortality rate for specific initial densities, and 2) the change in total mortality as initial density is increased. An important aspect of the latter relationship is the maximum initial density for which experimental mortality is the same as control mortality. This density is hereafter referred to as the critical density.

Gambusia were tested at 29 different densities involving approximately 10,000 fish with mean weights of 0.165 ± 0.093 g (♀) and 0.127 ± 0.021 g (♂). For *Notemigonus*, 108 densities were tested, representing approximately 3500 fish with a mean weight of 1.37 ± 0.20 g. For *Carassius*, 12 densities were tested, representing 60 fish with a mean weight of 8.57 ± 1.71 g.

OBSERVATIONS

The behavior and survival of fishes when there was no detectable oxygen in the water column demonstrated the existence of oxygen near the water surface. The gradient shown in Fig. 1 is for the diffusion of atmospheric oxygen into pure water as calculated from Fick's Second Law. Under experimental conditions, as well as natural ones, the action of the reducing agent responsible for the elimination of oxygen must be accounted for. The reducing agent, in this case the sulfite ion, has a concentration profile which is inversely related to the oxygen profile. At points near the interface, oxygen enters rapidly from the air by diffusion, thus oxidizing all of the reducing agent and saturating the surface film

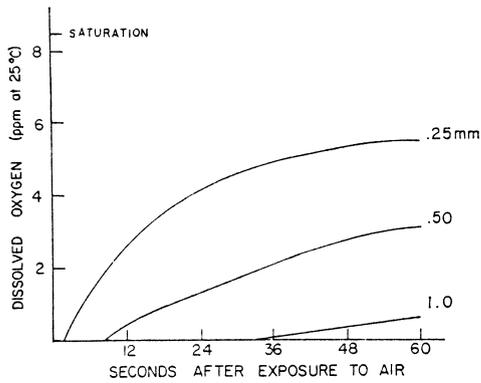


Fig. 1. Theoretical rate of diffusion of atmospheric oxygen into water. Depth below surface indicated in mm.

with oxygen. The maximum depth at which a biologically significant concentration of oxygen occurs is determined by the diffusion rates and concentrations of oxygen and the reducing agent. The limiting effect of the low oxygen-water diffusion rate is evident in Fig. 1.

None of the experimental species were observed to gulp air or pass bubbles from the opercula, despite the reduction of subsurface oxygen to zero. None of the fishes released air bubbles when trapped below the surface after breathing at the surface.

Surfacing resulted from the reduction of dissolved oxygen and became progressively more continuous as the oxygen content of the water declined (Table 1). All fishes except *Carassius* remained at the surface when the subsurface oxygen approached zero. *Carassius*, periodically assumed a stationary position at the surface, then moved to the bottom and remained inactive.

For all fishes except *Carassius*, surfacing was accompanied by continuous movement parallel to the interface. Each species assumed a characteristic posture for swimming at the surface. The cyprinodontoids (*Gambusia*, *Poecilia*, *Fundulus*) swam with the long axis of the body almost parallel to the water surface, whereas the other experimental fishes maintained a large angle of inclination between the body axis and the water surface (Fig. 2).

All fishes except *Carassius* died before the dissolved oxygen reached zero when they were prevented from surfacing. *Carassius* survived for an average of 40 min after the

TABLE 1. BEHAVIOR OF SELECTED FISHES IN RESPONSE TO A DECLINE IN DISSOLVED OXYGEN CONCENTRATION.

Species	Inclination of Body Axis with Surface (degrees)	Approximate O ₂ Maximum for Surfacing (ppm)	Comments
<i>Fundulus notatus</i>	5-10	0.5	No sign of distress at 0.0 ppm subsurface O ₂ .
<i>Gambusia affinis</i>	5-10	2.5	Tendency to stay at the surface, despite O ₂ .
<i>Poecilia reticulata</i>	5-10	0.5	Identical in surface behavior to <i>Gambusia</i> .
<i>Notemigonus crysoleucas</i>	20-25	1.0	Vigorous swimming at the surface.
<i>Brachydanio rerio</i>	20-25	0.5	Similar in surface behavior to <i>Notemigonus</i> .
<i>Lepomis macrochirus</i>	30-35	0.5	Reversal of pectoral fin movement at the surface.
<i>Lepomis cyanellus</i>	30-35	0.4	Little movement at the surface.
<i>Micropterus salmoides</i>	30-35		Mortality shortly follows sporadic surfacing.
<i>Carassius auratus</i>	30-35		Surfaces only for short periods, reduced activity.

oxygen content of the water reached zero. All fishes except *Micropterus* survived oxygen elimination for at least 1 hr when they were permitted to surface.

The replacement of air by nitrogen over a tank containing *Poecilia* and *Brachydanio*, both exhibiting surfacing behavior, immediately resulted in their distress and death, even though neither species was using atmospheric oxygen directly.

In the first test of survival ability, a concentration of 750 ppm sodium sulfite was needed to keep *Notemigonus* continuously at the surface for 24 hr. *Gambusia* required 3150 ppm. *Carassius* could not be induced to remain at the surface continuously, and was arbitrarily treated with 750 ppm sodium sul-

fite in the determination of surface area requirement.

Surface area requirement in the absence of dissolved oxygen was different for each of the three representative species. Test densities of *Carassius* as high as 100 mg of fish per cm² surface area showed no mortality during the 24 hr test period. Despite crowding, *Carassius* had no tendency to disperse over the tank surface, but remained aggregated and stationary at the surface or motionless on the bottom. Mortality rates for different initial densities of *Gambusia* and *Notemigonus* indicate that, at densities sufficient to cause mortality, a new stable density of fish is reached after 14 hr (Fig. 3). *Gambusia* experience almost no mortality up to

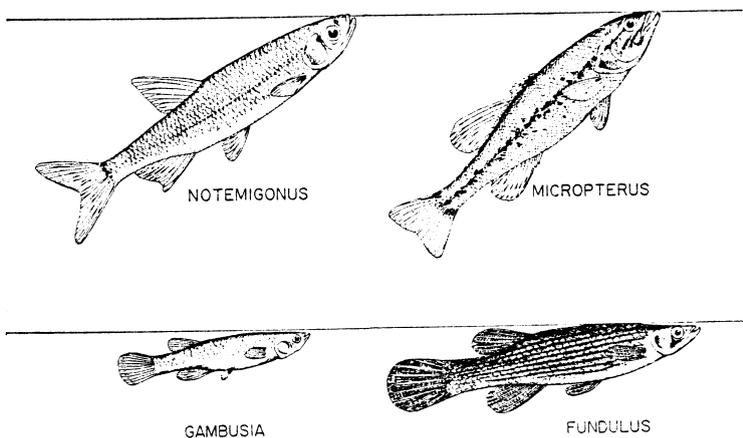


Fig. 2. Orientation of different fishes at the atmosphere-water interface in water depleted of dissolved oxygen.

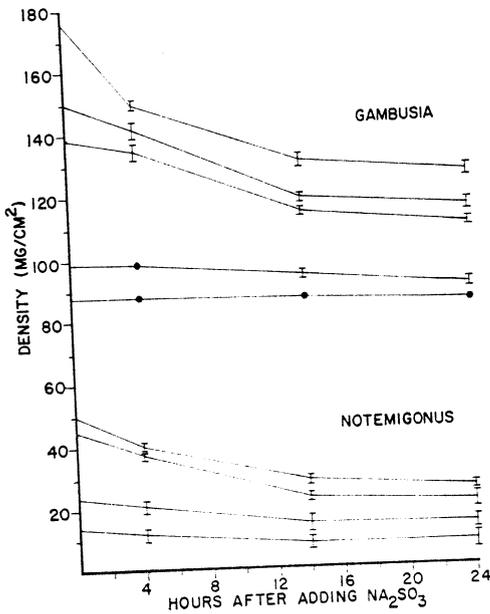


Fig. 3. Changes in survival leading to a stable survival density of *Gambusia* and *Notemigonus* subjected to a vertical oxygen gradient severe enough to cause continuous surface breathing (3150 ppm Na_2SO_3 for *Gambusia*, 750 ppm Na_2SO_3 for *Notemigonus*). Where replicates differ, ranges are indicated. Solid dots represent 100% survival.

a critical density of 100 mg/cm^2 , whereas the critical density for *Notemigonus* is about 10 mg/cm^2 (Fig. 4). Control tanks had almost no mortality.

DISCUSSION

Survival and behavior indicate that the test species represent four categories of adaptation for survival in waters depleted of dissolved oxygen: 1) unadapted, 2) physiologically adapted, 3) partially anatomically adapted, 4) fully anatomically adapted.

Micropterus, representing category 1, was unable under experimental conditions to extend its survival time by using surface water. *Micropterus* did respond to oxygen depletion by vigorous surface activity, and it is possible that under conditions less severe than those studied the surfacing behavior of this fish would be of some value.

Carassius, representing category 2, was unique in its failure to remain continuously at the surface when oxygen was eliminated from the water column. *Carassius* is known to have physiological characteristics which adapt it for surviving low concentrations of dissolved oxygen (Fry and Hart, 1948). Blažka (1958) has shown that *Carassius* can survive anoxia for extended periods at low temperatures (5°C), but dies in several hours at higher temperatures (16°C) when no oxygen is present. Blažka inferred from his experiments that anaerobic metabolism in *Caras-*

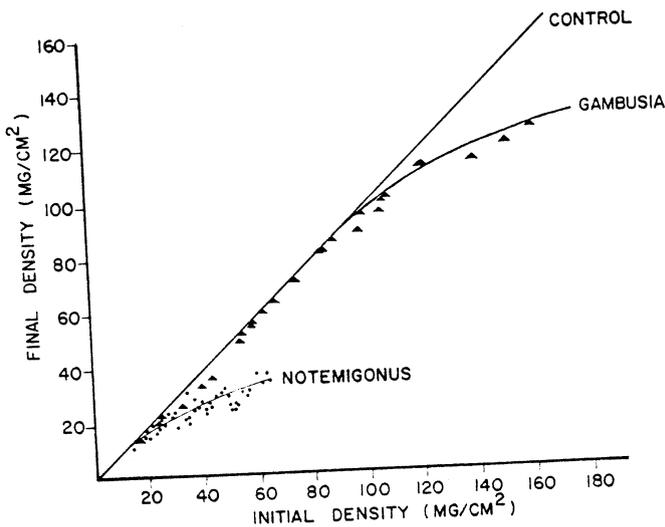


Fig. 4. Comparison of survival of *Gambusia* and *Notemigonus* after 14 hr, showing critical densities when subjected to a vertical oxygen gradient severe enough to cause continuous surface breathing (3150 ppm Na_2SO_3 for *Gambusia*, 750 ppm Na_2SO_3 for *Notemigonus*).

sius is not accompanied by the accumulation of intermediary metabolites which require subsequent oxidation. This interpretation of physiological tolerance in *Carassius* is supported in part by Smit (1965). The present study shows that *Carassius* is also able to use the oxygenated water of the surface film in surviving anoxia at higher temperatures. However, the large terminal mouth of *Carassius* is not suited for the selective use of a thin surface layer. Instead of moving at the surface to facilitate the intake of surface water, *Carassius* maintains a low oxygen demand by inactivity, thus requiring only the small amount of oxygen that can be supplied by its limited intake of oxygenated water.

Notemigonus, *Brachydanio*, and *Lepomis*, representing category 3, are all able to withstand the elimination of subsurface oxygen when the vertical oxygen gradient is not severely reduced. The immediate distress and death of category 3 fishes that are prevented from surfacing in the absence of dissolved oxygen suggests that these fishes are similar to *Micropterus* in lacking physiological tolerance to oxygen depletion. Although more thorough investigation might reveal some differences in the minimum dissolved oxygen requirements of *Micropterus* and the fishes of category 3, it is probable that the distinction between these two groups can be attributed mainly to difference in mouth size. *Micropterus*, because of its large mouth, is unable to selectively draw water from the surface.

Notemigonus, *Brachydanio*, and *Lepomis* have terminal mouths and must therefore incline the body axis at the surface to contact the oxygen bearing surface water. These fishes move awkwardly at the surface because of their inclined posture, and expend much energy to keep their mouths at the surface while swimming parallel to it. They are here considered to be suited to survive periods of oxygen depletion, but not adapted to occupy a habitat frequently or continuously lacking dissolved oxygen in the water column.

Fundulus, *Gambusia*, and *Poecilia*, representing category 4, are of particular interest due to their ability to withstand the most severe experimental conditions without apparent stress. The dorsally oriented mouths and flattened heads of these fishes account for their ability to effectively use oxygen rich water at the atmosphere-water interface without greatly altering their usual swim-

ming posture. These forms are here considered to be adapted to habitats characterized by long or continuous absence of oxygen in the water column.

An increase in percent mortality as fish density is increased for *Notemigonus*, of category 3, and *Gambusia*, of category 4, indicates that density dependent factors affect the use of the oxygen source in these fishes. Two such factors were recognized: truncation of the oxygen bearing layer, and mutual interference in surface behavior.

When fish are absent from tanks containing sodium sulfite, equilibrium between oxygen diffusion from the atmosphere and the action of the reducing agent in the water maintains an oxygen gradient at the water surface. The theoretical consideration of surface dynamics (Fig. 1) indicates that a biologically significant oxygen concentration would not occur at depths greater than 2 or 3 mm if oxygen were being continuously eliminated from below. The addition of fish decreases the thickness of the oxygen bearing layer, since oxygenated surface water is either mixed with oxygen-free water below or is used in respiration. A constant oxygen supply occurs only in a surface layer thin enough to be renewed by diffusion between disturbances. When the fish are crowded beyond their critical density, the oxygen bearing layer is truncated so severely that the fish can no longer restrict their breathing to it. The critical densities of *Gambusia* and *Notemigonus* thus indicate the relative abilities of these fishes to use the surface water oxygen source.

When crowded, both species are also affected by mutual interference in surface behavior. At the critical density (100 mg/cm²) *Gambusia* almost crowd each other from the surface. On the other hand, the critical density for *Notemigonus* (10 mg/cm²) would allow each fish ample room at the surface, if movement were minimal. However, since *Notemigonus* moves vigorously at the surface and must simultaneously maintain a large angle of surface inclination, mutual interference occurs at lower densities. When interference occurs, each fish periodically loses contact with the oxygen source. At high densities both species are therefore less efficient in using the surface oxygen source because of mechanical interferences, and this effect is greater for *Notemigonus* than for *Gambusia*.

Although the determinations of surface area requirement were not designed to distinguish between the two density dependent factors, it seems probable that truncation of the oxygen bearing layer played the greatest role in causing mortality. The uniformity of the results, particularly for *Gambusia*, supports this conclusion. In any case, the difference in critical densities for the two species reflects the marked advantage of *Gambusia* in using the stratum of water closest to the interface.

When the density of fish exceeds that which the area of surface can support, there is high mortality during the early hours of the experiment. Low or insignificant mortalities during the last hours of the experiment indicate that the adjustment of density to surface area has occurred.

An attempt was made to preserve uniformity of size in the test species, since mouth size varies with size of fish. Since the oxygen bearing surface layer is very thin, it is virtually unavailable to large fish of any species in the same sense that it is unavailable to the small *Micropterus*. Less severe instances of oxygen depletion would be accompanied by a surface source of greater vertical extent, which might be of use to larger fish. In cases of mild oxygen depletion, fishes with large mouths might also profit from the vertical mixing that results from their disturbance of the surface.

The importance of mouth size and morphology in the efficient use of oxygen bearing surface water is of particular interest with respect to the fishes of category 4, which typically inhabit waters where oxygen depletion is common. *Gambusia*, *Fundulus*, and *Poecilia*, which are morphologically well suited for using the surface water oxygen source, never become large. These fishes therefore retain their capacity to use oxygenated water at the interface for prolonged or indefinite periods, regardless of age. Since the volume of water required for respiration by larger fishes could not be supplied by a diffusion induced oxygen source at the surface, the small maximum size of such fishes as *Gambusia* might also be considered adaptive for using surface oxygen. This conclusion is supported in that fishes inhabiting swamps and other oxygen poor environments are generally either adapted for aerial respiration and show a wide range of sizes, or possess morphological features suitable for using

oxygenated water at the interface and attain small maximum sizes.

The importance of oxygen depletion to fishes in a natural environment would in part be determined by factors not considered above. At high temperatures, for example, the oxygen requirement of fishes would be higher and the saturation value for oxygen in water would be lower. On the other hand, fishes using surface water would profit to some extent from the increased diffusion rate of oxygen in water at higher temperatures. Also, the effect of wind on the water surface might be of considerable importance. The continuous movement of air and surface might induce a larger vertical oxygen gradient, but a rough surface would undoubtedly limit the ability of fishes to maintain the most effective relationship to the interface. It is of interest in this connection that disturbance of the water surface in swamp habitats is minimal.

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