Reprinted from COPEIA, 1955, No. 2, pp. 104-106, May 20
Printed in U.S.A.

Fish Respiration in the Natural Oxygen Gradient of an Anaerobic Spring in Florida

HOWARD T. ODUM AND DAVID K. CALDWELL

ON rare occasions one finds in nature an ecological circumstance so perfectly composed that it constitutes a natural experiment superior to any man-made construction that could be built at reasonable cost in the laboratory. Definite inferences can then be obtained with a small effort. Such natural experimental situations have additional value if they are permanent enough for data to be cumulative. In this communication some observations made in one of the remarkable Florida springs are used to draw conclusions about the respiration of fishes. It is hoped that others will be attracted to these permanent chemostatic gradients.

Beecher Springs, adjacent to the University of Florida Conservation Reserve, Welaka, Putnam County, emerges from a limestone aquifer without oxygen. As it flows downstream in its run (Fig. 1) this small stream picks up oxygen by diffusion and by photosynthesis of the algae. At a constant temperature diurnally and seasonally of 22° to 23°C., the physical and biological characteristics of this spring seem constant as surveyed 6 times over a period of 3 years. Extensive work on trace elements in another spring, Silver Springs, Marion County, indicates that a chemostatic condition is to be expected in the springs of the region. Thus Beecher Springs seems to constitute a permanent oxygen gradient in which organisms have become adjusted. White sulfur bacteria and blue-green algae dominate the boil with other algal types appearing downstream, and finally higher plants appear 300 meters from the boil. The algae have been studied by Larry A. Whitford, North Carolina State College, and will be the subject of a forthcoming paper by him. Midges (Tendipedidae and Heleidae) are found throughout the run and in part are the basis of the food chain leading to some of the fishes.

The oxygen values on the map (Fig. 1) were obtained with the Rideal-Stewart-Winkler method. Essentially similar values were recorded on June 6, 1952 and a year later on June 22, 1953. There are clearly eddies of water in the stream so that oxygen conditions at any one point fluctuate slightly with a range of about 1 ppm. Some idea of this variation from eddy to eddy can be obtained from the data in Table I. The analytical accuracy is about 0.1 ppm plus or minus.

The water is clear and the boil area smells strongly of sulfurous gases. Other properties are as follows: carbon dioxide, 10 ppm; pH, 7.2–7.7; chlorides, 166 ppm; phosphorus, 0.14 ppm; nitrate nitrogen, 0.02 ppm; alkalinity, 105 ppm ($CaCO_3$); hydrogen sulfide, 0.02 ppm.

The natural distribution of fishes in this natural gradient was observed with the aid of a face mask by swimming underwater where clear visibility is 30 meters or more. The observed species distribution is indicated in Fig. 1 and is listed below. The eddies of water permit local temporary microhabitats to acquire more oxygen so that fishes can move into the higher oxygen spots to replenish oxygen and thus can live in parts of the stream where the average oxygen may be below their physiological tolerances. Fig. 1 shows ecological tolerances under stream conditions by indicating the farthest

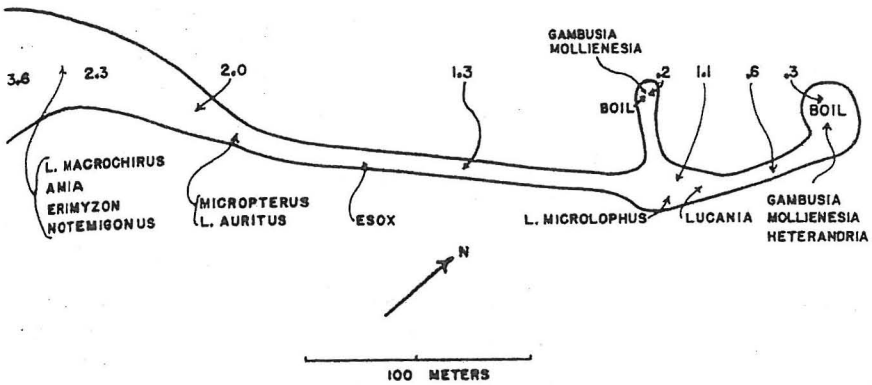

Fig. 1. Map of Beecher Springs, Welaka, Florida. Dissolved oxygen values are given in parts per million. The nearest that the fish were observed to come to the boil is indicated for 12 species in the middle of a sunny day (June 24, 1953).

point upstream that each species was observed to go. It is possible that the decrease of the small hydrogen sulfide concentration downstream is also involved. In order of distribution down the spring run the fishes represented are: eastern gambusia, *Gambusia affinis holbrooki* (Girard); sailfin molly, *Mollienesia latipinna* LeSueur; least killifish, *Heterandria formosa* Agassiz; redfin killifish, *Lucania goodei* Jordan; shellcracker, *Lepomis microlophus* (Günther); redfin pickerel, *Esox americanus* Gmelin; redbreast sunfish, *Lepomis auritus* (Linnaeus); largemouth bass, *Micropterus salmoides* (Lacépède); chubsucker, *Erimyzon succetta* (Lacépède); bluegill, *Lepomis macrochirus* Rafinesque; golden shiner, *Notemigonus crysoleucas* (Mitchill); and bowfin, *Amia calva* Linnaeus.

Experiments in Submerged Cages

Although Habs and Mueller (1948) found that the eastern gambusia lived readily in 0.3 ppm oxygen, the abundance of three cyprinodont species in the churning outflow headwaters (boil) where the oxygen is less than 0.3 ppm day and night seemed remarkable. These fishes are observed to break the surface continually. Mollys feeding on the bottom swim to the surface frequently before returning to the bottom. Although gulping by these fishes has long been observed, the writers do not know of a clear demonstration of the thresholds at which gulping is necessary for these widespread fishes. The following experiments were conducted within the springs.

Table I

Dissolved Oxygen, in Parts Per Million, in Beecher Springs, Florida

For 1953, three analyses for each station are preceded by the mean of these values. For stations, see Fig. 1

Station	VI:6:1952	VI:24:1953
Boil A	0.05	0.3 (0.27, 0.30, 0.50)
B	0.26	0.6 (0.50, 0.59, 0.65)
C	0.9	1.1 (0.40, 0.65, 2.16)
Boil D	1.3	0.2 (0.21, 0.13, 0.29)
E	1.7	1.3 (1.26, 1.31, 0.99)
F	1.1	2.0 (2.30, 1.80, 2.01)
G	...	2.3 (3.51, 1.15, 2.32)
H	3.6	...

Gambusia and *Mollienesia* taken from the boil were placed in the following situations:

1. Out of water in a dry box on land.
2. In cages in the boil at the surface so that fish could gulp at the surface.
3. In 4 cages submerged in the boil so that fishes could not get closer than 1 inch to the surface. One cage was near the surface and one was at a depth of 2 feet. A third cage included only *Gambusia* and a fourth only *Mollienesia*.
4. Submerged below the surface downstream where the oxygen was 1.3 ppm. (See Fig. 1.)

The amounts of time required for the fishes to die are summarized in Table II. Where oxygen was inadequate, distress was noticed within three minutes and most of the fishes were dead in about half the time necessary for all to succumb. Although *Gambusia* and *Mollienesia* are readily observed to pump the surface film over their gills in stagnant waters

TABLE II

FISH SURVIVAL EXPERIMENTS IN BEECHER SPRINGS, JANUARY 11, 1953

Experimental situation	Number of fishes	Duration in minutes	Oxygen tension, ppm	Percent survival
Cage in the boil at the surface of the water, fish gulping air*	37	160	0.28	92
Completely out of water*	30	22	In air	93
Cages completely submerged in boil:				
Gambusia, avg. weight: .23 gm.	27	11	0.28	0
Mollienesia, avg. weight: 0.37 gms.	27	22	0.28	0
Gambusia and *Mollienesia*:				
Below the surface 1 inch	24	19	0.28	0
Below the surface 2 feet	48	20	0.28	0
Cage completely submerged downstream*	20	25	1.3	100

* *Gambusia* and *Mollienesia*.

where oxygen is low, this is not the means of respiration in this turbulent but anaerobic stream. Definite gulping of air is observed. That gulping is necessary at these low tensions is demonstrated by the survival of those fishes with access to the surface. Since there was a rapid turbulent current cascading upward through the experimental cages an oxygenated surface film could not develop. When prevented from gulping, the fish drowned quickly. Those fishes kept out of water were quite alive at the end of 22 minutes since they demonstrated normal behavior when returned to the water.

Whereas the respiration of air by fishes has been much studied, the mechanism of aerial respiration in small cyprinodont fishes seems to have been neglected. Our dissections indicate that the Beecher Springs individuals do not differ from *Gambusia affinis holbrooki* elsewhere in having a closed swim bladder, a short intestine opening directly into a short oesophagus, and pseudobranchs (Vialli, 1926). These latter vascularized structures lining the pharynx may be the structures used as a respiratory surface for gulped air.

Another anaerobic spring in which obligate gulping by fish may be directly inferred is the large Warm Salt Spring in Sarasota County, Florida. Here, tarpon, *Tarpon atlanticus* (Valenciennes); gar, *Lepisosteus sp.*; southern variegated cyprinodon, *Cyprinodon variegatus variegatus* (Lacépède); sailfin mollys, and eastern gambusia are found in about half sea water salinity (17,800 ppm) and a temperature of 31°C. The oxygen content is between 0.00 and 0.81 ppm day and night. The tarpon were observed to roll and the smaller fishes to break the water continually. That tarpon and gars breathe with a vascularized air bladder is known from observations and dissections summarized by Babcock (1951) for the tarpon and Jones and Marshall (1953) for the gar.

These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Florida, NR 163–106.

LITERATURE CITED

BABCOCK, LOUIS L. 1951. The Tarpon. 5th ed. Privately published, 157 pp., illus.

HABS, H., AND G. MUELLER. 1948. Uber das Verhalten der Gambusien gegenuber dem Sauerstoffgehalt des Wassers. *Zeitschr. Hgy. u. Infektienskrankh. 128: 371–8.* (Abstract)

JONES, F. R., AND N. B. MARSHALL. 1953. The structure and function of the teleostean swim bladder. *Biol. Rev. 28: 16–83.*

VIALLI, M. 1926. Le pseudobranchie dei Pesci Arch. *Ital. Anat. Embriol., 23: 49–117* (Abstract)

DEPARTMENT OF BIOLOGY, UNIVERSITY OF FLORIDA, GAINESVILLE, FLORIDA.