THE MINIMUM OXYGEN REQUIREMENTS OF FIVE SPECIES OF FISH UNDER QUIESCENT CONDITIONS

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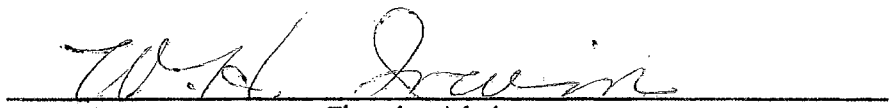

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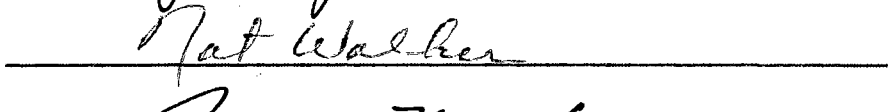

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INTRODUCTION

Many of the factors that influence the survival of fish, i.e. oxygen, carbon dioxide, temperature, hydrogen ions, numbers of organisms present, and others, are not fully understood. The main objective of the project, herein reported, was a study of the oxygen concentrations necessary for certain species of fish under quiescent conditions. The oxygen used in the metabolism of fish varies from the minimum required to maintain life to the maximum they are able to tolerate. Quiescent and active conditions were determined by observing the activity of the fish. Quiescent conditions are those in which the fish remain near the minimum metabolic rate most of the time. Active conditions are those in which the fish remain near the maximum metabolic rate most of the time.

A second objective was to determine recognizable signs indicative of oxygen want for the fish. Westfall (1945), Merkens and Downing (1957), and others, have found that some fish can tolerate high concentrations of certain substances only under high oxygen tensions. All of the substances in the water are not known under varying laboratory conditions. If the characteristic signs of oxygen deficiency occurred at oxygen levels normally tolerated by the fish, oxygen could be added to determine it's effect, and a chemical analysis of the water performed to determine, if possible, the limiting factor.

Four species of fish were obtained in ponds and streams near Stillwater, Oklahoma, and one species was raised in the laboratory. Observations were made concerning the habits and reactions of the fish

as they were captured, transported, stored, and used in experiments. The study was conducted in the Aquatic Biology Laboratory, Oklahoma State University.

REVIEW OF THE LITERATURE

Respiration in fish has received considerable attention since the turn of the century. Studies of the minimum oxygen requirements of a species are extremely difficult, because oxygen requirements vary with the life history stage, and composition of the environment. Oxygen consumption of fish is known to vary with (1) the species, (2) within a species, and (3) within one individual from period to period (Clausen, 1936). The minimum oxygen tolerance (the ability of fish to survive indefinitely in a defined environment), and resistance (the ability of fish to survive for a limited period in a defined environment which will eventually produce death), of some of the common fish has been studied, some under field and some under laboratory conditions.

Many observations have been made during winter, when extremely low levels of dissolved oxygen often accompany the ice and snow. The oxygen demands of fish are reduced in cold water because of reduced metabolism. Cooper and Washburn (1946), and Moyle and Clothier (1959) observed that fish survived oxygen tensions of less than 1 part per million in winter. Greenbank (1945) found that some fish survived below 1 ppm for several days but believed his figures inadequate to be used as a minimum tolerance limit. Other investigators have reported both higher and lower oxygen requirements during the winter months concomitant with various environmental conditions.

During the spring, summer, and fall months, many studies concerning the minimum oxygen requirements of fish have been performed. More (1942)

worked out the oxygen requirements of nine species of fish by placing them in wire cages and lowering the cages in a lake to depths of known oxygen content. He found that some species could tolerate oxygen tensions lower than 1 ppm but that in general, oxygen tensions of less than 3.5 ppm were fatal within twenty four hours. Katz and Gaufin (1953) found that thirty species of fish survived 1 ppm at night when "adequate" concentrations of dissolved oxygen were maintained during the day. Jahoda (1947) studied a trout stream in which the volume had been reduced to pools. He observed the pools and found trout fingerlings surviving 1.1 ppm. Pearse and Achtenberg (1920) observed that Perca flavescens commonly entered stagnant water below the thermocline, where the dissolved oxygen was well below 1 ppm, and concluded it was doubtful that they fed for more than a few minutes. Barney and Anson (1920) reported that Gambusia affinis lived and reproduced in ponds with a dissolved oxygen content of 0.26 ppm.

Among the many studies performed to determine the effects of selected environmental and physiological processes on the minimum oxygen requirements of fish, Fry and Hart (1948), Alabaster et al. (1957), and Downing and Merkens (1957) found an increased oxygen demand with increased temperature. Wells (1913), and Black, Fry, and Black (1954) observed that the oxygen demand increased when the carbon dioxide content increased. Fry, Black, and Black (1947) found the ability of fish to utilize oxygen in the presence of carbon dioxide increased as the temperature increased. Pruthi (1927), Wiebe et al. (1934), and Townsend and Cheyne (1944) found the ability of fish to withstand low oxygen tensions varied with the pH level. Packard (1905) found that he could increase the tolerance of two species of marine fish to low oxygen

levels by increasing the alkalinity. Black (1940) found that those species whose blood had a high affinity for oxygen were able to tolerate low oxygen levels. Root (1930) observed that carbon dioxide reduced the affinity of the blood for oxygen. Krough and Leitch (1919), and Irving, Black, and Safford (1941) found that temperature and carbon dioxide affected the ability of the hemoglobin to bind and release oxygen. Prosser et al. (1957) concluded that Carassius auratus could be adjusted to low oxygen levels of approximately 0.35 ppm by an increasing affinity of the blood for oxygen and a lowering of the oxidative activity of the tissues.

Some investigators have performed experiments to see what effect the gases of the swimbladder have on the ability of fish to withstand low oxygen tensions. Safford (1940) found that the ability to extract oxygen and carbon dioxide from the swimbladder varied with the species, however, Hall (1924), and Black (1942) observed that the swimbladder gases were probably of no significant value in permitting fish to tolerate low concentrations of dissolved oxygen.

In conclusion, the voluminous literature concerning the minimum oxygen requirements of fish indicates that at oxygen tensions of 3 ppm mortality due to hypoxia, is not to be expected under ordinary conditions. In fact, oxygen tensions of 1 ppm, and less, can be tolerated for long periods by even the more sensitive fish, if the other environmental factors are favorable. Because at the present time our knowledge concerning favorable environments is incomplete, and the nonlethal effects of low oxygen tensions may lower the resistance of the fish to disease, parasites, predators, reduction of the food supply, etc., Ellis (1944), Tarzwell (1958), and others, have recommended that to

support a good fish fauna in the field 5 ppm of dissolved oxygen should be the minimum concentration.

MATERIALS AND METHODS

The fish tested for their minimum oxygen requirements were; Pimephales promelas Rafinesque, the fathead minnow; Hybognathus nuchalis Agassiz, the silvery minnow; Gambusia affinis (Baird and Girard), the mosquito fish; and Lebistes reticulatus (Peters), the guppy. In the process of collecting, some Notropis girardi Hubbs and Ortenburger, the Arkansas River shiner, were obtained. The latter species was also tested with the principle study animals. The names of the fish are those approved by the American Fisheries Society (A List of Common and Scientific Names of Fishes from the United States and Canada, Second Edition).

A fine mesh seine was employed to capture P. promelas from a farm pond near Stillwater, Oklahoma, and H. nuchalis and N. girardi from the Cimarron River near Perkins and Coyle, Oklahoma. Gambusia affinis were collected with dip nets from a sewage pond near Lake Carl Blackwell, nine miles west of Stillwater, Oklahoma. Lebistes reticulatus were raised in the laboratory. The principle species were relatively easy to capture from August, 1959, through January, 1960. Notropis girardi were often difficult to locate, being either present in large numbers or completely absent. Hybognathus nuchalis and N. girardi were more easily injured while handling than the other species. Temperature changes of water and high concentrations of fish presented the only transportation problems. "Tail rot" was a troublesome problem with all except L. reticulatus. Water from the laboratory taken to the field to receive the fish, in which they remained overnight, seemed to reduce

mortality and to hasten adjustment to the laboratory environment.

The fish were stored in the laboratory in conditioned water, i.e. tap water that had stood in an open tank for not less than one week. The water was treated with Terramycin to prevent tail rot (Irwin, 1959). All visibly sick individuals were removed as soon as observed. The period required to become accustomed to laboratory conditions ranged from two to five days and varied among species and with the individuals. The fish were fed a mixture of commercial poultry feed and powdered egg (Irwin, personal communication). No attempt was made to separate species or to size specimens while in storage.

TABLE I

TOTAL LENGTHS OF SPECIMENS USED IN THE EXPERIMENTS

Species	Range (mm)	Range that contained at least 50% of the specimens (mm)
L. reticulatus	6-34	20-29
G. affinis	20-55	25-34
P. promelas	25-59	40-49
H. nuchalis	20-70	40-59
N. girardi	20-40	25-34

Two types of containers were used in the tests. A glass aquarium 12-1/8 inches long by 6-3/8 inches wide by 7-1/8 inches deep, and a polyethylene container 11-1/2 inches long by 7-1/2 inches wide by 12 inches deep. Preparatory to testing, the containers were filled with conditioned water and aeration begun. The pH, temperature, and oxygen content of the water were measured after the fish were introduced

(Standard Methods for the Examination of Water and Wastewater, Eleventh Edition). A Beckman 180 pocket pH meter was used to obtain pH readings, and a standard centigrade thermometer was used for measuring temperatures. Oxygen samples were collected with an Irwin Sampler (Welch, 1948), from within one inch of the surface. Oxygen determination was made by use of the Azide modification of the Winkler method and the iodine content was measured in a Bausch and Lomb Spectronic 20 Colorimeter. A portion of the oxygen sample was titrated with sodium thiosulphate and used to adjust the Spectronic 20.

A variety of techniques have been employed to obtain waters with low oxygen tensions. Hart (1944), and Burdick et al. (1954) sealed fish in containers and allowed them to remove oxygen by respiration. Burdick et al. (1957) using the sealed jar method found Perca flavescens could tolerate less than 1 ppm dissolved oxygen. Paton (1902), and Shelford (1918) boiled water to remove the oxygen. Wilding (1939) used the boiling method and found that some fish could tolerate oxygen tensions of less than 1 ppm. Westfall (1945) using an "air rebreather", found that Carassius auratus tolerated 0.9 ppm for two hours. In order to maintain a flow of water of a constant oxygen content a variety of devices, utilizing pure nitrogen gas to remove the oxygen, have been employed. Gardner (1926), Graham (1949), Fry (1951), and Whitmore et al. (1960) are only a few who have utilized such a method.

Low oxygen tensions under laboratory conditions are generally due to a failure of the aerating equipment and subsequent use of the available oxygen by (1) fish, (2) bacterial action on organic wastes, and (3) respiration or decomposition of plants. Low oxygen levels often occur rapidly in the laboratory but usually are soon detected. In order

to more nearly simulate a reduced oxygen situation under laboratory conditions, and because of the financial limitations imposed by the construction of an apparatus similar to the one described in Merkens (1957), such a device was not employed. To remove the dissolved oxygen in the test containers pure nitrogen gas was bubbled through the water. The rate of removal was determined with a trial and error method of manipulating the number of release stones and nitrogen flow. A glass plate was placed in such a way as to cover approximately ninety nine percent of the top of the test container and helped to maintain a more constant oxygen tension in the water. Dissolved oxygen was measured when the fish first showed signs of distress and when they died. Death was considered to be indicated by the cessation of all respiratory movements. The excess water obtained from the sampling method was discarded as surface disturbances changed the dissolved oxygen concentration. It was usually possible to remove the dead fish with a pair of long handled forceps without appreciably distrubing the oxygen content of the water. The dead fish were then measured.

Exploratory tests were run to determine the reactions of the test animals to low oxygen tensions, to learn the approximate tolerance levels for each species, and to measure the resistance of the fish to low oxygen concentrations. A series of experiments were then performed varying the numbers of test animals, temperature, speed of oxygen removal, and time the fish were exposed to the various oxygen tensions. The test periods ranged from two to over twenty four hours (see appendix).

REACTIONS TO LOW OXYGEN TENSIONS

Some general reactions of the specimens to low oxygen tensions were (1) increased opercular movements, (2) a tendency to favor the surface, (3) a loss of ability to maintain a more or less horizontal position, and (4) an apparent total loss of equilibrium. Opercular movements usually increased in rate until, or after equilibrium was lost and then decreased, becoming irregular gasps (see Table II).

Reactions to low oxygen concentrations under conditions of an extremely rapid decrease were generally different than those produced by a moderate rate of oxygen reduction. Under rapid reduction, reactions were similar to those reported by Douglas (1959) using a toxic effluent. All species exhibited a characteristic pattern of violent movements. The most violent were specimens of L. reticulatus. After losing equilibrium; they would sink to the bottom, recover temporarily, dart toward the surface, often leaping two to six inches out of the water, fall back into the water; sink, lay on the bottom, and die. The behavior of G. affinis specimens was not as violent as L. reticulatus but they would dart around the surface often leaping out of the water. The reactions of specimens of P. promelas were less violent than G. affinis. They would dart wildly around the test container, including both the top and the bottom, periodically attempting to leave the unfavorable environment by leaping from it. Hybognathus nuchalis and N. girardi individuals were more placid than any of the other species, although they too would occasionally dart around and attempt to break

TABLE II

REACTIONS OF FISH TO "NORMAL" AND REDUCED OXYGEN TENSIONS IN THE LABORATORY

Species	Behavior at oxygen tensions of 1-10 ppm	Behavior at oxygen tensions of 0-2 ppm*
L. reticulatus	Calm; utilized all areas of the test vessel.	Favored surface; increased opercular rate; gradually assumed vertical position; lost equilibrium; sank; recovered; darted toward the surface; lost equilibrium; sank to the bottom; died on the bottom.
G. affinis	Calm; utilized all areas of the test vessel.	Favored surface; increased opercular rate; gradually assumed vertical position; kept moving at the surface; lost equilibrium; turned on back; died at the surface.
P. promelas	Calm; favored lower areas of the test vessel but utilized all.	Favored surface; grouped; increased opercular rate; gradually assumed vertical position; lost equilibrium; turned on back; died at the surface.
H. nuchalis	Calm; favored lower areas of the test vessel but utilized all.	Favored surface; grouped; increased opercular rate; gradually assumed vertical position; lost equilibrium; turned on back; died at the surface.
N. girardi	Calm; utilized all areas of the test vessel.	Favored surface; grouped; increased opercular rate; gradually assumed vertical position; lost equilibrium; turned on back; died at the surface.

*All species would temporarily recover equilibrium frequently before dying.

the surface and escape the unfavorable environment.

The resistance of the five species of fish to oxygen tensions of less than 1 ppm was observed to be (ascending order), H. nuchalis and N. girardi (no noticeable difference), P. promelas, G. affinis, and L. reticulatus. Two experiments, that contained all of the test species, under fairly uniform conditions were tested statistically and indicated that (1) there was a significant difference among the five species, and (2) there was no significant difference between the two experiments. Lebistes reticulatus was significantly more tolerant of oxygen tensions of less than 1 ppm than were P. promelas, H. nuchalis, and N. girardi, but exhibited no significant difference when compared to G. affinis. Gambusia affinis was not significantly different from P. promelas but did exhibit a significant difference when compared to H. nuchalis and N. girardi. Pimephales promelas, H. nuchalis, and N. girardi did not differ significantly from each other (Table III).

The numbers of fish surviving oxygen tensions of 1.0-0.5 ppm under various temperature ranges were tested statistically, and indicated that (1) there was a significant difference among the temperature ranges, and (2) there was a significant difference among the species at the higher temperatures (Table IV). At temperatures of 18-20°C, and 22-26°C there was no significant difference among the species. At temperatures of 28-31°C G. affinis was significantly different from P. promelas and N. girardi, and P. promelas was significantly different from H. nuchalis. At temperatures of 32-33°C L. reticulatus was significantly different from P. promelas. The analysis also reveals that L. reticulatus and G. affinis had not approached their high temperature-low oxygen tolerance limits, while P. promelas and H. nuchalis apparently had. Sufficient

TABLE III

STATISTICAL ANALYSIS OF THE RESISTANCE OF THE FISH TO OXYGEN TENSIONS OF 1.0-0.5 PPM AT 19-20°C

Experiment	Percent Survival A	B	C	D	E	df	Chi-Square
IX	92.6	79.2	68.2	54.5	55.6	4	14.9421 **
X	85.7	70.6	52.9	46.4	44.4	4	20.8596 **
					Sum	8	35.8017 **
					Pooled	4	35.0018 **
					Heterogeneity	4	0.7998
			A compared to B			1	2.4756
			A compared to C			1	10.9279 **
			A compared to D			1	21.4568 **
			A compared to E			1	22.0298 **
			B compared to C			1	3.0405
			B compared to D			1	9.5381 **
			B compared to E			1	9.9281 **
			C compared to D			1	1.838
			C compared to E			1	2.0136
			D compared to E			1	0.0040

**Chi-square significant at the .01 level

A = *L. reticulatus* B = *G. affinis* C = *P. promelas*

D = *H. nuchalis* E = *N. girardi*

Results

(1) There was a significant difference exhibited among the species.

(2) There was no significant difference between experiments IX and X.

(3) Species A was significantly different from C, D, and E.

(4) Species B was significantly different from D and E.

(5) Species C, D, and E exhibited no significant differences from each other.

TABLE IV

STATISTICAL ANALYSIS OF THE PERCENT SURVIVING OXYGEN TENSIONS OF 1.0-0.5 PPM UNDER VARIOUS TEMPERATURE RANGES

Species	Percent Survival 18-20°C	22-26°C	28-31°C	32-33°C	df	Chi-Square
A	61.7	96	---	100	2	10.3195 **
B	59.1	100	100	---	2	12.9074 **
C	55.8	97.4	73.2	0	3	90.8904 **
D	45.7	100	3	---	2	95.3009 **
E	57.4	100	---	---	1	11.5296 **
				Sum	10	220.9478 **
				Pooled	3	333.4201 **
				Heterogeneity	7	-112.4723 **
				Temperature range 18-20°C	4	2.6843
				Temperature range 22-26°C	4	0.1412
				Temperature range 28-31°C	2	85.4208 **
				B compared to C	1	4.468 *
				B compared to D	1	91.3494 **
				C compared to D	1	64.6724 **
				Temperature range 32-33°C	1	100. **

*Chi-square significant at the .05 level
**Chi-square significant at the .01 level

A = L. reticulatus B = G. affinis C = P. promelas

D = H. nuchalis E = N. girardi

Results

(1) The temperature ranges at which species A, B, C, D, and E were tested differed significantly.

(2) The test for heterogeneity indicated a significant difference among the species.

(3) At temperatures of 18-20°C and 22-26°C there was no significant difference among the species.

(4) At temperatures of 28-31°C B was significantly different from C and D; and C was significantly different from D.

(5) At temperatures of 32-33°C A was significantly different from C.

data were not available for N. girardi.

All species were exposed to an oxygen tension of 1 ppm (± 0.5 ppm), for approximately 18 hours and exhibited no visible signs of distress from oxygen deficiency (Experiment VIII). In Experiment VII all species were exposed to oxygen tensions of 0.62 (± 0.3 ppm), for approximately 22 hours. During that period three small L. reticulatus died (15.8% of the total number), and one P. promelas (5.6% of the total number). Although none of the G. affinis, H. nuchalis, and N. girardi died all P. promelas, H. nuchalis, and N. girardi exhibited signs of distress at the time of termination of the experiment. The analysis indicates that a large number of the fish were able to live at oxygen tensions of 0.42-0.5 ppm, under the given environmental conditions (Table V). The occurrence of no deaths at or above the 0.9 ppm level (Table VI), reveals that at 1.0 ppm all test species should be expected to live under quiescent conditions, in a pH range of 7.4-8.6 and a temperature range of 19-33°C.

The total number of fish upon which the above conclusion was based were 258 L. reticulatus, 150 G. affinis, 207 P. promelas, 167 H. nuchalis, and 86 N. girardi.

Following the recommendations of Tang (1930) all measurements have been presented for the purpose of aiding other investigators (see appendix).

TABLE V

SUMMARIZATION OF ALL TESTS OF THE NUMBERS OF FISH REMAINING ALIVE AT THE OXYGEN LEVELS AT WHICH THE TESTS WERE TERMINATED

	Oxygen ppm	Species A	B	C	D	E
Alive at experiments inception	8.0	18	15	16	16	13
Alive at experiments termination	0.9	18	15	16	16	13
Alive at experiments inception	3.7-8.0	70	53	73	67	45
Alive at experiments termination	0.5	58	41	30	35	26
Alive at experiments inception	8.0	27	24	22	33	18
Alive at experiments termination	0.42	25	19	15	18	10

A = L. reticulatus B = G. affinis C = P. promelas

D = H. nuchalis E = N. girardi

TABLE VI

SUMMARIZATION OF ALL TESTS OF THE NUMBERS OF FISH DEAD AND ALIVE AT VARIOUS OXYGEN LEVELS

Species	Oxygen ppm .08	.24	.29	.32	.40	.42	.45	.50	.56	.62	.66	.72	.80	.86
A (dead)	-	55	22	55	5	2	3	4	-	5	-	-	6	-
(alive)	-	55	77	122	129	164	167	229	-	234	-	-	240	-
B (dead)	18	35	-	-	5	-	5	7	-	5	-	-	-	-
(alive)	18	53	-	-	58	-	82	130	-	135	-	-	-	-
C (dead)	-	34	7	6	22	16	8	11	11	7	15	6	-	3
(alive)	-	34	41	47	69	100	108	149	160	167	182	188	-	191
D (dead)	-	10	1	-	5	13	15	8	30	15	-	-	1	-
(alive)	-	10	11	-	16	47	62	105	135	150	-	-	151	-
E (dead)	-	10	-	-	-	-	15	4	-	8	-	-	-	-
(alive)	-	10	-	-	-	-	35	65	-	73	-	-	-	-

A = L. reticulatus B = G. affinis C = P. promelas

D = H. nuchalis E = N. girardi

DISCUSSION

The method of oxygen removal employed undoubtedly affected the relative concentrations of other dissolved gases in the water, because bubbling nitrogen through water removes dissolved gases more or less readily (Dr. T. E. Moore, Professor of Chemistry and Dr. E. E. Kohnke, Associate Professor of Physics, Oklahoma State University, personal communications). In water certain dissolved gases could become concentrated and might prove toxic to fish, especially at low oxygen tensions. The presence of carbon dioxide as a lethal factor can be approximated by pH measurement. Shelford, and Allee (1913), Powers et al. (1938), and Fry (1957) considered carbon dioxide to be more significant in respiration than oxygen. The lethal effects of all the dissolved gases have not been determined. Therefore, results obtained from experiments using nitrogen to remove oxygen from the water must be applied to laboratory or field conditions with discretion.

Belding (1929), and others, have found that visible signs of oxygen distress varied with the environmental conditions. Downing (1954), and others, have found that the ability of fish to withstand low oxygen tensions varied with the chemical composition of the water. Therefore, the use of visible signs of distress because of low oxygen tensions must also be used with discretion. At times a complete chemical analysis and further testing for the presence or absence of conditions required by fish will be necessary.

Because of the human and mechanical errors inherent in the method

of oxygen determination employed no attempt was made to fix the minimum oxygen requirements under 1 ppm. Although the collecting, handling, and storing techniques employed may have resulted in a biased population of test animals, making it difficult to estimate to what degree the fish tested were representative of the population sampled, it was felt that such a bias always operates, to a greater or lesser degree, when wild fish are transplanted to a laboratory environment.

It was noted that placing a large number of fish in the test containers seemed to lower the tolerance of the larger specimens of P. promelas and H. nuchalis to low oxygen tensions. Carbon dioxide and oxygen tensions apparently were not limiting factors. Since the larger specimens died when a larger population was present, even though the oxygen tension was higher, it seems some other factor must have been involved. The controversal "group effect" of Schuett (1933), in which fish at times used less oxygen per individual in groups than they did when they were alone, may also be an explanation. For a review of the effects of numbers on physiological functions see Allee (1934).

When the fish with terminal mouths assumed a vertical position at the surface of the water their mouths were extremely close to the surface film. After equilibrium was lost and they turned on their backs their mouths were further away from the surface by several millimeters. Fish with ventral mouths would have the situation reversed. Because the surface area of water appears to contain the most oxygen a fish with a terminal mouth has an advantage over one with a ventral mouth while it's power of equilibrium is still present.

It would seem from the literature, and from personal observations, that oxygen, by itself, should rarely be a limiting factor in the upper

layer of water when in contact with the atmosphere. It is necessary that some oxygen be available, and interactions of oxygen with the other environmental factors are extremely important. Marsh (1908) concluded that water could be too "pure" and additional matter other than oxygen is necessary to maintain life. Clausen (1936) observed that fish of warm, slow-moving waters, were able to tolerate extremely low oxygen tensions if given access to the upper one to five inches of water. Odum and Caldwell (1958) studied a natural hot spring in Florida to observe which species inhabited the areas of low oxygen content. They found that Gambusia affinis tolerated 0.3 ppm if given access to the surface of the water. Difficulty was encountered throughout the present study in trying to keep oxygen out of the water. Dissolved oxygen was never found completely removed during any of the tests, presumably because the apparatus employed was not efficient enough to overcome the counter effects of diffusion at the water's surface.

The fish tested represented two orders and two families. Order Cypriniformes, family Cyprinidae, was represented by P. promelas, H. nuchalis, and N. girardi. Order Cyprinodontiformes, family Poeciliidae, was represented by L. reticulatus and G. affinis. The analysis indicates that (1) the low oxygen tolerances of the members within either family were not statistically significant, and (2) there was a significant difference among the members of one family and the members of the other family (Table III). The analysis indicates that the poeciliids tolerated low oxygen tensions at high temperatures better than the cyprinids (Table IV). The poeciliids (1) tended to spend more time in the surface layer of water than did the cyprinids, and (2) possessed "uplifted" terminal mouths compared to the terminal to sub-terminal mouths of the cyprinids.

The inherent respiratory capabilities of the fish, their habits, and external morphology suggests that resistance to oxygen tensions of less than 1 ppm may have taxonomic implications at either the order or family level.

Our knowledge of the inherent respiratory requirements of fish, and the effects of the environment on them, is fragmentary, incomplete, and contradictory. Because a knowledge of the minimum oxygen requirements of fish is important in establishing water quality criteria a pressing need is the determination of the oxygen requirements of fish during their normal activities, i.e. feeding, growing, and breeding.

Another practical application for a knowledge of the minimum oxygen requirements of fish would be the establishment of required minimum oxygen tensions in bioassay work. The present use of the bioassay at the Aquatic Biology Laboratory, Oklahoma State University, is in determination of degree of toxicity of oil refinery effluents to fish. It is not used to measure the lethal effects of oxygen depletion. An effluent may have an oxygen demand which in itself can distress or kill fish. Bubbling oxygen through the effluent to satisfy the oxygen demand may expel some of the toxic substances. A knowledge of the minimum oxygen requirements of the test animals would allow the addition of only enough oxygen to maintain the "normal" physiological functions of the test animals, concomitant with the existing environmental conditions.

SUMMARY

1. Experiments were performed concerning the minimum oxygen requirements of five species of fish under quiescent conditions and the results are presented.
2. All specimens of L. reticulatus, G. affinis, P. promelas, H. nuchalis, and N. girardi were able to live at oxygen tensions of 1.0 ppm.
3. Reactions of the fish to low oxygen tensions varied with (1) the speed of oxygen removal, and (2) the species.
4. The effects of temperature on the ability of the fish to tolerate oxygen tensions of less than 1 ppm were tested statistically and the results are presented.
5. The resistance of the fish to oxygen tensions of less than 1 ppm was tested statistically and the results are presented.
6. The difficulties encountered in acquiring and applying a knowledge of the minimum oxygen requirements of fish are discussed.
7. Areas for future research are suggested.

LITERATURE CITED

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APPENDIX

EXPERIMENT I

Date	Time	Temp. C.	pH	D.O. ppm	Observations
11-30-59	0810	--	--	---	14 H. nuchalis introduced in glass aquarium; no oxygen added.
12-3-59	1300	31	8.4	2.46	Nitrogen flow started.
	1330	--	--	1.23	Increased operculer rate; favored surface.
	1400	--	--	1.16	At surface for longer periods.
	1930	--	--	0.42	Some lost equilibrium; recovered; moved to bottom for brief periods; returned; turned on back.
	2010	--	--	0.72	1 H. nuchalis died.
	2150	--	--	0.56	1 H. nuchalis died.
	2230	--	8.4	0.56	5 H. nuchalis died.
					1 H. nuchalis died.
	2300	--	--	0.56	2 H. nuchalis died.
					3 H. nuchalis died.
12-3-59	2340	29	8.5	0.56	1 H. nuchalis died.

EXPERIMENT II

Date	Time	Temp. C.	pH	D.O. ppm	Observations
12-6-59	0730	--	--	--	19 H. nuchalis introduced in glass aquarium; no oxygen added.
	1330	30	8.2	0.56	Increased opercular rate; fish favored surface; nitrogen stripping begun.
	1500	--	--	0.32	Some began to lose equilibrium.
	1545	--	--	0.80	1 H. nuchalis died.
	1830	--	--	0.56	13 H. nuchalis died.
	1900	--	--	--	4 H. nuchalis died.
	2230	31	8.4	0.29	1 H. nuchalis died.

EXPERIMENT III

Date	Time	Temp. C.	pH	D.O. ppm	Observations
12-6-50	0730	--	--	--	18 G. affinis introduced in glass aquarium; no oxygen added.
	1330	30	8.2	4.20	Slow nitrogen flow; large bubbles.
	1500	--	--	3.40	
	1830	--	--	2.31	
	2230	--	--	2.09	
	2300	31	--	--	Nitrogen flow stopped.
12-8-59	1830	29	8.4	1.42	Nitrogen flow started.
	1900	--	--	0.56	Favored surface; increased opercular movement but fish
	1925	--	--	0.56	left surface for long periods and seemed to be able to
	2015	--	--	0.56	tolerate this tension with no trouble; nitrogen flow
	2045	--	--	0.56	increased.
	2215	29	8.2	0.08	18 G. affinis lost equilibrium and died.

EXPERIMENT IV

Date	Time	Temp. C.	pH	D.O. ppm	Observations
12-8-59	1830	29	8.2	2.40	41 *P. promelas* introduced in glass aquarium; nitrogen flow started.
	1900	--	--	0.80	Increased opercular rate; fish favored surface.
	1915	--	--	0.72	Some began to lose equilibrium; tended to group at surface.
	1945	--	--	0.56	1 *P. promelas* died.
	1955	--	--	0.56	10 *P. promelas* died.
	2005	--	--	0.40	17 *P. promelas* died.
	2020	--	--	0.29	7 *P. promelas* died.
	2106	28	8.4	0.32	6 *P. promelas* died.

EXPERIMENT V

Date	Time	Temp. C.	pH	D.O. ppm	Observations
12-9-59	0730	--	--	--	28 L. reticulatus introduced in glass aquarium; no oxygen added.
	1010	26	7.9	4.0	Nitrogen flow started.
	1020	--	--	0.96	Increased opercular rate.
	1045	--	--	0.56	Favored surface.
	1105	--	--	0.56	Losing equilibrium; activity violent; some jumped from water.
	1130	--	--	0.29	22 L. reticulatus died.
	1145	26	7.9	0.80	6 L. reticulatus died.

EXPERIMENT VI

Date	Time	Temp. C.	pH	D.O. ppm	Observations
12-16-59	1200	--	--	--	9 P. promelas, 55 L. reticulatus introduced in glass aquarium; no oxygen added.
	1445	33	8.4	1.70	Nitrogen flow started.
	1522	--	--	0.90	P. promelas and L. reticulatus favored surface; increased opercular rate; some P. promelas lost equilibrium.
					3 P. promelas died.
	1555	--	--	0.72	6 P. promelas died.
	1610	--	--	0.32	L. reticulatus lost equilibrium; violet activity culminated by wild jumping from water.
	1630	32	8.3	0.32	55 L. reticulatus died.

EXPERIMENT VII

Date	Time	Temp. C.	pH	D.O. ppm	Observations
1-13-60	1015	24	8.6	8.00	19 L. reticulatus, 18 P. promelas, 14 G. affinis, 16 H. nuchalis, and 12 N. girardi introduced in polyethylene container; started nitrogen flow.
	1125	--	--	2.65	
	1430	--	--	1.42	
	1505	--	--	0.72	H. nuchalis and N. girardi favored surface; increased opercular movement.
	1930	--	--	0.62	Increased nitrogen flow.
					3 L. reticulatus (small) died.
	2230	--	--	0.62	
	2300	--	--	0.40	H. nuchalis and N. girardi began to assume a vertical position; some P. promelas began showing signs of distress.
1-14-60	0730	--	--	0.86	
	1100	--	--	0.50	1 P. promelas died.
	1245	24	8.5	0.50	Experiment stopped: H. nuchalis and N. girardi stayed on surface; G. affinis and L. reticulatus appeared normal.

EXPERIMENT VIII

Date	Time	Temp. C.	pH	D.O. ppm	Observations
1-13-60	1015	24	8.6	8.00	18 L. reticulatus, 16 P. promelas, 15 G. affinis, 16 H. nuchalis, and 13 N. girardi introduced in polyethylene container; started nitrogen flow.
	1125	--	--	4.50	
	1430	--	--	1.92	
	1515	--	--	0.86	H. nuchalis and N. girardi tend to favor surface: reduced nitrogen flow.
	1610	--	--	1.56	
	1800	--	--	1.42	
	1845	--	--	1.23	
	2230	--	--	1.16	
	2300	--	--	1.05	
1-14-60	0730	--	--	1.56	
	1100	--	--	1.42	
	1245	24	8.5	0.90	H. nuchalis and N. girardi tend to favor surface but no other signs of distress noted; experiment stopped.

EXPERIMENT IX

Date	Time	Temp. C.	pH	D.O. ppm	Observations
1-16-60	0810	19	8.2	8.00	22 *P. promelas*, 33 *H. nuchalis*, 24 *G. affinis*, 27 *L. reticulatus*, and 18 *N. girardi* introduced in glass aquarium; started nitrogen flow.
	0945	--	--	0.86	
	1100	--	--	0.86	Some *G. affinis*, *L. reticulatus*, and *P. promelas* at surface; all *H. nuchalis* and *N. girardi* at surface.
	1210	--	--	0.62	Some specimens of all species lost equilibrium.
	1245	--	--	0.50	7 *P. promelas*, 15 *H. nuchalis*, 5 *G. affinis*, 2 *L. reticulatus*, and 8 *N. girardi* died.
	1700	--	8.2	0.42	Stopped nitrogen; left fish without oxygen.
1-23-60	0800	20	7.8	2.31	Remaining fish survived the 7 day period on oxygen obtained by diffusion in the surface layer of water.

EXPERIMENT X

Date	Time	Temp. C.	pH	D.O. ppm	Observations
1-16-60	0810	19	8.2	8.00	17 G. affinis, 17 P. promelas, 21 L. reticulatus, 27 N. girardi, and 28 H. nuchalis introduced in polyethylene container; started nitrogen flow.
	0945	--	--	0.62	
	1100	--	--	0.56	Some G. affinis, L. reticulatus, and P. promelas individuals at surface; all H. nuchalis and N. girardi at surface. Some specimens of all species lost equilibrium.
	1210	--	--	0.42	5 G. affinis, 8 P. promelas, 3 L. reticulatus, 15 N. girardi, and 14 H. nuchalis died.
	1245	--	--	0.50	Stopped nitrogen flow.
1-23-60	0800	20	7.7	3.09	Remaining fish survived the 7 day period on oxygen obtained by diffusion in the surface layer of water.

EXPERIMENT XI

Date	Time	Temp. C.	pH	D.O. ppm	Observations
1-23-60	0845	19	7.8	2.40	36 *P. promelas*, 8 *H. nuchalis*, 10 *N. girardi*, 30 *G. affinis*, and 50 *L. reticulatus* introduced in polyethylene container; nitrogen flow started.
	1000	--	--	1.16	
	1030	--	--	1.16	
	1115	--	--	0.96	*H. nuchalis*, *N. girardi*, and some *P. promelas* began to favor the surface.
	1140	--	--	0.42	*N. girardi*, *H. nuchalis*, and some *P. promelas* assumed vertical position.
	1145	--	--	--	7 *P. promelas* and 3 *H. nuchalis* died; *G. affinis* followed by *L. reticulatus* began showing behavior indicating oxygen want.
	1300	19	7.8	0.24	10 *N. girardi*, 30 *G. affinis*, 29 *P. promelas*, 5 *H. nuchalis*, and 50 *L. reticulatus* died.

EXPERIMENT XII

Date	Time	Temp. C.	pH	D.O. ppm	Observations
1-23-60	0845	18	7.8	3.70	38 *P. promelas*, 23 *H. nuchalis*, 6 *N. girardi*, 30 *L. reticulatus*, and 22 *G. affinis* introduced in glass aquarium; nitrogen flow started.
	1000	--	--	1.64	
	1030	--	--	0.96	All species favored surface; some *H. nuchalis* and *N. girardi* began to lose equilibrium.
	1140	--	--	0.66	Some *P. promelas* lost equilibrium and 2 died.
	1200	--	--	--	13 *P. promelas* died.
					Some *G. affinis* and *L. reticulatus* lost equilibrium.
	1300	--	--	0.42	9 *P. promelas* died; 2 *L. reticulatus* died; 5 *H. nuchalis* died.
	1400	--	--	0.42	5 *H. nuchalis* died.
	1605	18	7.8	0.50	10 *P. promelas*, 8 *H. nuchalis*, 7 *G. affinis*, 4 *N. girardi*, and 4 *L. reticulatus* died.
					Experiment stopped; all the remaining *H. nuchalis* and *N. girardi* were on their backs almost dead; the remaining *P. promelas*, *G. affinis* and *L. reticulatus* individuals showed no ill effects.

EXPERIMENT XIII

Date	Time	Temp. C.	pH	D.O. ppm	Observations
1-25-60	1435	24	7.5	4.90	5 H. nuchalis, 5 P. promelas, 5 L. reticulates, and 5 G. affinis introduced in polyethylene container; nitrogen flow started.
	1525	--	--	0.42	H. nuchalis and P. promelas favored surface and lost equilibrium; G. affinis and L. reticulatus come to surface; violent activity.
	1620	24	7.4	0.40	5 L. reticulatus, G. affinis, H. nuchalis, and P. promelas died.

Experiment XIV

Date	Time	Temp. C.	pH	D.O. ppm	Observations
1-25-60	1435	22	7.6	7.00	5 H. nuchalis, 5 P. promelas, 5 L. reticulates, and 5 G. affinis introduced in glass aquarium; nitrogen flow started.
	1525	--	--	0.42	H. nuchalis and P. promelas favored surface and lost equilibrium. Violent characteristic activity; all lost equilibrium.
	1640	22	7.5	0.24	5 L. reticulatus, G. affinis, H. nuchalis, and P. promelas died.

VITA

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