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CERTAIN ASPECTS OF GILL RESPIRATION OF GARS

(PISCES: LEPISOSTEIDAE)

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JOHN C. LANDOLT

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CERTAIN ASPECTS OF GILL RESPIRATION OF GARS

(PISCES: LEPISOSTEIDAE)

APPROVED BY

Loren E. Pierce

Hubert Frings

Ronald L. Perkins

Elroy L. Rice

DISSERTATION COMMITTEE

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CERTAIN ASPECTS OF GILL RESPIRATION OF GARS

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CHAPTER I

INTRODUCTION

The behavior of coming to the surface of the water to gulp air by members of the genus Lepisosteus has been described by many observers (Poey, 1858; L. Agassiz, 1859; A. Agassiz, 1879; Wilder, 1876, 1877; Mark, 1890; Morris, 1892; Suttkus, 1963) and such behavior has generally been related to respiratory considerations.

The respiratory function of the swimbladder and the phenomenon of aerial breathing in gars has been studied by several investigators. Potter (1927) found that longnose (L. osseus) and shortnose (L. platostomus) gars that had been deprived of the ability to gulp air into the swimbladder died in a matter of hours when placed into deoxygenated water, but survived for several days in well aerated water. On the other hand, fish could live up to twenty days in oxygen depleted water if they were allowed to gulp air. These results were essentially duplicated by McCormack (1967) working with a different species of gar (L. platyrhinchus).

In recent years, aerial respiration in gars has been the object of various studies conducted by investigators at the University of Oklahoma. Saksena (1963) devised a method of remotely recording the rates of aerial

breathing of longnose gar, and spotted gar. Increases in rates of aerial breathing were correlated with increasing temperature, higher levels of activity and feeding. Rates of aerial breathing were generally slightly higher at night than during daylight hours. Winston (1967) observed many of these same responses in similar experiments involving alligator gar L. spatula. Both he and Saksena felt that the rate of aerial breathing was a function of the metabolic rate of the gars under the various experimental conditions.

Renfro (1968) using longnose, spotted and alligator gars in a variety of experiments concerning aerial respiration, reported a number of observations. Young-of-year longnose and spotted gars, deprived of atmospheric gas exchange, exhibited average oxygen consumptions at rates almost ten times greater than those of adult animals tested by Potter. These juvenile fishes were tolerant of dissolved oxygen concentrations as low as 2.45 parts per million (ppm). Using aerial breathing rates as an index of metabolic activity, he found that spotted gar require as much as 54 hours to recover from handling and that alligator gar require between 48 and 72 hours to adjust to temperature changes 5-10 F. Renfro observed no ill effects on gars deprived of aerial gas exchange as long as the dissolved oxygen concentration of the ambient water was maintained at or above 5 ppm at 21 C. The hydrostatic nature of the swimbladder of gars was also demonstrated.

To date, the capability of gars to take up oxygen by way of their gills has been little investigated. The purposes of the present study were two-fold. The first was to measure metabolic rates, branchial respiratory volumes and per cent uptake of available oxygen using an experi-

mental design which limited the access of gars to atmospheric oxygen. Such experiments were conducted at several different temperatures and over a range of moderate to high dissolved oxygen concentrations in the ambient water.

The second objective was to investigate the structure and arrangement of the gill apparatus of gars and to estimate the amount of respiratory surface area afforded by these structures.

CHAPTER II

MATERIALS AND METHODS

All gars used in this study were collected from Lake Texoma, an impoundment of the Red and Wachita rivers on the border between Texas and Oklahoma. Adult fishes (285-820 g) were obtained from gill-nets or by electro-fishing. Young-of-year gars used for the gill area studies were collected by dipnet and seining. The gars were maintained in large concrete or metal tanks in well aerated water. The fishes were fed regularly a diet of silversides (Menidia audens) or commercial bait minnows. Adult gars were weighed to the nearest five grams and a color-coded dart tag was inserted into the musculature at the caudal base of the dorsal fin for identification purposes. Young-of-year gars were weighed to the nearest gram.

At the time of weighing and tagging, gars to be used in the metabolism experiments were equipped with a polyethylene cannula sleeve to which a gill effluent cannula was later attached. This process involved drilling a small hole through the opercular bone toward its posterior margin, just large enough to permit insertion of a one inch length of P.E. 200 Clay-Adams Intramedic tubing through the hold and out through the opercular slit. The proximal end of the tubing was heated with a match flame to create an expanded flare. This flared end was drawn back flush with the internal surface of the operculum and fixed in place by a cuff posi-

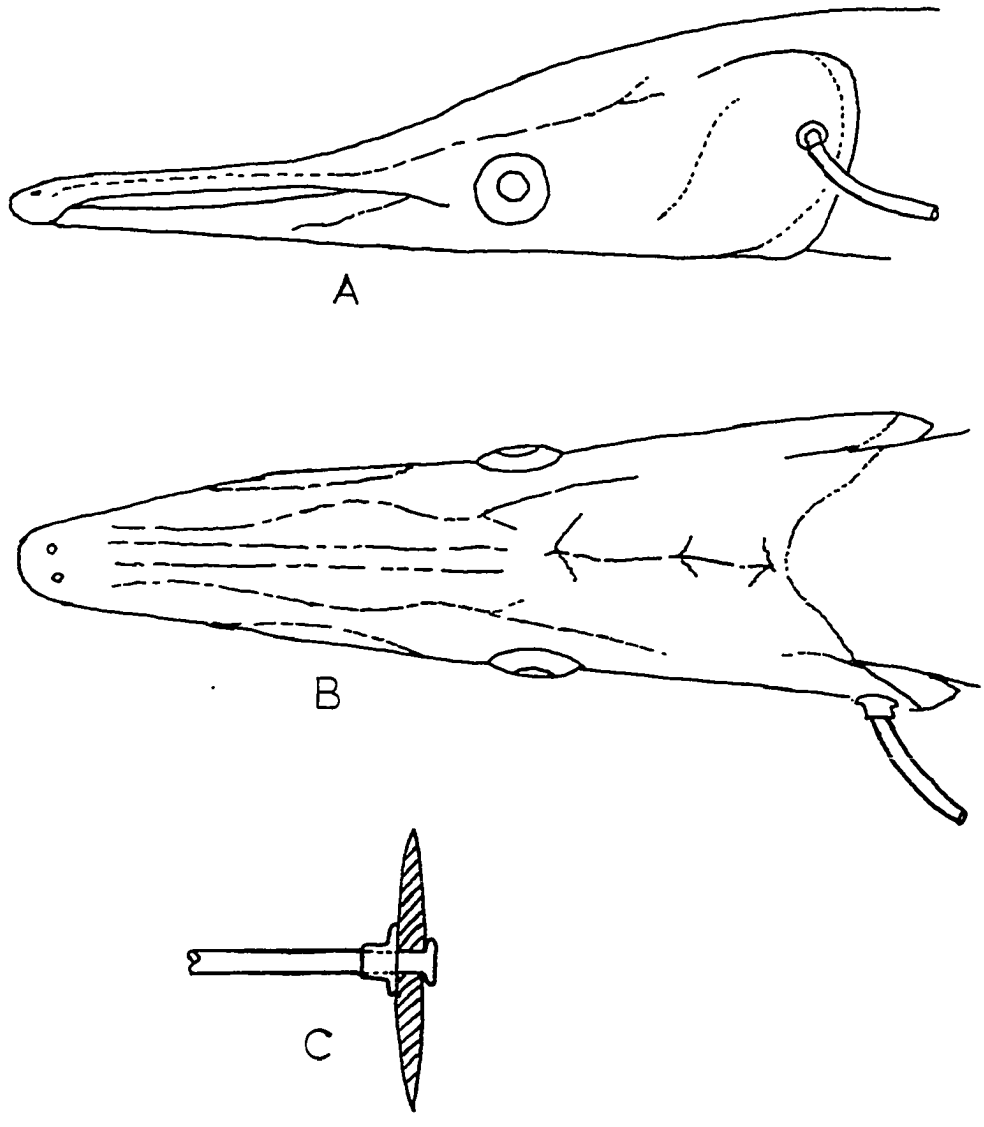

Figure 1. Placement of the cannula sleeve through the opercular bone of a gar (A, side view; B, top view). Part C shows the detail of the internal, terminal flare and the external positioning cuff.

tioned against the external surface of the opercular bone (Figure 1). The placement of this sleeve did not appear to interfere with the normal opercular movements of the fishes.

The opercular effluent cannula itself was of P.E. 90 Clay-Adams polyethylene tubing. The diameters of the cannula and cannula sleeve were such that by inserting the former into the latter a rather snug connection was achieved with little or no leakage probable.

The design of the experimental respirometer was a modification of that described by Saunders (1962), and is shown in Figure 2.

The horizontal holding compartment was a one meter section of plexiglass tubing with an inside diameter of 10.2 cm. Two smaller tubes (I.D. = 0.95 cm) were fixed along the outside of the holding compartment 180 degrees apart. A series of small holes was drilled to communicate the small tubes with the holding compartment. The lateral tubes opened into a vertical standpipe. Water was pumped from a reservoir through a glass wool filter to the overflow container and then into the vertical standpipe where it was mixed. The rate of flow through the entire system was regulated by a screw clamp at the effluent end and was measured by a flowmeter or by timed filling of a vessel of known volume. The gill effluent cannula, which was used to sample water that the fish passed over its gills, exited through a port in the wall of the holding compartment.

A stirrer, rotated by a small electric motor on the standpipe, created an internal circulation within the holding compartment by drawing water from the holding compartment through the small tube connected below the stirrer blades, which was replaced by water mixed with that coming from a large reservoir *via* the small tube connected to the standpipe just above the stirrer blades.

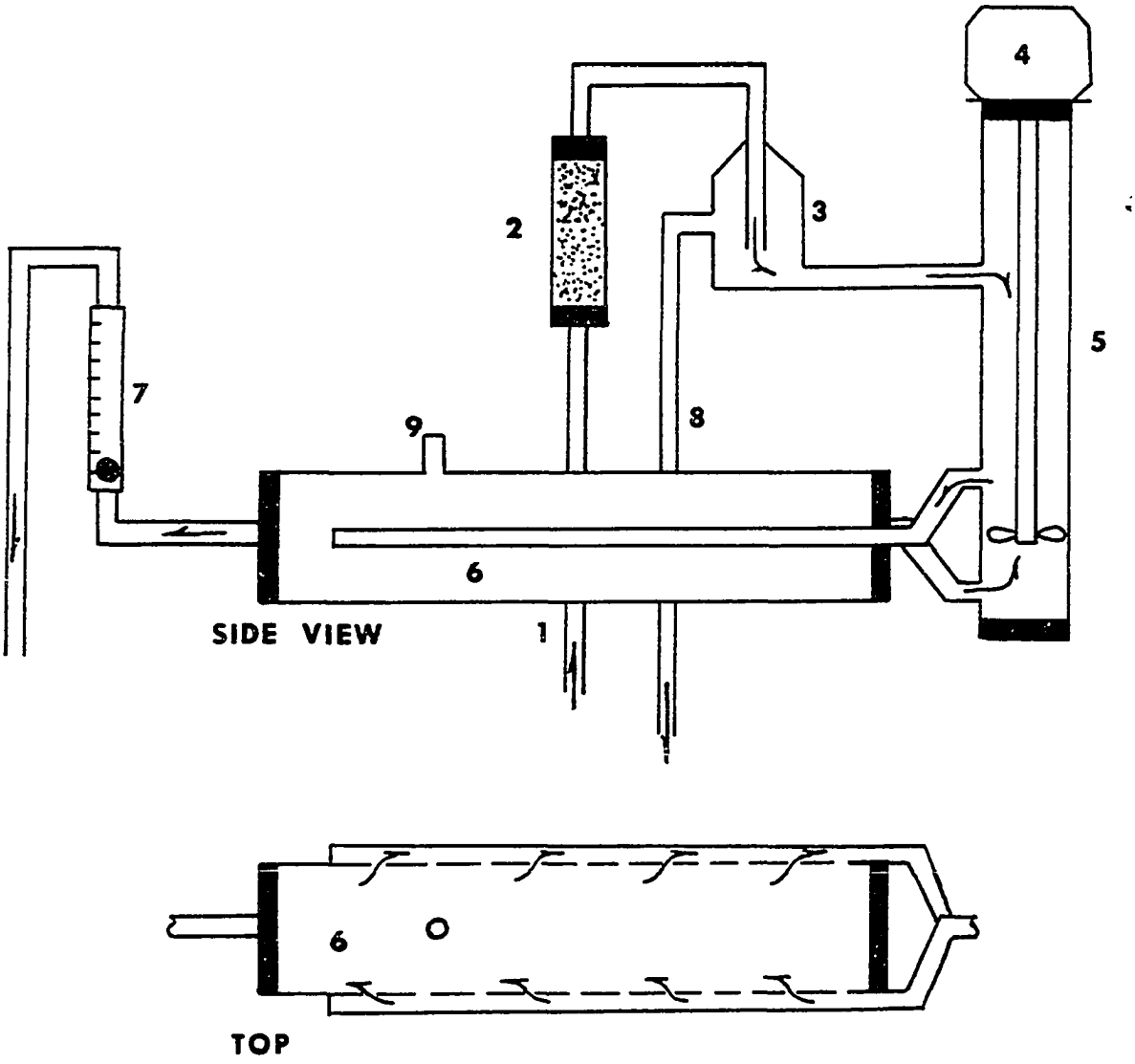

Figure 2. Schematic diagram of the respirometer chamber: 1, inlet from large reservoir of aerated water; 2, glass wool filter; 3, overflow reservoir; 4, stirrer motor; 5, vertical standpipe; 6, horizontal holding compartment; 7, flowmeter; 8, overflow return; 9, exit port for opercular cannula. Arrows denote direction of water flows.

Experimental Procedure

The general procedure for the experiments was similar to that followed by Saunders (1962). Gars that had previously been weighed and equipped with the opercular cannula sleeve were connected to the gill effluent cannula and placed into the holding compartment of the respirometer. Water was pumped into the system from the large reservoir completely filling the holding compartment. The rate of flow was set at a rather high value (1-2 liters/min). A period of twelve hours was allowed for the fish to recover from handling and to adjust to confinement in the chamber. At the end of this time, the flow rate was set at a specified level (70-1000 ml/min), and successive samples of water leaving the holding compartment were collected and the dissolved oxygen concentrations of these samples determined. This was done until the dissolved oxygen level in the effluent water stabilized. When this point was reached, opercular breathing rates were recorded and samples of water delivered by the opercular cannula taken. The flow rate of water through the system was then adjusted to a different level and the process repeated.

In initial experiments conducted at 20 C, a series of shortnose and spotted gars were tested. Because the holding compartment was completely filled with water, attempts to perform aerial breathing motor patterns were apparently frustrated. This appeared to have an excitatory effect upon the animals which persisted for a period of 10 to 14 hours after tests were begun.

It was discovered that this excited state was greatly alleviated by permitting an air space to remain above the animal in the respirometer,

which allowed the gar to perform its aerial breathing behavior. A series of tests under this condition was conducted at the same temperature, on the assumption that the fish would remove most, if not all of the oxygen in the air space during the 12 hour adjustment period. Such depletion could not be verified and therefore, an atmosphere of nitrogen gas was utilized in all subsequent experiments.

Longnose and shortnose gars of suitable size became unavailable during the course of the study. Most of the results of investigations of gill respiratory characteristics are therefore based upon experiments conducted using spotted gar.

Computation Formulas and Symbols

The formulas used to calculate the various gill respiratory factors are identical to those reported by Saunders (1962), with the exception of the symbolism employed. Definitions of symbols used in the present study are as follows:

C_{rO_2} = Concentration of dissolved oxygen in the reservoir water (cc O_2/liter).

C_{iO_2} = Concentration of dissolved oxygen in water leaving the system at equilibrium (ie. that of water inspired by the fish). (cc O_2/liter)

C_{eO_2} = Dissolved oxygen concentration of water that had been passed over the gills as sampled by the opercular cannula. (cc O_2/liter)

$\dot{V}_w$ = Flow rate of water through the respirometer (ml/min).

$\dot{V}_g$ = Respiratory volume (ml/sec).

$\dot{V}_{O_2}$ = Oxygen consumption (cc O_2/hr).

Under equilibrium conditions:

$$\dot{V}_{O_2} = (C_{rO_2} - C_{iO_2})\ (\dot{V}_w)\ (60\ \text{min/hr})\ (1\ \text{liter}/1000\ \text{ml})$$

$$\dot{V}_g = \frac{(C_{rO_2} - C_{iO_2})\ (\dot{V}_w)\ (1\ \text{min}/60\ \text{sec})}{C_{iO_2} - C_{eO_2}}$$

$$\%\ \text{Uptake of available oxygen} = \frac{(C_{iO_2} - C_{eO_2})\ X\ 100}{C_{iO_2}}$$

Dissolved Oxygen Determinations

The azide modification of the Winkler method of dissolved oxygen determination was utilized. The reagents were prepared according to the outlines reported by the American Public Health Association (1955).

Samples (150 ml) of system effluent water were titrated against 0.025 N sodium thiosulfate delivered by a 50 ml blue-line burette. The 6 ml samples of gill effluent water were titrated against 0.0025 N thiosulfate delivered by a micrometer burette of 2.0 ml capacity with units of 0.01 ml. These small samples were acidified with ortho-phosphoric acid rather than with concentrated sulfuric acid to reduce the possible loss of iodine due to heating (Fox and Wingfield, 1938).

The thiosulfate solutions were standardized frequently and checked against common water samples to make sure they rendered comparable determinations.

Temperature

Gars, at least in Lake Texoma, may be exposed to a wide range of seasonal environmental temperatures; from less than 50 F during the winter to at least 90 F during the summer months (Grinstead 1965). This fact, along with the information available on the aerial respiratory behavior of

gars with respect to temperature changes, suggested that experiments concerning gill respiratory capabilities should be conducted at several different temperature levels. Temperatures used in the present study were 12 C, 20 C and 30 C.

Experimental animals were maintained at these selected temperatures for periods of at least two weeks before being tested in the respirometer. Gars with a recent thermal history different from any particular test level were brought to that temperature at a rate of change of 1.0 C per day, before the two week adjustment period was begun. Beamish (1964) used a similar "acclimation" procedure in metabolic studies of several species of fishes. This procedure was compatible with the results of acclimation studies by Renfro (1968) involving alligator gar.

Gill Area Determinations

In this portion of the study, gill material from longnose, shortnose and spotted gars was examined.

The sites of functional gas exchange of the gill of most fishes are the epithelial surfaces of the secondary lamellae of the gill filaments of the branchial arches (Hughes and Shelton, 1962; Lagler, Bardach and Miller, 1962). To obtain estimates of the total area of the secondary lamellae of gars, a combination of the methods reported by Gray (1954), Hughes (1966) and Saunders (1962) was employed.

The four gill arches from the left side of the branchial chamber were carefully dissected from freshly sacrificed animals. This gill material was placed immediately into a solution of 10% formalin. All counts and measurements were made within several hours after removal of the gill arches.

The gill filaments are arranged in two rows on each arch, the anterior and posterior hemibranchs. The number of filaments of each hemibranch on each of the four arches was counted. This total was doubled to yield the number of filaments of the entire gill network. Average filament length was calculated on the basis of the length of every tenth filament of the anterior hemibranch of each arch, as measured by a calibrated ocular micrometer of a dissecting microscope. The number of secondary lamellae contained in selected one millimeter segments of these same filaments was counted. Several secondary lamellae from regions about one third and two thirds of the distance from the base were dissected from filaments located near the dorsal, middle and ventral parts of each gill arch. These representative lamellae were drawn in outline on calibrated graph paper with the aid of a camera lucida affixed to a compound microscope. The areas of the lamellae were estimated by counting the number of squares of the graph paper inclosed by the outlines. Since both sides of the plate-like lamellae are exposed to the ventilation current, these areas were doubled.

The total gill area of the fish examined could then be calculated by multiplying together the values of total number of filaments, mean filament length, mean number of secondary lamellae per millimeter filament length and their average areas.

There is a well developed pseudobranch that is intimately attached to the inner surface of each opercular bone of gars. It is similar in structure to a single hemibranch with what appear to be secondary lamellae attached to its filaments. The pseudobranch is covered by a thin transparent membrane. Because of difficulty in dissecting these structures and their uncertain role in gas exchange in the usual sense (Lagler et al., 1962), their contribution to the total gill area of these fishes was not considered.

CHAPTER III

RESULTS

Respirometer Experiments

Atmosphere Effects

Initial determinations of metabolic rate, gill respiratory volume, per cent utilization of available oxygen and observations of opercular breathing rate were performed using shortnose and spotted gars at 20 C, with no atmosphere present in the holding compartment of the respirometer chamber. These observations and determinations suggested that these fishes were additionally stressed by the absence of an atmosphere which prevented aerial breathing behavior. Comparisons of the data from these animals with those from other gars tested at the same temperature, but with either a conditioned air or nitrogen atmosphere tended to support this hypothesis. Animals deprived of an atmosphere generally had much higher rates of opercular movements, higher values of respiratory volume, lower levels of per cent utilization and higher metabolic rates than did the fishes provided with either type of atmosphere (Table 1). All data were accumulated after comparable periods of confinement in the respirometer and at similar levels of dissolved oxygen in the ambient waters.

Paired t-tests (Table 2) of the means of metabolic rates of fishes under each experimental condition indicated that animals tested without

Table 1. Mean values of respiratory volume ($\dot{V}_g$) in ml/sec, per cent uptake of available oxygen (%U), opercular breathing rates (OR) in breaths/min and metabolic rates ($\dot{V}_{O2}$/kg) of shortnose (L.p.) and spotted (L.o.) gars at 20 C under various atmosphere conditions in the respirometer. Numbers of observations are in parentheses.

Species	Weight (g)	No Atmosphere				Conditioned Air Atmosphere				Nitrogen Atmosphere			
		$\dot{V}_g$	%U	OR	$\dot{V}_{O2}$/kg	$\dot{V}_g$	%U	OR	$\dot{V}_{O2}$/kg	$\dot{V}_g$	%U	OR	$\dot{V}_{O2}$/kg
L.p. #1	640	8.54 (3)	39.5	49.3	52.07	3.96 (4)	48.0	37.5	41.28				
L.p. #2	740	12.54 (2)	30.3	46.0	53.60								
L.p. #3	625	7.64 (4)	29.4	51.2	46.90	2.05 (4)	79.0	20.2	29.13				
L.p. #4	570					1.64 (4)	54.0	23.0	43.47				
L.o. #1	550	10.56 (2)	31.4	57.0	69.10								
L.o. #2	820	13.72 (2)	22.9	45.5	43.76								
L.o. #3	795	8.79 (4)	45.4	42.8	57.92								
L.o. #4	570					1.77 (4)	75.6	19.5	39.85	2.39 (4)	66.6	21.2	35.14
L.o. #5	290					1.82 (4)	59.0	28.0	49.65	1.21 (5)	52.4	24.8	36.75
L.o. #6	500					8.70 (4)	31.4	33.5	59.10	8.20 (4)	23.0	28.0	45.14
L.o. #7	480									2.36 (4)	64.0	29.5	48.47
Grand Means		10.30	33.15	48.6	53.46	3.32	57.8	27.0	43.75	3.54	51.5	25.9	41.10

Table 2. Results of paired t-tests of the overall mean values of oxygen consumption ($\dot{V}_{O2}$/kg) of experiments using various atmosphere conditions involving shortnose and spotted gars at 20 C.

Paired Atmosphere	Mean $\dot{V}_{O2}$/kg	Standard Error of Mean	N	t	df	P
No Atmosphere	53.46	± 2.55	17	3.089	39	<0.01
Conditioned Air	43.75	± 2.55	24			
No Atmosphere	53.46	± 2.55	17	3.442	32	<0.01
Nitrogen	41.10	± 2.53	17			
Conditioned Air	43.75	± 2.55	24	0.719	39	$0.40<P<0.50$
Nitrogen	41.10	± 2.53	17			

an atmosphere had significantly higher levels of oxygen consumption than those maintained under either a conditioned air or nitrogen atmosphere ($P<0.01$), and further, that there was no significant statistical difference in the metabolic rates between fishes investigated under the latter two conditions ($0.40<P<0.50$).

Temperature Effects

The results of experiments conducted at 12 C, 20 C and 30 C concerning the various gill respiratory components are summarized in Appendix Table 1.

At 12 C, rates of oxygen consumption of spotted gar averaged about 20 cc O_2/kg/hr (Figure 3). This mean value would be considerably lower, if the results obtained from one fish (#11) were omitted. This particular animal died two days after its use in the experiment and examination of its gills revealed that it carried a heavy infestation of microfilarial blood parasites. The diseased condition of this fish may have had an effect upon its metabolic rate as measured in the respirometer, which was approximately twice those measured for any other fish at this temperature. Oxygen consumptions measured in the other gars ranged from about 5 cc O_2/kg/hr to almost 23 cc O_2/kg/hr.

Within the group of spotted gar tested at 20 C, overall oxygen consumption was about 41 cc O_2/kg/hr. The highest rate measured at this temperature was almost 62 cc O_2/kg/hr. Potter (1927) reported a mean value of 36.9 cc O_2/kg/hr of oxygen consumption for an undisclosed number of longnose and shortnose gars of comparable size at about 20 C, apparently unrestrained with regard to aerial gas exchange. Ranges of oxygen consumption were not given in his report.

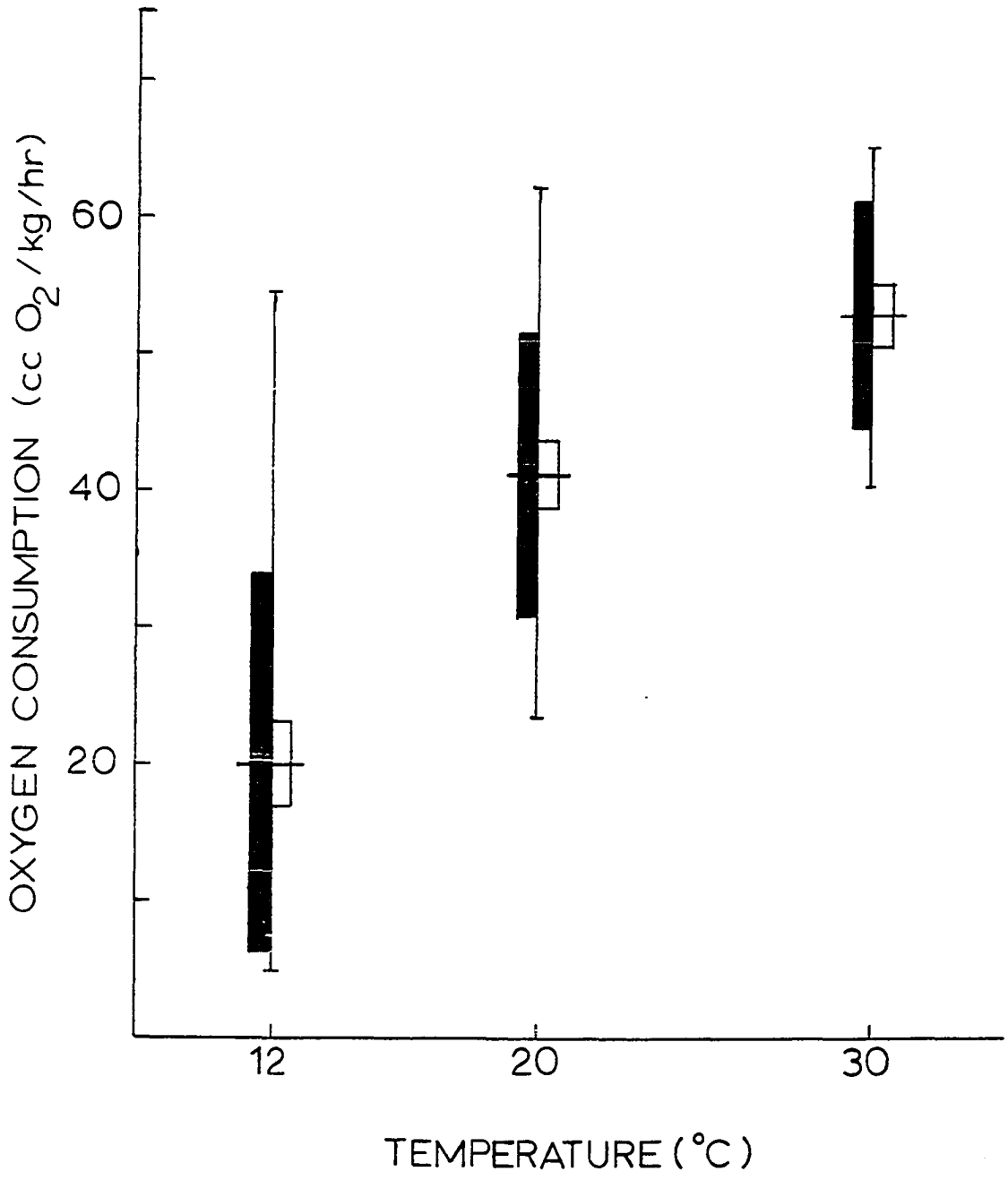

Figure 3. Results of oxygen consumption measurements of spotted gar at the selected test temperatures. Vertical lines represent the observed range, the horizontal line is the mean, the solid bar is the standard deviation and the open bar is the standard error.

Metabolic rates of three spotted gar tested at 30 C in the respirometer ranged from 40 to 65 cc O_2/kg/hr with an overall average of about 53 cc O_2/kg/hr.

The relationship of oxygen consumption of spotted gar to temperature indicates that under the conditions and procedures used in these experiments, metabolic rates almost doubled after a long term change from 12 C to 20 C (Q_{10} = 1.7). A somewhat smaller increase in the rate of oxygen consumption accompanied the temperature change from 20 C to 30 C (Q_{10} = 1.3). There were overlaps in individual observations of oxygen consumption of fish tested at the different temperatures.

The opercular breathing rates of spotted gar increased with increment of temperature. The mean rates of fish at 12 C ranged from 11.5 to 24.5 breaths per minute (B/min) with the highest values again exhibited by fish #11. The range of mean opercular breathing rates of gar at 20 C was from 21.2 to 29.5 B/min, and at 30 C the rates had increased to a range of from 32.2 to 43.0 B/min.

In general, the opercular breathing rate of each fish tested increased as the level of dissolved oxygen in the ambient water of the respirometer decreased.

Estimates of respiratory volume of the experimental animals were obtained by calculating the volume of ventilation water that would be required to deliver to the animal the amount of oxygen ($\dot{V}_{02}$) at the measured differences in dissolved oxygen concentration between inspired and expired water.

Mean respiratory volumes (ml/sec) for gar tested at 12 C ranged from 0.26 to 5.45. At 20 C, the range in respiratory volume was from 1.21 to 8.20 and at 30 C, from 2.85 to 21.60 ml/sec.

The stroke volumes of the opercular breaths were approximated by dividing the respiratory volume by the breathing rate using comparable time intervals. Within the group of fish tested at 12 C, most values for stroke volume fell between one and seven milliliters per breath (ml/B) with values as low as 0.55 ml/B and as high as 24.6 ml/B recorded. At 20 C, stroke volumes between 2 and 10 ml/B predominated, with one value as high as 35.5 ml/B. The range in stroke volumes of fish tested at 30 C was from 3.6 to 45.1 ml/B.

Ranges of mean values of per cent utilization of available oxygen at 12 C, 20 C and 30 C were 15-75%, 23-64% and 17-48% respectively.

Effects of Dissolved Oxygen Concentration

In all of the respirometer experiments, the dissolved oxygen concentration of ambient water at equilibrium was determined by the metabolic rate of the fish and by the rate of flow of water through the system. Water entering the respirometer from the reservoir was at or near the temperature saturation point with respect to oxygen concentration. Since smaller amounts of this saturated water entered the system as the rate of flow was reduced, there was less oxygen available to the fish. Increase in temperature also reduces the solubility of oxygen in water.

In order to attain equivalent ranges of ambient oxygen concentrations at the three different temperatures, flow rate levels were set at progressively higher levels for tests conducted at the higher temperatures.

Within each temperature group, ranges of ambient dissolved oxygen concentrations were quite comparable. In general, as the ambient oxygen

level in the respirometer fell, increases in respiratory volume, opercular rates and oxygen consumption were recorded with accompanying decreases in values of per cent uptake of oxygen (Appendix Table 1). These trends, however, were not always consistent.

Among groups, the effects of temperature upon the metabolic rates of the animals and upon oxygen solubility produced slightly lower levels of ambient oxygen concentration in experiments conducted at progressively higher temperatures, even with compensatory adjustments in the flow rate.

It is possible, therefore, that the changes that occur in the various parameters of gill respiration and oxygen consumption with change in temperature may be partially due to these small differences in ranges of dissolved oxygen.

Gill Structure

The morphology of the gill apparatus of the gars examined in this portion of the study is similar to that of most fish species that have been previously described (Hughes and Shelton, 1962; Saunders, 1962; Lagler et al., 1962; Gray, 1954).

The first and second arches are virtually the same size with about the same number of gill filaments which are the same average length. The third and fourth arches exhibit progressive reductions in both of these respects. There is a corresponding reduction in relative size of the gill slits between the arches and the slit posterior to the fourth arch is almost non-existant. There are slightly fewer filaments on the posterior hemibranch of each arch than on the anterior one.

A striking feature of the gills of gars is the presence of an interbranchial septum joining the filaments of opposing hemibranchs of each

arch. The interbranchial septum extends from the base to a distance of one-half to one-third the filament length. The filaments of the anterior and posterior hemibranchs are spaced alternately along the gill arches (Figure 4).

The presence of such a septum is not uncommon in bony fishes (Fry, 1957). This feature would prevent interdigitation of the secondary lamellae of adjacent filaments as has been described by Hughes (1966) and would distort water flow in the proximal region of the filaments. Presumably, water that has passed over the lamellae in this area courses along the septum outward and exits between the tips of the filaments of the anterior and posterior hemibranchs of each arch.

The length of individual secondary lamellae is proportional to width of the gill filament at any particular point. The filaments are slightly narrower at the bases than in the middle. The filaments taper to a point at their distal ends. The height of the lamellae is fairly constant along the length of the filaments, except at the extreme tips. The secondary lamellae are typically oval in shape throughout, but become more circular near the distal ends of the filaments.

Microscopic examination of unstained secondary lamellae and sections of them stained with eosin and Harris' hematoxylin have shown that the capillary blood space of these structures consists of well developed, parallel channels arching from one end of the lamella to the other. These channels appear to be formed by rows of pillar cells between the epithelial surfaces of the lamellae (Figure 5). There is little indication of anastomosis between the capillary channels. In several preparations that included sections of the basal element of the gill filament,

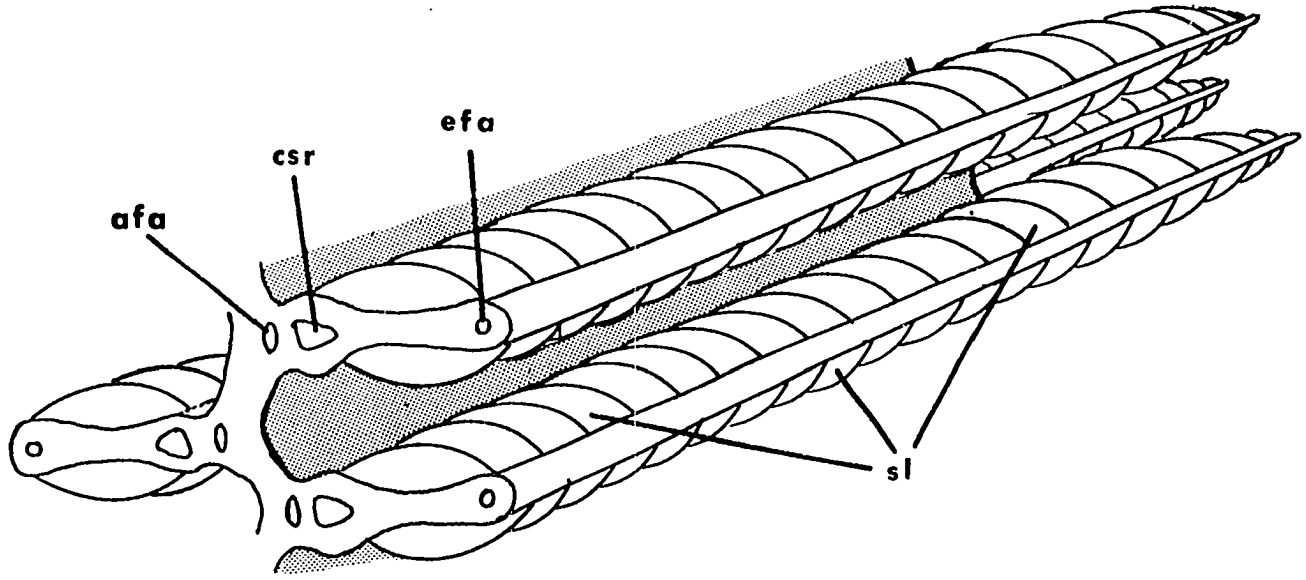

Figure 4. Schematic diagram showing three gill filaments dissected from a gill arch. The exposed basal ends are on the left. The stippled areas indicate the position and extent of the interbranchial septum. (afa - afferent filamental artery; csr - cartilagenous supporting rod; efa - efferent filamental artery; sl - secondary lamallae).

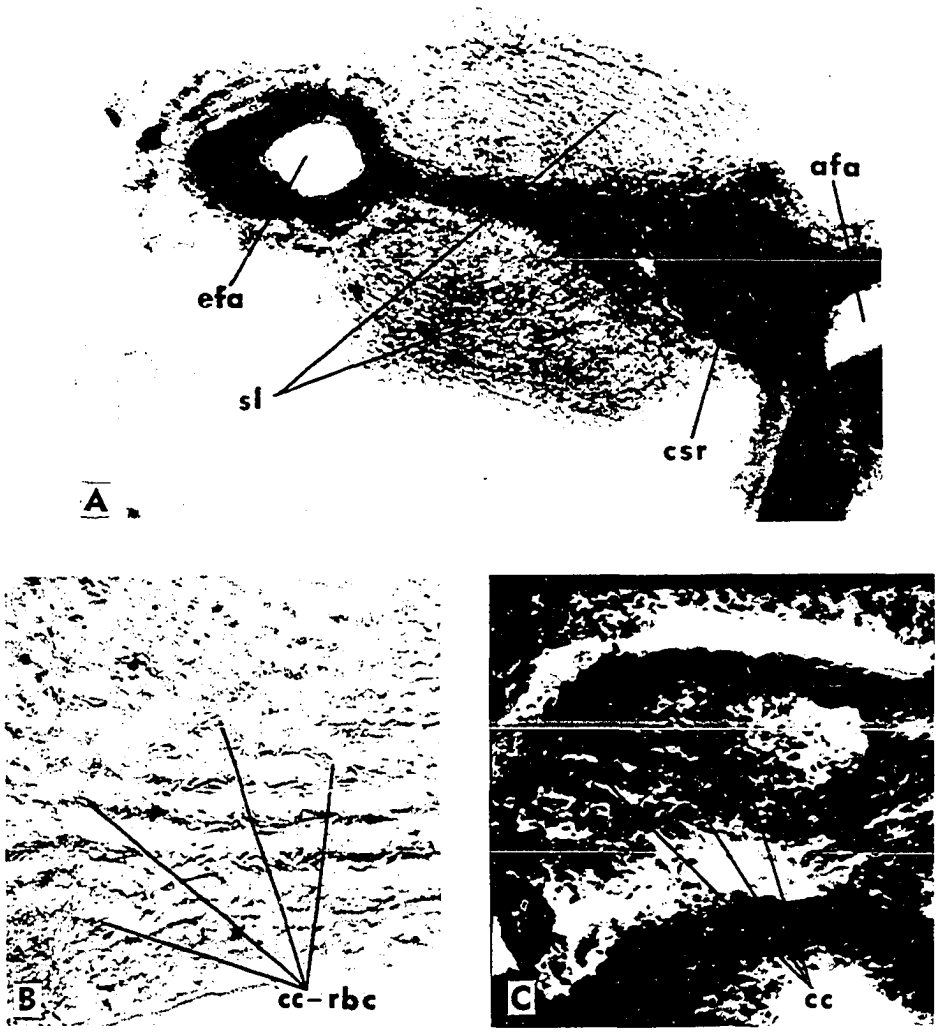

Figure 5. Photomicrographs of the structure of the secondary lamellae. A, cross-section of a gill filament between lamellae; (sl) exposing the afferent (afa) and efferent (efa) filamental arteries and the cartilagenous supporting rod (car) of the filament; B, portion of a lamella at higher magnification showing the parallel capillary blood channels (cc) filled with oval red blood cells (rbc); C, section through a lamella.

numerous red blood cells were in a large space within the basal element itself. Vascular connections between this space and either the capillary channels or the main afferent and efferent filamental arteries could not be established.

There were no detectable differences in the structure and arrangement of the gill apparatus among the three species of gars examined.

Gill Dimensions

The results of the various counts and measurements made to determine the gill surface area of specimens of longnose, shortnose and spotted gars are contained in Table 3.

All fishes examined, regardless of size or species had approximately one thousand gill filaments. This would indicate that the total complement of gill filaments develops at a fairly early stage (before two months of age) and that very few, if any, gill filaments are added after this time.

On the other hand, the data indicate that during growth of these fishes, the filaments grow longer, more secondary lamellae develop, the secondary lamellae increase in size, and become less closely spaced along the gill filament.

The ratio of total lamellar area to body weight is not constant during development, but decreases with increase in body size in all three species of gars.

Table 3. Results of gill area studies

Species	Body Weight (g)	Total No. Filaments	Mean Fil. Length (mm)	Mean 2nd. Lamellae/ mm Filament	Mean Area 2nd. Lamella (mm^2)	Total No. 2nd. Lamellae	Total Area 2nd. Lamellae (mm^2)	Total Area Body Weight (mm^2/g)
L. platostomus	600	977	6.2	27.0	0.29	$16.35X10^4$	47415	79.02
	520	1097	5.4	26.5	0.26	$15.70X10^4$	40820	78.50
	400	1095	5.0	26.2	0.26	$14.34X10^4$	37284	93.21
	350	996	5.2	26.1	0.25	$13.52X10^4$	33800	96.60
	285	1098	4.5	26.6	0.19	$13.07X10^4$	24833	87.13
	61	1020	2.5	28.0	0.11	$7.12X10^4$	7832	127.34
L. oculatus	700	999	5.8	27.3	0.30	$15.82X10^4$	47460	67.80
	480	1036	5.3	27.1	0.25	$14.96X10^4$	37400	77.91
	340	1084	5.0	26.8	-	$14.50X10^4$	-	-
	290	1036	4.3	27.0	0.18	$12.03X10^4$	21654	74.66
	33	980	2.2	31.0	0.07	$6.82X10^4$	4774	144.66
L. osseus	720	1063	6.0	26.6	0.25	$16.97X10^4$	42425	58.92
	450	998	5.5	27.4	0.26	$15.01X10^4$	39026	86.72
	35	990	2.2	33.0	0.08	$7.04X10^4$	5632	163.24
	26	982	2.0	33.2	0.07	$6.63X10^4$	4641	178.50

CHAPTER IV

DISCUSSION

The original experimental design of the respirometer experiments prevented entirely the capability of gars to extract oxygen from an atmosphere *via* the swimbladder, by completely filling the holding compartment with water during the entire course of the tests. The preliminary experiments, conducted in this fashion, revealed that the absence of an atmosphere had an excitatory effect upon the activity of the gars which was reflected by oxygen consumption values and opercular breathing rates significantly in excess of what might be considered as "normal".

The provision of an atmosphere, even one consisting initially of pure nitrogen would permit a certain amount of atmospheric oxygen to become available to the fish in the respirometer. Oxygen will diffuse across the air-water interface at a rate determined by the surface area of the interface and by the partial pressure difference between oxygen dissolved in the water and that of the oxygen concentration of the atmosphere above the water.

Thorpe and Crisp (1947) investigated the phenomenon of plastron respiration of certain aquatic insects, a mechanism which involves many considerations parallel to those imposed by the existence of an air-water interface in the respirometer during the various experiments. Several hemipteran and coleopteran aquatic insects are capable of deriving their

oxygen requirements from the amounts of this gas that diffuse from the water across the air-water interface of a bubble trapped by a network of hair-like bristles in the abdominal region. As oxygen is removed from the bubble through respiration, it is replaced by diffusion from the surrounding water. Thorpe and Crisp have represented this diffusion process mathematically by the following relationship:

$$\frac{dO}{dt} = A\, i_o\, (P_1 - P_2),$$

where dO/dt is the rate of oxygen diffusion, A is the surface area of the interface, i_o is the "invasion coefficient" of oxygen across the interface and P_1 and P_2 are the tensions of oxygen on either side of the interface. The value of the invasion coefficient presented by these workers is 0.029 cc O_2 at N.T.P./cm^2/min/atmosphere.

With respect to the conditions of this relationship that may prevail in the respirometer A = ca. 500 cm^2, P_1 = 0.2 atm O_2 in water at saturation, and P_2 = 0 atm O_2 in atmosphere intially. Intergrating this formula to determine the time required for diffusion of oxygen to create a partial pressure of this gas in the atmospheric space to 99% of the partial pressure of oxygen in the water at equilibrium yields a value of about 28 minutes, if the initial partial pressure of oxygen in the space is zero.

Potter (1927) calculated that a 600 g gar has a swimbladder volume capacity of 50 cc and that at each aerial breath, about 50% of the oxygen contained by that volume is extracted. If the partial pressure of oxygen in air is 160 mm Hg, then at each breath, the fish may take up as much as 5 cc O_2 (50 cc X 160/760 X 0.5). The volume of the air in the respirometer was approximately one liter, therefore the removal of

this much oxygen from the atmosphere would reduce the partial pressure of oxygen to about 97.5%. The time required for this reduction in partial pressure to return to 99% of the equilibrium value would be only about six minutes.

After oxygen equilibrium has been established, there will be no net movement of oxygen across the interface except in response to removal of oxygen from the atmosphere through air breathing by the fish. Oxygen so removed from the atmosphere will be rapidly replaced from the water flowing through the system, and so equilibrium will be quickly re-established following an aerial breath. It is therefore reasonable to assume that measurements of dissolved oxygen concentrations taken between aerial breaths of gars in the respirometer and after the time required for oxygen equilibrium to become re-established would reflect realistic values of gill respiratory characteristics. Values of per cent uptake would be unaffected since these measurements were taken directly.

Although aerial gas exchange was not entirely prevented by the experimental design, it is felt that it was minimized by the fact that equilibrium partial pressures were never more than 130 mm Hg (0.17 atm) and were frequently as low as 0.11 atm.

It cannot be denied that the fishes in the respirometer were able to extract some of their oxygen requirements from "air" in the space in the chamber and therefore the results of these experiments concerning oxygen consumption, respiratory volume and stroke volume of opercular breaths may be perhaps modified by this situation.

For purposes of analysis and comparisons of gill respiratory characteristics, it was assumed that during periods between aerial breaths,

uptake of oxygen from the water maintaining equilibrium was accomplished by gill oxygen uptake alone. All measurements were made after six minutes following any aerial breaths.

Metabolic Rates

Because oxygen may diffuse from the water flowing through the respirometer into the space permitted even though that space was intially pure nitrogen, the close similarity of responses between groups of gars tested under "conditioned air" and initial nitrogen may be explained by the fact that by the time measurements were taken, oxygen was at comparable equilibration levels between air and water in the respirometer chamber, and not, as had been originally supposed, due to similar negligible amounts of oxygen in the space. The significantly elevated metabolic rates and concomitant increased levels of opercular breathing rates and respiratory volumes measured in gars subjected to a no-atmosphere situation may be due, in part, to relatively hypoxic overall conditions (no oxygen available for aerial exchange), but it is felt that these responses are primarily due to generally increased activity level of the animals when unable to perform aerial breathing motor patterns. Any hydrostatic function of the swimbladder would be also impaired, which also might produce higher levels of activity. Potter (1927) noted that opercular breathing rates of gars whose pneumatic duct, joining the pharynx with the swimbladder had been plugged, were one-third higher than those of unaltered control animals. Renfro (1968) found no significant difference in rates of aerial breathing in spotted gar at about 30 C before and after several hours confinement below the surface of the water at a dissolved oxygen concentration of 5.5 ppm and that fish so confined

for as long as 96 hours exhibited "no ill effects".

The standard or basal metabolic rates of fishes are difficult to measure (Fry 1957). This level may be approached by designing experiments which restrict the movements of the animals and minimize excitatory influences. As Fry suggests, this condition may reflect a routine metabolism of the fish. Many of the studies directed toward investigating the effects of temperature upon metabolic rate have involved conditions conducive to standard or routine physiological states (Winberg, 1956; Fry, 1957). The majority of the investigations reviewed by these authors indicate that exposure to increasing temperatures of a wide variety of fishes causes increases in oxygen consumption. Such increases typically follow Krogh's "normal curve" which reflects a general doubling of standard metabolic rate with 10 C increments in temperature ($Q_{10} = 2$). Both Fry and Winberg report some diminution of Q_{10} values, however, with increasing temperature. Beamish (1964) has reported this trend in thermally acclimated bullheads, suckers and carp.

According to Fry, the effects of temperature upon active rates of oxygen consumption may be quite different. Active metabolic rates may level off, or even decline at increased temperature.

Although every attempt was made to maintain the gars in this study at resting states, spontaneous activity was exhibited by fishes in the respirometer. The animals were able to maneuver a short distance forward and backward in the respirometer and to engage in aerial breathing movements. It is possible that the metabolic rates may have exceeded, at times, routine levels.

The decrease in Q_{10} of metabolic rates from 20 C to 30 C compared

to the value at the 12 C to 20 C range may therefore represent a normal pattern with regard to routine metabolism or perhaps is influenced by active metabolic considerations which may produce a leveling off or decline at the 30 C point.

Saksena (1963) found that rates of aerial breathing increased with increment in temperature, but that almost invariably there was a decrease somewhere in the 20 C to 30 C range. Renfro (1968) correlated these observations to the fact that acclimation to temperature change, as reflected by the rate of aerial breathing, seemed to require almost twice as long in this range of temperature as at lower levels.

Based upon Renfro's results it was assumed that the two week period for temperature acclimation in this study would be adequate, however, two spotted gar tested after a considerably shorter term change (12 hours) from 20 C to 30 C exhibited metabolic rates within the range of values obtained from fish maintained at 30 C for the two week period.

The amount of water that a fish must move past its gills to obtain necessary amounts of oxygen for its metabolic processes is determined by the concentration of oxygen in the water relative to the requirements of the animal. Increase in respiratory volume in fishes usually accompanies lowered dissolved oxygen concentration (Gerald and Cech, 1969; Spitzer, Marvin and Heath, 1969). Over the moderate ranges of dissolved oxygen concentrations used in the experiments with spotted gar, such a relationship is not pronounced except in those animals tested at 30 C. Since the supply of animals was limited during certain periods of the study, moderate to high levels of dissolved oxygen were purposely maintained at all three experimental temperatures to preclude the possibility of kill-

ing any fish due to asphyxiation. The effect of hypoxia was not one of the realistic aims of this study. It is probable that differences in the ranges of respiratory volumes among temperature groups is due to the level of oxygen consumption while fluctuations of metabolic rate and respiratory volume observed within groups and among separate determinations for individual fish may be due to changes in dissolved oxygen concentration, variation in spontaneous activity and perhaps experimental error.

Values of per cent utilization of oxygen by gill uptake by spotted gar are quite comparable to those summarized by Gerald and Cech (1969) for a variety of aquatic organisms (mostly fishes). Figure 6 shows the relationship of per cent utilization to respiratory volume of gar tested at the three temperatures. Such inverse relationships have been demonstrated for trout (van Dam, 1938), brown bullheads, white suckers and carp (Saunders, 1962). The slopes of these log-log relationships are well within the ranges of those calculated by Saunders.

It should be noted that at respiratory volumes less that 1-2 ml/sec, there appears to be a relative constancy of per cent utilization at fairly high levels. This may be due to the relative richness of oxygen in the ventilation water relative to the amount of oxygen being removed by the fish (Appendix Table 1).

Values of per cent utilization of spotted gar are generally much higher than measurements reported for the South American lungfish, Lepidosiren paradoxa (Johansen and Lenfant, 1967) or for the African form, Protopterus aethiopicus (Lenfant and Johansen, 1968).

Changes in respiratory volumes of fishes may be accomplished by an alteration of the opercular breathing rate and/or by changes in ampli-

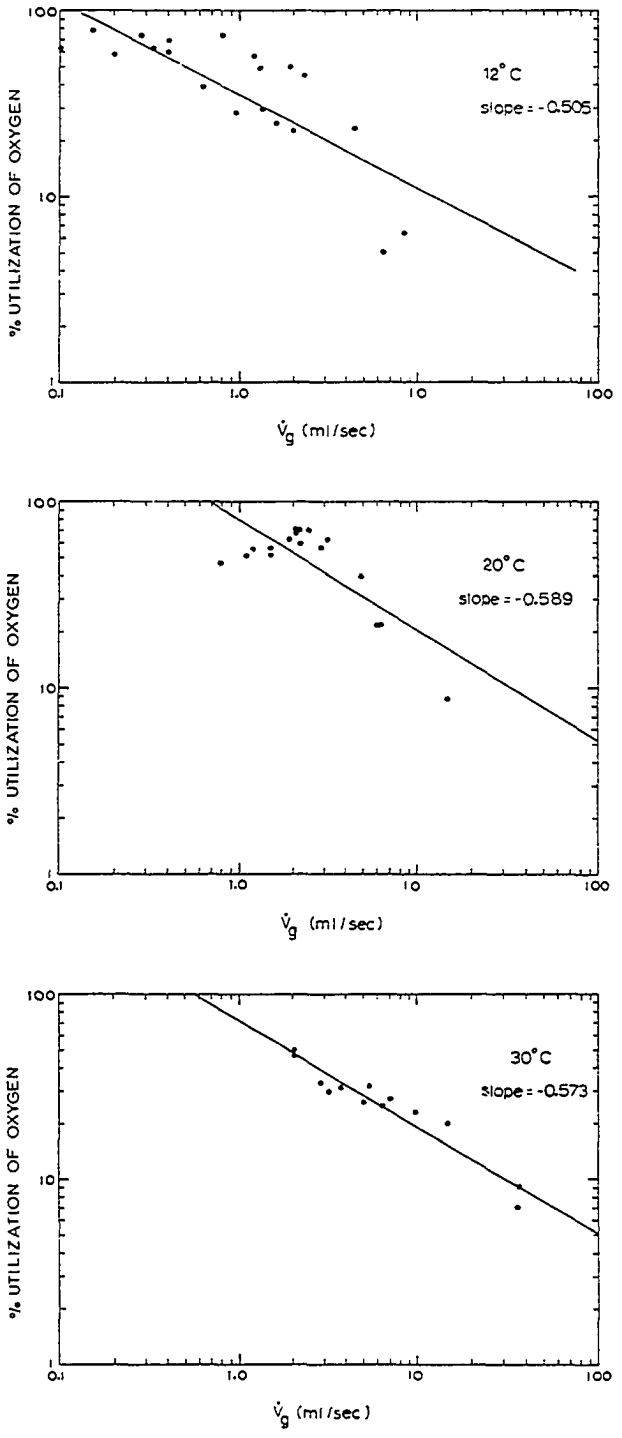

Figure 6. Log-log relationships of repiratory volumes ($\dot{V}_g$) to corresponding values of per cent utilization measured for spotted gar at three test temperatures. Regression lines derived by least squares method.

tude (stroke volume) (Spitzer, et al., 1969). Spotted gar seem to rely primarily upon changes in stroke volume to regulate respiratory volume, with rate changes of secondary importance (Figure 7). If stroke volume remained constant over a range of respiratory volumes, the slopes of the regression lines would approximate zero and if stroke volume were inversely related to respiratory volume, the slopes would be negative. On the basis of the results obtained 80% to 95% of any increase noted in respiratory volume was accomplished by increment in stroke volume. In this respect, spotted gar are similar to elasmobranchs (Ogden, 1945; Hughes and Umezawa, 1968), trout (van Dam, 1938), Neoceratodus (Johansen, Lenfant and Grigg, 1967) and juvenile Ictalurus punctatus (Gerald and Cech, 1969).

Gill Area

With the exception of the work of Price (1931), Hughes (1966), Muir (1969), Muir and Hughes (1969) and to some extent that of Gray (1954), previous measurements of the gill area of various species of fishes have not considered the change in the ratio of total gill area to body size. In most other studies, only one or two specimens provided the basis of such measurements.

Difficulty in comparison also arises from the fact that several approaches and techniques have been used to make estimates of the gill surface areas of fishes. Hughes (1966) states that the measurements made by Byczkowska-Smyk (1957, 1958, 1959) may be greatly inflated due to errors in estimating the areas of individual secondary lamellae. George and Dubale (1941) and Dubale (1951) using a number of gill-breathing and air-breathing Indian fishes ignored the areas of secondary lamellae in

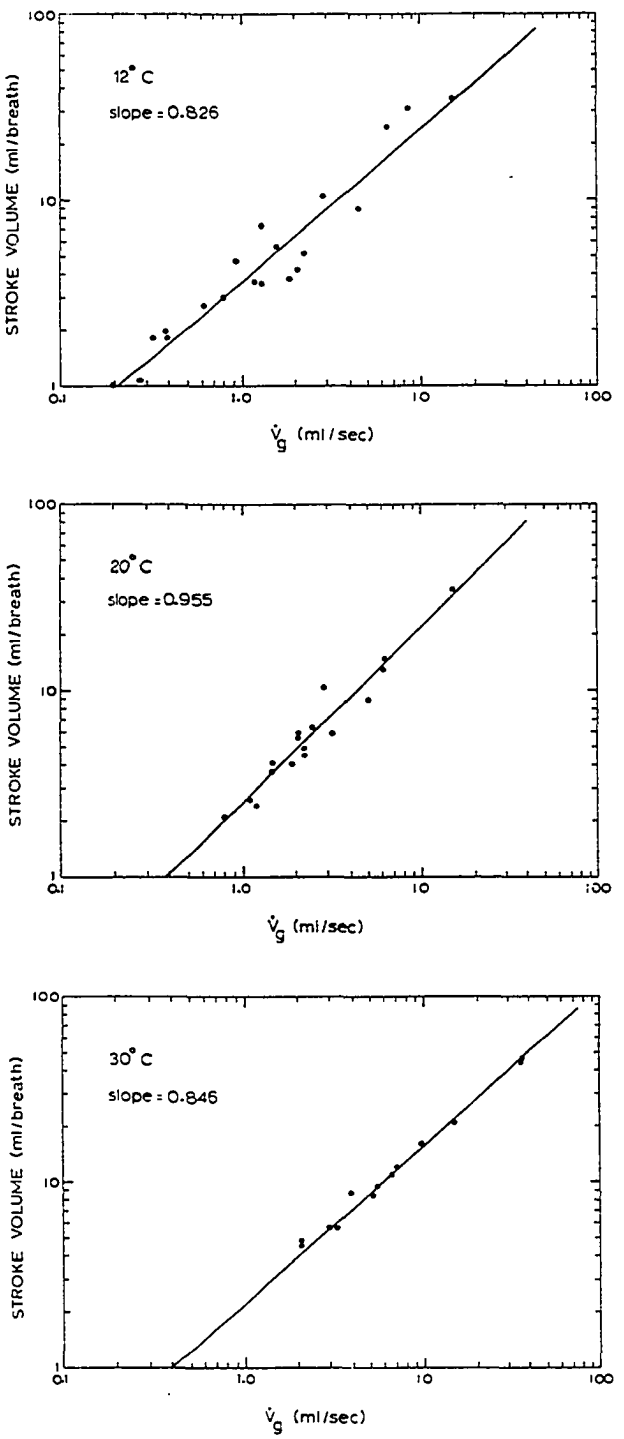

Figure 7. Log-log relationships of stroke volume (ml/breath) to respiratory volume ($\dot{V}_g$) for spotted gar at three test temperatures.

estimating gill area. These workers reported that fishes that possess accessory means of respiration have about half the total gill surface area of strictly gill breathing forms.

Observations of gill areas of lungfishes (Dipnoi) are not quantitative. Grigg (1965) and Lenfant, Johansen and Grigg (1967) report that Neoceratodus forsteri has well developed gills and is apparently an obligatory gill breather although having an air breathing capability (this form does not aestivate). Lenfant and Johansen (1968) describe the gills of Protopterus aethiopicus as "coarse and atrophied". Johansen and Lenfant (1967) report that the gills of Lepidosiren paradoxa are greatly reduced with "gill arches 1 and 2 --- almost devoid of gill filaments". These latter two species are known to aestivate in mud cocoons during periods of seasonal drouth. Lepidosiren appears to be an obligatory air breather under most environmental conditions. Other obligate air breathers, for example the electric eel (Electrophorus electricus) have been described as having degenerate gill structure (Johansen, 1968).

Price (1931), working with smallmouth bass (Micropterus dolomieu) found that the ratio of total gill area to body weight decreased with increase in body weight and that the relationship between these factors, plotted on log-log coordinates suggested a straight line with a slope of 0.785. Muir (1969) reported a similar situation with respect to three species of tunas, a series of marine fishes studied by Gray (1954) and analysed by Ursin (1967) and for small numbers of individuals of some freshwater species. In a more detailed investigation of the tuna data, Muir and Hughes (1969) demonstrated that there also appear to be rather consistent log-log relationships between body weight and the spacing of

secondary lamellae along the gill filaments, the average area of individual secondary lamellae and total filament length.

Double logarithmic plots of the gill area components obtained from individuals of three species of gars are represented in Figure 8. Regression analyses by least squares method was performed to obtain the indicated regression lines and formulas. The general formula for all of these relationships is :$Y = \underline{a}W^{\underline{b}}$, where Y is the value of each particular gill area component, $\underline{a}$ is the value of that factor represented by one gram fish, W is body weight in grams and $\underline{b}$ is the regression coefficient (slope). The logarithmic form of this equation is: $\log Y = \log \underline{a} + \underline{b} \log W$.

Regression lines could be fitted to the values of the various gill area factors plotted against body weight for each species of gar separately, but F tests of homogeneity of the slopes of the derived lines indicated that the data might be combined (Table 4).

Muir and Hughes (1969) summarize the results of some of the studies concerning relative amounts of gill surface area for wide size ranges of individual fish of several species. These values and equivalent data for gars are listed in Table 5. Tunas have more than 10 times the gill area of gars at the one gram level and values of Gray's intermediates and smallmouth bass are respectively, three and two times as great. At 100 g and 1000 g levels, the magnitude of these differences increases. For one gram fishes, the gill surface areas of the gars and the roach are almost identical. Due to the steeper slope of the regression line of the roach (0.90), the gill area of this fish is much greater than that of gars at the larger size levels.

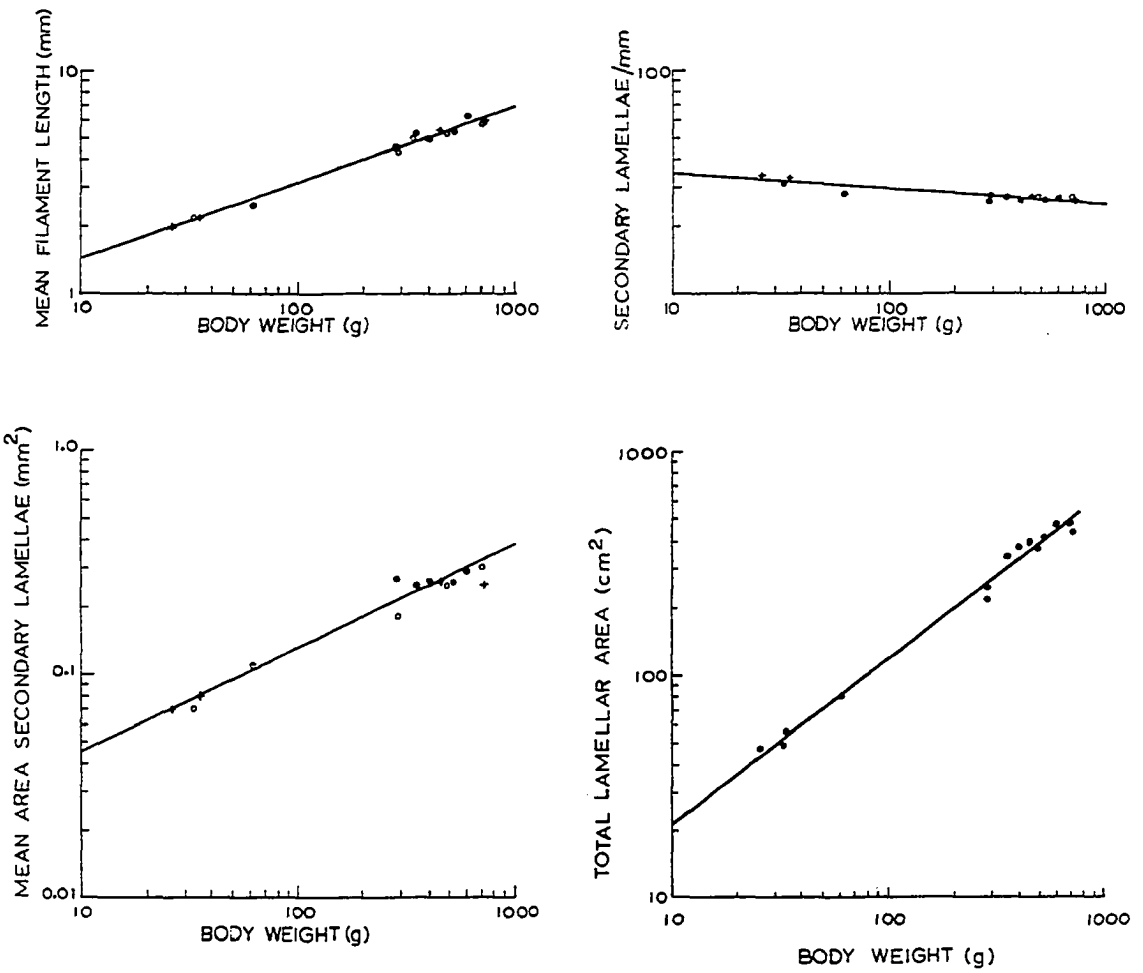

Figure 8. Log-log plots of the relationships of total lamellar area and gill area components to body weight for spotted gar (open circles), shortnose gar (closed circles) and longnose gar (+). Straight lines derived by least squares method.

Table 4. Values of a (Y intercept-one gram), b (slope) and 95% confidence limits of b for regression equations by least squares relating various gill area factors to body weight for three species of gars. The P values are the results of F tests of homogeneity of slopes among species.

A. Mean gill filament length (mm)

	a	b	95% C. L.
L. platostomus	0.5281	0.380	$0.2953 < b < 0.4647$
L. oculatus	0.7330	0.316	$0.1533 < b < 0.4787$
L. osseus	0.6655	0.339	$0.2285 < b < 0.3713$
		$P > 0.25$ n.s.	
Combines	0.6569	0.340	$0.3087 < b < 0.3713$

B. Mean number of secondary lamellae/mm filament length

	a	b	95% C. L.
L. platostomus	31.35	-0.0280	$-0.0672 < b < 0.0112$
L. oculatus	35.95	-0.0454	$-0.0871 < b < -0.0037$
L. osseus	41.75	-0.0686	$-0.1002 < b < -0.0370$
		$P > 0.05$ n.s.	
Combined	38.75	-0.0603	$-0.0767 < b < -0.0439$

Table 4. continued

C. Mean area of individual secondary lamellae ($mm^2 \times 10^{-2}$)

	a	b	95% C. L.
L. platostomus	1.887	0.427	$0.245 < b < 0.609$
L. oculatus	1.318	0.473	$0.192 < b < 0.754$
L. osseus	1.865	0.411	$0.249 < b < 0.573$
		$P > 0.50$ n.s.	
Combined	1.653	0.441	$0.328 < b < 0.554$

D. Total lamellar area (cm^2)

	a	b	95% C. L.
L. platostomus	2.952	0.796	$0.6975 < b < 0.8951$
L. oculatus	3.342	0.754	$0.5866 < b < 0.9210$
L. osseus	4.787	0.698	$0.5268 < b < 0.8700$
		$P > 0.10$ n.s.	
Combined	3.936	0.738	$0.6694 < b < 0.8066$

Table 5. Comparison of total gill areas (cm^2) of gars with other species of fish (Muir and Hughes, 1969) at size intervals, based upon regression lines.

	Body Weight		
	1 g	100 g	1000 g
Skipjack tuna	52.18	2620	18400
Yellowfin and bluefin tunas	40.25	2000	14380
Gray's intermediates*	13.92	600	3990
Smallmouth bass	8.65	330	1960
Roach	3.98	190	1290
Combined-gars	3.94	120	660

*Several species of marine fishes that Gray classified as intermediate in general activity and relative amount of gill surface area.

Using values for one gram fishes, tunas have approximately 10 times the total filament length of gars and smallmouth bass. Bass exhibit 1.3 times the total filament length of gars. At this size level, gars have about 40 secondary lamellae/mm filament length compared to 80/mm in bass and up to 120/mm in tunas. The lowest value reported by Gray was 22/mm for a 305 g toadfish.

Average areas of individual secondary lamellae of gars (one gram) are intermediate between values for tunas (0.009 mm^2) and bass 0.04 mm^2).

In general, active species of fish have more secondary lamellae, which are smaller, but more closely spaced, than more sluggish forms (Muir and Hughes, 1969). By these criteria, gars would fall in the sluggish category, which conforms to the general behavior usually described for these animals (Hubbs and Lagler, 1947), although they may be quite active while feeding and spawning.

Gars possess greatly reduced gill surface areas compared to equivalent size, highly active species, but only slightly less gill area than some sluggish forms; e.g., carp, brown bullhead and white sucker (Saunders, 1962). These latter three species have approximately the same numbers of secondary lamellae/mm filament length as do gars.

Theoretical Limits of Oxygen Diffusion Through The Gills

The rate at which oxygen may diffuse from the ventilation fluid into the blood perfusing the secondary lamellae of the gills may be described by the formula: $\dot{V}_{02} = (D)(A)/d \cdot \Delta P_{02}/760$; where D is the diffusion constant of oxygen (3.4×10^{-5} cc 0_2 $cm/cm^2/min/$ atmosphere at 20 C in

pure water), A is the gill surface area in cm^2, d is the average diffusion distance between water and blood and $\Delta P_{02}/760$ is the ratio of the mean partial pressure difference in mm Hg of oxygen between water and blood at the gills (ΔP_{02}) to the pressure of pure oxygen at S.T.P. Realistically, the value of the diffusion constant would be somewhat less than that stated, since the ventilation and perfusion fluids are not pure water and part of the diffusion medium is epithelial or connective tissue of the secondary lamellae. The value d has been estimated as being from one-half (Hughes, 1966) to one-quarter (Saunders, 1962) the distance between secondary lamellae.

Water and blood flows at the respiratory surfaces of fishes have been described as counter-current (van Dam, 1938; Hazelhoff and Evenhuis, 1952), so that partial pressure gradients between water and blood are maximized along the lengths of the secondary lamellae, thus facilitating oxygen diffusion.

A 500 g gar has a total effective gill surface area of about 385 cm^2, and d, equal to one-quarter the distance between adjacent lamellae is about 1.9×10^{-3} cm. Oxygen consumptions of spotted gar at 20 C were generally between 0.3 and o.4 cc O_2/min. Minimal values of ΔP_{02} ranging from 33 to 44 mm Hg must be present to permit this magnitude of oxygen diffusion through the gills. Higher rates of oxygen consumption would require larger values of ΔP_{02}.

No information exists as to the changes that actually may occur in the partial pressure of oxygen in the blood of gars as it moves through the capillary blood space of the secondary lamellae. Smith (1968) reports that the oxygen capacity of whole blood of spotted gar is almost

16 vol % (160 cc O_2/1000 ml) at 150+ mm Hg, which is quite high for fish blood, but lower than some values that have been reported. However, Smith constructed oxygen dissociation curves for hemoglobin solutions of gar blood which seem to indicate saturation of hemoglobin at oxygen partial pressures in excess of 20 mm Hg, but at concentrations of only about 6 vol %. Smith offers no explanation for these results.

According to Rahn (1966), ventilation/perfusion ratios of fishes have been calculated in a range of 15-20, that is, the amount of water that a fish passes over its gills is 15-20 times greater than the volume of blood perfusing these structures per unit time. If it is assumed that all blood must pass through the gills in each circuit, then the perfusion rate is equal to cardiac output. The generalized gar at 20 C described previously might exhibit respiratory volumes in a range of 1 to 3 ml/sec or 60 to 180 ml/min, which would reflect cardiac outputs ranging from 12 to 3 ml/min depending upon the v/p ratio. Application of the indirect Fick principle: cardiac output = $\dot{V}_{O2}$/(concentration of oxygen in arterial blood minus that of venous blood) (Florey, 1966); to those theoretical values of cardiac output, at oxygen consumptions of 0.3 to 0.4 cc O_2/min, yields estimates of the oxygen concentration difference of blood on either side of the gill circulation of 2 to 13 vol %. If it is further assumed that the partial pressure of oxygen of blood just entering the gills from the heart is essentially zero (concentration = 0), and that in transit through the secondary lamellae the blood picks up enough oxygen to meet the demands of the animal's metabolic activity, a partial pressure increase to only about 10 mm Hg may occur if the perfusion rate is as high as 12 ml/min.

Theoretically, a 500 g gar may be able to extract all of its oxygen requirements for "routine" metabolism at 20 C via gill diffusion alone if the partial pressure of oxygen in the ambient water is in excess of about 60 mm Hg (2.5 cc O_2/l at S.T.P.). It should be stressed that these computations are based upon questionable observations of blood-oxygen relationships of gars and upon estimates of cardiac output and changes in oxygen partial pressure of blood perfusing the gills that are without actual empirical support with specific regard to gars.

Fish may respond to hypoxic conditions in several ways (hypoxia referring to the supply of oxygen relative to the metabolic demands of the animal). The fish may pump greater amounts of water over the gills, maintaining a higher relative partial pressure gradient by reducing the per cent uptake of oxygen, although this would increase the energy expenditure of the animal. Fish may exhibit a reduction in activity and oxygen consumption, and there are some forms, e.g. catfish and carp, that may shift to a greater dependence upon anerobic processes in response to temporary, severe hypoxia (Gerald and Cech, 1969), or by moving to an area with a higher oxygen concentration.

Gars and other air-breathing fishes have yet another avenue open to them if exposed to hypoxic waters. These forms may take in atmospheric oxygen, the partial pressure of which is usually quite high, through accessory respiratory surfaces.

CHAPTER V

SUMMARY

Gars deprived of access to an atmosphere exhibited elevated, long-term levels of activity and oxygen consumption which were alleviated by the presence of an air-water interface. Such a provision permits a certain degree of aerial gas exchange regardless of the initial composition of gasses in that atmosphere.

At 12 C, metabolic rates of spotted gar averaged about 20 cc O_2/kg/hr, with respiratory volumes usually less than 2 ml/sec. Values of per cent utilization of available oxygen in the ambient water were as high as 80%, with most measurements greater than 30%.

Fish tested at 20 C exhibited mean metabolic rates of about 41 cc O_2/kg/hr, respiratory volumes generally between 1 and 3 ml/sec and per cent utilization as high as 72%.

At 30 C average values of metabolic rates, respiratory volumes and per cents utilization were 53 cc O_2/kg/hr, 3 to 22 ml/sec and less than 40% respectively.

Per cents utilization were inversely proportional to respiratory volumes. Increase in respiratory volume was accomplished primarily by increase in stroke volume of the opercular breaths, with opercular rate having a minor effect.

The structure of the gills of gars is similar to that of other teleosts. The gills do not exhibit the extreme degrees of atrophy that exists in some of the lungfishes and other air-breathing forms.

The gill components of gars follow the same patterns of development during growth in size that has been demonstrated in a variety of strictly gill-breathing fishes and gill area is comparable in size to that of some sluggish forms.

Although the gill area of gars is greatly reduced compared to more active species, it appears to be large enough and distributed in such a way as to permit sufficient oxygen diffusion to meet the routine requirements of these animals at moderate dissolved oxygen concentrations.

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APPENDIX

Appendix Table 1. Respiratory characteristics of spotted gar tested in the respirometer with an atmosphere present at 12 C, 20 C and 30 C.

Fish No. (at 12 C)	Body Weight (g)	C_{iO_2} (cc/l)	C_{eO_2} (cc/l)	$\dot{V}_g$ (ml/sec)	%U	$\dot{V}_{O_2}$ (cc O_2/kg/hr)	OR (B/min)	Stroke Vol. (ml/B)
#5	290	6.36	2.56	0.20	59.7	9.42	12	1.00
		6.36	2.25	0.10	64.5	5.07	11	0.55
		6.06	2.21	0.33	63.5	15.68	11	1.80
		5.64	2.17	0.39	61.5	16.70	12	1.95
#7	480	5.93	4.47	1.58	24.7	17.29	17	5.58
		5.84	3.51	0.62	39.9	10.81	14	2.66
		5.72	4.09	0.94	28.5	11.46	12	4.70
		5.00	3.51	1.33	29.7	14.76	11	7.25
#10	420	5.82	1.73	0.40	70.3	13.92	15	1.60
		5.77	1.45	0.28	74.9	10.20	16	1.05
		5.80	1.18	0.15	79.7	6.09	13	0.69
		4.42	1.13	0.80	74.4	22.61	16	3.00
#11 *	310	5.52	2.76	1.31	49.9	41.79	22	3.57
		5.22	2.23	1.20	57.2	41.38	20	3.60
		4.51	2.49	3.24	44.7	54.55	27	5.20
		3.82	1.92	1.86	49.7	40.81	29	3.85
#12	600	6.18	5.87	6.55	5.1	12.34	16	24.56
		5.68	5.32	8.69	6.3	18.56	17	30.67
		5.17	3.97	2.01	23.1	14.39	29	4.16
		3.32	2.60	4.54	23.9	22.24	30	9.08

* Fish #11 died two days after its use in the experiment

Appendix Table 1. continued

Fish No. (at 12 C)	Body Weight (g)	C_{iO_2} (cc/l)	C_{eO_2} (cc/l)	$\dot{V}_g$ (ml/sec)	%U	$\dot{V}_{O_2}$ (cc O_2/kg/hr)	OR (B/min)	Stroke Vol. (ml/B)
#4	570	4.22	1.84	2.91	56.4	43.58	18	10.60
		3.89	1.23	2.08	68.3	34.81	21	5.94
		3.28	0.99	2.51	69.9	36.15	24	6.28
		2.79	0.79	2.06	71.9	26.00	22	5.62
#5	290	5.03	2.23	1.50	55.7	51.99	22	4.09
		4.90	2.35	1.48	52.0	46.33	24	3.70
		4.90	2.58	0.81	47.4	23.36	23	2.11
		4.52	2.20	1.09	51.2	31.23	25	2.62
		3.28	1.67	1.18	55.9	30.84	30	2.36
#7	480	4.68	1.32	2.21	71.7	55.53	27	4.91
		4.28	1.64	3.14	61.6	61.97	32	5.89
		4.16	1.69	2.23	59.3	41.20	30	4.46
		3.86	1.55	1.92	63.4	35.18	29	3.97
#6	500	4.83	4.41	15.38	8.7	46.37	26	35.49
		4.42	3.63	6.34	21.5	45.24	25	15.22
		4.24	3.31	6.09	21.9	40.71	28	13.05
		3.36	1.82	5.00	40.0	48.24	33	9.09

Appendix Table 1. continued

Fish No. (at 12 C)	Body Weight (g)	C_{iO_2} (cc/l)	C_{eO_2} (cc/l)	$\dot{V}_g$ (ml/sec)	%U	$\dot{V}_{O_2}$ (cc O_2/kg/hr)	OR (B/min)	Stroke Vol. (ml/B)
#5	290	4.18	2.75	3.91	30.9	59.56	27	8.69
		4.18	2.65	3.03	33.4	49.82	32	5.68
		3.81	1.90	2.14	50.2	50.56	34	3.78
		3.62	1.92	2.06	47.0	43.32	34	3.64
		3.35	2.35	3.26	29.7	40.06	34	5.75
#7	480	4.03	2.98	5.17	25.9	40.32	36	8.62
		3.81	2.86	6.71	25.0	47.77	36	11.18
		3.39	3.16	36.90	6.8	63.74	50	44.28
		2.84	2.60	37.60	8.9	64.95	50	45.12
#15	410	3.53	2.69	5.56	32.1	55.20	35	9.53
		3.21	2.34	7.20	27.2	55.16	35	12.34
		2.86	2.20	9.90	23.0	57.03	38	15.63
		2.18	1.75	15.20	19.9	57.75	41	22.24

The partial pressure of oxygen = 0.76/α X the concentration of oxygen (cc O_2/l), where α is the solubility or "Bunsen" coefficient of oxygen in water. Values of α at 12 C, 20 C and 30 C are 0.0364, 0.0310 and 0.0261 respectively.