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GRADUATE COLLEGE

ASPECTS OF RESPIRATORY ADAPTATION IN THE BOWFIN, *AMIA CALVA*

A DISSERTATION

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ASPECTS OF RESPIRATORY ADAPTATION

IN THE BOWFIN, *AMIA CALVA*

APPROVED BY

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TABLE OF CONTENTS

Page

Chapter

LIST OF TABLES

LIST OF ILLUSTRATIONS

CHAPTER I

Figure | Page

None

CHAPTER II

CHAPTER III

ASPECTS OF RESPIRATORY ADAPTATION IN THE BOWFIN *AMIA CALVA* L.

CHAPTER I

INTRODUCTION

Historical-Taxonomic Features

The bowfin is a large predatory Holostean fish in the order *Amiiformes*. This order is archaic and dates from the Upper Permian to the recent. Bowfins were numerous in the Jurassic but today only the monotypic family *Amiidea* survives (Young, 1962). This species was described by Linnaeus (1766) in the type locality of Charleston, South Carolina and named *Amia* (meaning ancient fish) and *calva* (meaning smooth). The bowfin is known by a variety of other non-scientific names; dogfish, grindle, mud-fish, cotton fish, black fish, grinnel, speckeled cat, lake lawyer, scaled ling, green bass, scaley catfish, poisson-castor, choupiquel (Wilder, 1875 b; Robinson, 1875; Scott and Crossman, 1973). Taxonomically, Amiids are transitional between the closely related gars (order, Lepisostiformes) and teleosts (Breder and Rosen, 1966).

General Description

In terms of body form and size, bowfins resemble teleosts in the Salmonidae group (large trout and salmon). The general

body shape is torpedo-like and soft cycloid scales cover the body except for the head and fins. The scales lack ganoin; a substance common to the scales of the gar and certain other primitive fish. The head is conic and encased with heavy dermal bones (Allis, 1889 a). The jaw structure and oral compartment is composed of several bones which are alveolus and contain numerous canine-like teeth (Shutfeldt, 1885). Unique structural characteristics include a gular plate found within the mandibular angle (Lagler, 1956) and a serrated throat appendage (Wilder, 1877 b). The fins are spineless and well vascularized. The dorsal fin arches bow-like over the back, the caudal fin is rounded and abbreviated heterocercal and the pectoral, pelvic and anal fins are fan-like and fleshy based (Wilder, 1877 c). The lateral line is complete and contains 64-68 scales (Allis, 1889 b).

The respiratory system is bimodal consisting of gills and a dorsal air sac. The gills are well developed with short knob-like rakers and have a specialized interlamellar septum that protects and supports the lamellae (Bevelander, 1934). The air sac is single, bifid in front, compartmentalized, well vascularized and capable of gas exchange (Goodrich, 1930). The external nares open from tubular extensions (Legendre, 1954).

The bowfin shows marked sexual dimorphism. Females are larger than males and possess a light-gray spot in the base of the caudal fin. Males have a darker caudal spot surrounded by a yellow orange halo. Both sexes are dark olive dorsally, show a mottled reticulated pattern of lime and yellow laterally and are cream

white ventrally. The anal and paired fins are light with greenish edges. The dorsal fin is olive with horizontal dark bars, whereas, the caudal fin is light green with interrupted vertical dark bands. During the spring spawning season, males show an enhancement of coloration in the orange and green hues (Zahl and Davis, 1932).

Distribution

The bowfin is restricted to eastern North America (Moore, 1968) but closely related fossil forms are known from the Tertiary of Europe and Asia (Scott and Crossman, 1973). Bowfins are distributed northward to lower Quebec, Ontario and Vermont, westward throughout most of Minnesota, South Dakota, Nebraska, Kansas, Oklahoma and Texas and eastward to the southwest Appalachians and along the Atlantic coastal states from Connecticut to Flordia. Bowfin populations are largest and most concentrated in southeastern United States. In Oklahoma, the bowfin is found in the southeast tributaries and low gradient portions of Arkansas, Poteau, Kiamichi, Little, Mountain Fork and Red River (Ortenberger and Hubbs, 1926; Hubbs and Ortenberger, 1929; Finnel, 1955).

The bowfin shows preference for swampy, vegetated bays and backwaters of lakes and rivers which are relatively clean and unpolluted. Because this type of habitat is disappearing due to draining, filling and dredging, the future of this last representative of the Amiid group is uncertain.

Life History Review

Early studies of the bowfin state that spawning occurs

from late April to early June after males construct large circular nests in shallows where rotted vegetation is abundant (Dean, 1899; Reighard, 1900; Reighard, 1902). The water temperature during these activities ranges from 16°to 19°C (Reighard, 1903). Females produce large numbers of eggs which become adhesive and turn dark gray after release (Dean, 1895). Males then protect and care for the nest, eggs and young (Doan, 1938). Eggs hatch after 8-10 days (Whitman and Eycleshymer, 1897) into 8 mm larvae with snout tipped adhesive organs that permit attachment to vegetation (Dean, 1895; Reighard and Phelps, 1908; Mansueti and Hardy, 1967). Parental care by adult males ceases at the 102 mm stage and by early fall young of the year are 127-229 mm in length (Carlander, 1969).

Age and growth studies of the bowfin are meager due to difficulties in applying standard scale aging techniques (Cooper and Schafer, 1954). Northern populations reach sexual maturity between three and five years when females and males are 610 and 457 mm long, respectivley (Cartier and Magnin, 1967). These authors also reported that maximum size is reached at about 870 mm and 6800g in Quebec waters. The longevity of this fish is about 12 years in natural waters and up to 30 years in captivity (Carlander, 1969).

The bowfin is a voracious predator feeding on small fishes, crayfish, amphibians, insects and small shrimp (Breder, 1928; Scott, 1938; Lagler and Hubbs, 1940; Berry, 1955; Dillard, 1965). An average daily ration for young of the year is equivalent to 6-7% of body weight (Herting and Witt, 1968). Since the fish

codominates with sunfishes, basses, gars and other game fish carnivors as a top order predator, it is usually considered a pest and of little commerical or recreational use (Scott and Crossman, 1973).

Dissertation Topic Development

A significant aspect of bowfin life history is survival ability in low quality waters that show daily and seasonal fluctuations in temperature and in oxygen and carbon dioxide content. The capability of the bowfin to survive has been documented under such extreme conditions as warm, hypoxic pools and partially dried mud holes (Dence, 1933), estivation burrows (Gowanlock, 1933; Neill, 1959), brackish intertidal zones (Mansueti and Hardy, 1967) and starvation during 20 months in captivity (Smallwood, 1916). Few North American freshwater fish can withstand such physiologically demanding conditions.

The survival capabilities of the bowfin indicate well developed adaptations especially in the respiratory system. Since Amia is the only survivor of its group, it is essential to the field of comparative physiology to identify the functional mechanisms of this system and to describe how they contribute to survival under environmental stress. Knowledge of these mechanisms would also add much to the understanding of the Holostean fish group and lower vertebrate respiration.

A relationship between the adaptability of bowfins in marginal aquatic habitats and respiratory function was first established when Wilder (1875 a) observed that the bowfin could rise to the surface of hypoxic water and breathe air. Later

Wilder (1977a) described the gas exchange process that occurred in the air sac. Black (1940) then showed how certain blood characteristics could contribute to respiratory adaptiveness. Johansen et al. (1970) published a more detailed work concerning the aerial breathing process, blood respiratory characteristics and gill function. The latest account of bowfin respiratory behavior described quantitative aspects of the aerial breathing process (Horn and Riggs, 1973). Recently, Weber et al. (1976) presented a study of molecular characteristics of Amia hemoglobin.

It was the purpose of this dissertation to carry out additional study on the bowfin respiratory system in areas that were neglected or not treated in detail in previous investigations. The topics investigated are discussed in the following chapters which are written in the style required for publication in a particular professional journal. Chapter I is introductory material and will not be published. Chapter II describes selected habitats in Oklahoma where the bowfin is easily collected and will not be published. Chapter III presents several gill and blood characteristics for the bowfin and is designed for publication in Comparative Biochemistry and Physiology. Chapter IV will be presented to Respiration Physiology and concerns morphometric and pharmacological aspects of the bowfin air sac.

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CHAPTER II

CHARACTERISTICS OF BOWFIN HABITATS IN SOUTHEASTERN OKLAHOMA

Introduction

This chapter describes three bowfin habitats in the Red River floodplain of McCurtain County, Oklahoma. The local drainage systems of this region are low gradient, shallow and weedy (Webb, 1970). Several oxbow lakes and sloughs have formed from cutoffs of the Red River and are in various stages of maturity. Several farm ponds have been constructed near these areas and often interconnect with the natural waters during flooding.

The variety of aquatic habitats in southeastern McCurtain county produces a diverse environmental situation which includes Cypress swamp habitat. However, this region of Oklahoma is changing because the Red River no longer floods lowland areas as extensively due to upstream flood control. The reduction in flooding has stabilized the floodplain leading to increased maturation of lowland aquatic habitats. Draining of local wetland has intensified, thus, habitats for many floral and faunal types unique to this part of Oklahoma are disappearing.

This habitat description documents selected conditions during 1971 and portions of 1972 when the bowfin habitats of McCurtain County had extreme fluctuations in high water, summertime drought and farming activity.

Methods and Materials

The habitats selected for this study were (1) Derryberry Pond, a farm impoundment constructed within swamp-like Jenkins Reily Slough (2) Waterfall Creek, a sluggish, densely vegetated stream and (3) Deadman Lake, a mature oxbow lake. These bodies of water typify the major types of aquatic conditions in southeastern McCurtain County, Oklahoma (Figure 1).

A water analysis was conducted between January, 1971 and ended April 1972. Samples were collected monthly at each site in an open water area about two meters from shore. Weekly samples were taken only when significant changes in water level and productivity occurred. Sampling was conducted in the top 0.5 m water zone with a 1l Kemmerer sampler between 1200 and 1500 hours.

Water samples were analyzed for temperature, dissolved oxygen, pH and alkalinity. Air and water temperatures were recorded with a YSI Telethermometer (Yellow Springs, Ohio). Dissolved oxygen was determined using the unmodified Winkler method (APHA, 1971). All other conditions were measured by titration or colorimetry using the materials and procedures of a Hach Chemical Company (Ames, Iowa) water analysis laboratory kit, Model DREL.

Records of on site air temperatures and general weather conditions were supplemented with U.S. Weather Bureau records from Station 34-4451-09 in Idabell, Oklahoma.

Results and Discussion

Climatic Conditions.

Air temperature and rainfall patterns for the study area were summarized for the period January 1972 - December 1972 (Figure 2). The monthly mean temperature ranged from 7°C to 27°C for 1971 and from 6°C to 27°C for 1972. The annual mean temperature was 17°C for both 1971 and 1972 and reflected a mild temperature condition compared to other parts of the United States. The minimum temperatures were reached during the month of January while maximum temperatures were reached in July and August. The highest air temperature recorded during this study was 39°C on July 16, 1971, whereas, the lowest single air temperature was - 12°C on January 5, 1972.

The total annual rainfall was 121 cm for 1971 but dropped to 92 cm for 1972 (Figure 2). In december, 1971, 30 cm of rain fell and caused flooding of the study areas. The year of 1972 had a lowered rainfall pattern and some drying occurred in the shallow regions of the study habitats. Rainfall had a noticeable influence on the limnological features and are discussed in the following sections.

Flora and Fauna.

Each habitat supported a rich flora but Waterfall Creek

and Deadman Lake had the highest plant densities. Typical plant populations consisted of pond weed (Potamogeton), water primrose (Jussiaea), coontail (Ceratophyllum), arrowhead (Sagittaria), yellow pond lilly (Nuphar), yellow nelumbo (Nelumbo), cattail (Typha), bur-reed (Sparganium), water plantain (Alisma), duckweed (Lemna, Spirodella) and button bush (Cephalanthus). Derryberry Pond was free of most of these vegetation types except near the end of the study when water primrose began to develop rapidly.

The most common fish that occurred in association with Amia were: spotted gar (Lepisosteus productus), yellow bullhead (Ictalurus natalis), blackstripe topminnow (Fundulus notatus), mosquitofish (Gambusia affinis), piratepercn (Aphredoderus sayanus), pigmy sunfish (Elassoma zonatum), black crappie (Pomoxis nigromaculatus), warmouth (Chanobryttus coronarius), bluegill (Lepomis macrochirus), and largemouth bass (Micropterus salmoides). Common turtles in the area were: snapping turtle (Chelydra serpentina), mud turtle (Kinosternon subrubrum), pond slider (Pseudemys scripta). Common snakes were: common water snake (Natrix sipedon), and cottonmouth (Agkistrodon piscivorus).

Water Temperature.

Thermal conditions in the three study areas were similar, thus, only data from Derryberry Pond were plotted (Figure 3). The lowest surface water temperature of 8 °C was recorded January 28, 1971 while the highest temperatureof 35 °C was recorded August 9, 1971.

Water temperature patterns at other depths revealed frequent stratification. On other occasions, the water was holomictic (Hutchinson, 1957). These conditions were due to the shallow depth of the habitats, surface vegetation and periodic mixing by wind (Table 1).

The water temperature at any level did not fall below 4°C which is common for most bodies of water having a shallow depth and location between the 33'd and 34'th latitudes of North America (Wetzell, 1975; Respess et al., 1972; Nelson and Harp, 1972).

Dissolved Oxygen.

At the beginning of the study (January 28, 1971), all three study areas had similar dissolved oxygen concentrations but the range of values (8.6-9.4 mg/l) was below the predicted oxygen capacity of 11.7 mg/l (Figure 3). As the study progressed into February 1971, oxygen concentrations dropped slightly but during March and April, dissolved oxygen rose to super-saturated conditions. On April 29, 1971, the water of Derryberry Pond, Waterfall Creek and Deadman Lake had dissolved oxygen concentrations of 9.2, 10.0 and 10.0 mg/l, respectively, which gave saturation levels of 119% and 110%. These conditions were attributed to phytoplankton and higher plant activities (Hutchinson, 1957). Dissolved oxygen concentrations declined during June 1971 with Waterfall Creek and Deadman Lake showing the most pronounced change. On June 23, 1971, Waterfall Creek had 6.8 mg/l oxygen, whereas, Deadman Lake had 0.8 mg/l oxygen (the lowest value recorded in this study). These

lower concentrations were due to thick mats of pond weed, Potomogeton and duckweed, Lemna, on the water surface which virtually eliminated light penetration to the lower water zones. Thus, photosynthetic activity could not replace losses by community respiration and the systems gradually became hypoxic. Because of the summertime oxygen loss due to the abundance of aquatic plants, each study area was classed as eutrophic according to Reid (1961). Deadman Lake approached a dystrophic condition due to exaggerated oxygen losses and accumulation of partially decomposed plant material in the benthic zone. During the latter part of July 1971, heavy rains caused each habitat to fill to capacity. This change, combined with heavy wind and wave action, caused disruption and displacement of the floating plant zone. This increased light penetration led to increased dissolved oxygen content. On July 30, 1971, Derryberry Pond had 14.3 mg/l oxygen (193% saturated) (the highest value recorded in this study), Deadman Lake had 5.8 mg/l oxygen (78.4% saturated) and Waterfall Creek had 9.6 mg/l oxygen (130% saturated). These conditions were maintained into the early part of August 1971, but as plants gradually recovered the water surface, oxygen concentrations decreased. As the study extended into the fall and winter months, all dissolved oxygen concentrations were similar and approached predicted saturations. During the following spring, dissolved oxgyen concentrations approached the previous spring conditions.

The periodic low oxygen conditions in Deadman Lake created respiratory problems for the aquatic organisms. Lind (1974) stated

that most aquatic vertebrates are stressed at the 3 mg/l dissolved oxygen level. This stress value was reached on several occasions and forced fish and amphibians to the surface. Bowfin (Amia calva) and spotted gar (Lepisosteus occulatus)were so active at the surface that open channels were created due to displacement of the floating vegetation. These surface water openings increased the survival of these fish and also provided a temporary refuge for other fish, salamanders and turtles.

Hydrogen Ion Concentration.

At the beginning of the study, the pH in all three habitats was close to neutral (Figure 4). As sampling progressed into spring and summer, the pH of Derryberry Pond and Waterfall Creek steadily increased but Deadman Lake displayed an irregular pattern. High pH conditions were prevalent in each habitat during the fall. On December 16, 1971, heavy rain fell in the area causing flooding of all study sites. Consequently, the water pH of Derryberry Pond, Waterfall Creek and Deadman Lake decreased from the respective alkaline values of 8.5, 8.4 and 7.5 to the slightly acidic conditions of 6.6, 6.7 and 6.9. During the following months of February, March, and April 1972, pH increased in all study areas except Deadman Lake(Figure 4).

Overall, Derryberry Pond and Waterfall Creek showed the greatest variation; Derryberry Pond gave a pH range of 6.7 (March 27, 1971) to 9.1 (July 29, 1971), whereas, the Waterfall Creek pH range was 6.7 (recorded December 16, 1971) to 9.3 (recorded

August 16, 1971 as the highest value in the study). Deadman Lake trended toward acidic conditions which was probably due to decomposition products from the lower portions of the submerged plant zone. Humic acids are known to be released under such conditions (Wetzel, 1974) and lower pH. Deadman Lake had a more alkaline pH on days when the surface was relatively free of vegetation which allowed increased light penetration to the submerged plant zone. Thus, CO_2 removal from the water increased and caused a relative increase of bicarbonate and alkalinity. The pH range for this body of water was 6.0 (recorded on June 8, 1971 as the lowest pH value in the study) to 7.5 (detected on July 29, 1971 and November 21, 1971).

The pH condtions observed during the study were not unusual for these types of habitats and are comparable to similar bodies of water in this part of the United States (Respess et al., 1972).

Alkalinity.

The alkalinity conditions were variable (Figure 4). Derryberry Pond had the highest alkalinity which was not unexpected due to pH conditions. Overall, the alkalinity was due to bicarbonate, but, from June 23, 1971 to November 21, 1971 pH conditions were such that some carbonate alkalinity was present. However, these concentrations were low and transient. The alkalinity for this pond ranged from 35 mg/l (December 16, 1971) to 136 mg/l (October 11, 1971).

Deadman Lake and Waterfall Creek had similar alkalinities. The lowest alkalinities for these two areas were 30 mg/l and 27 mg/l (both recorded December 16, 1971), respectively. The highest respective alkalinities were 86 mg/l (August 16, 1971) and 98 mg/l (August 16, 1971).

The differences in alkalinity between the three study sites were related to a watershed condition. Waterfall Creek and Deadman Lake are surrounded by woods and pastures which reduced runoff of soil chemicals. Derryberry Pond is surrounded by cotton, corn and soybean fields which are frequently limed and fertilized. Since runoff from farmland is higher, it would carry more alkaline materials.

On December 16, 1971 heavy rains flooded all habitats. At this time alkalinity declined to the lowest and most uniform concentrations of the study.

According to the National Technical Advisory Committee (1968), aquatic habitats should have bicarbonate levels between 90 mg/l and 180 mg/l in order to support a good fish fauna. Such a range is required to support primary production and a diverse set of food chains. Deadman Lake and Waterfall Creek were at the lower end of the scale throughout most of the year, however, Derryberry Pond fell within the above range. When seine-haul samples were compared between the study areas, Derryberry Pond had the highest number and diversity of fish species, as well as,

abundant turtles, larval amphibians, freshwater shrimp and other assorted invertebrates (snails, mussels, beetles, etc.). Bowfins were more prevalent in Derryberry Pond than in any of the other habitats.

The bicarbonate values for this study were similar to those summarized by Kingsbury (1968) for the lower cretaceous limnological province of Oklahoma. This province will produce dissolved bicarbonate as high as 184.4 ± 52 mg/l in regions with limestone outcropings and as low as 67.1 ± 15 mg/l where sandstone and shale outcrops occur (Kingsbury, 1968). The above range of bicarbonate encompassed the values described for the habitats (found in the extreme southeast region of the lower cretaceous province) evaluated in this study.

Other limnological conditions were not studied extensively and therefore are excluded from this chapter. However, Kingsbury (1968) using data supplied by Howard P. Clemens, Professor of Zoology at the University of Oklahoma, was able to show that the waters of several habitats near the bodies of water examined in this study were relatively soft and low in mineral content. Calcium, sodium, potassium, magnesium and sulfate averaged, respectively, 30.8. 7.4, 3.4, 3.8 and <50 mg/l for the limestone districts of the lower cretaceous province. Since the habitats of this study have a similar geology, it is likely that they also have soft and low mineral content waters. The few limnological determinations that were done with regard to the above ions tend to confirm this supposition.

SUMMARY

Except for occasional disturbance by cattle and farming activity which led to periodic nutrient enrichment, the three bowfin habitats investigated in this study had good water quality; the water was moderately soft and low in mineral content.

Because of extensive plant populations, Waterfall Creek and Deadman Lake showed more pronounced fluctuations in dissolved oxygen, pH and alkalinity. When dissolved oxygen was low, bowfin migrated to the water surface and rested a few inches below the water surface among tangled vegetation. This behavior reduced energy loss and the potential harmful effects of hypoxia and hypercapnea. Except for aerial breathing and feeding activity, the bowfin is not an active swimmer and spends much of its time resting on bottom substrate. If the bowfin did not rest in the surface water zone during low oxygen conditions, they would have to expend considerable energy in order to aerial breathe.

These observations lead to further questions regarding bowfin respiratory activity. The surfacing behavior of the bowfin indicates sensory capability for detecting hypoxia. The question of how well a bowfin can survive in a high temperature, hypoxic and open water habitat is raised. This was partially answered on noon August 23, 1971 in an isolated backwater area of Derryberry Pond

when seven dead bowfin were observed. The water was 33 °C and had 16.8 mg/l dissolved oxygen, a pH of 8.9 , 19 mg/l carbonate and 109 mg/l bicarbonate alkalinity. Moreover, there was a dense population of green algae which indicated eutrophy and a potential for nighttime development of hypoxia. At night, the bowfin would not have sufficient dissolved oxygen for gill uptake and would have to continually swim in order to aerial breathe. Apparently this did happen and placed a higher metabolic demand on the animals than could be met by the respiratory system causing them to die.

The low oxygen, high temperatures and increased metabolic activity was also compounded by the high pH of the water. At this pH, dissolved carbon dioxide is virtually absent creating a large blood-water carbon dioxide gradient. An increased blood pH could result to produce a non-functional hemoglobin-oxygen affinity shift. Some of the questions raised from this habitat survey regarding the respiratory physiology of Amia calva L. are discussed in the following chapters.

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LIST OF TABLES

Table 1. Vertical temperature* patterns in Derryberry Pond, Waterfall Creek and Deadman Lake.

Location	Depth (meters)	2-28-71	3-1-71	6-8-71	8-9-71	10-11-71	11-21-71
Derryberry Pond	Surface	16	10	31	32	23	13
	0.3	15	10	31	29	22	13
	0.6	14	10	30	28	21	13
	0.9	10	10	27	28	21	12
	1.2	10	10	26	28	21	10
	1.5	9	10	25	27	20	10
	1.8	9	9	24	26	-	-
	2.1	8	9	24	-	-	-
Waterfall Creek	Surface	18	-	31	34	23	13
	Bottom (1.4 m)	13	-	26	27	19	11
Deadman Lake	Surface	17	-	27	30	22	13
	Bottom (1.1 m)	13	-	18	26	18	10

* Temperatures expressed in degrees centigrade

Figure Legends

Figure 1. Map of southestern McCurtain County, Oklahoma showing study areas along Red River flood plain.

Figure 2. Climatological summary of mean monthly air temperature and rainfall for Idabell, Oklahoma (Elev. 460 feet, Lat. 53° N, Long. 94° 49' W) weather station number 34445109; February, 1971 to April 1972.

Figure 3. Surface water temperature, theoretical and actual dissolved oxygen concentrations of Derryberry Pond, Waterfall Creek and Deadman Lake, January 28, 1971 to April 10, 1972.

Figure 4. pH and alkalinity conditions in the surface waters of Derryberry Pond, Waterfall Creek and Deadman Lake, January 28, 1971 to April 10, 1972.

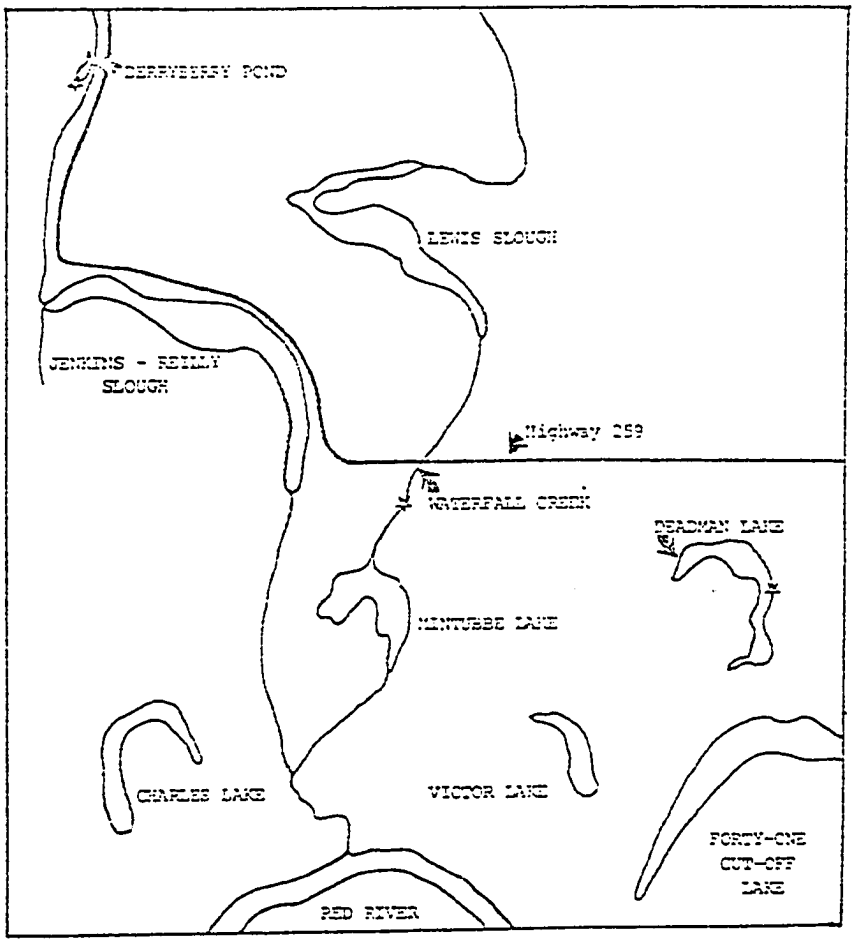
BERRYBERRY POND
LEWIS SLOUGH
JENKINS - REILLY SLOUGH
Highway 259
WATERFALL CREEK
DEADMAN LAKE
MINTUBBE LAKE
CHARLES LAKE
VICTOR LAKE
FORTY-ONE CUT-OFF LAKE
RED RIVER

Figure 1

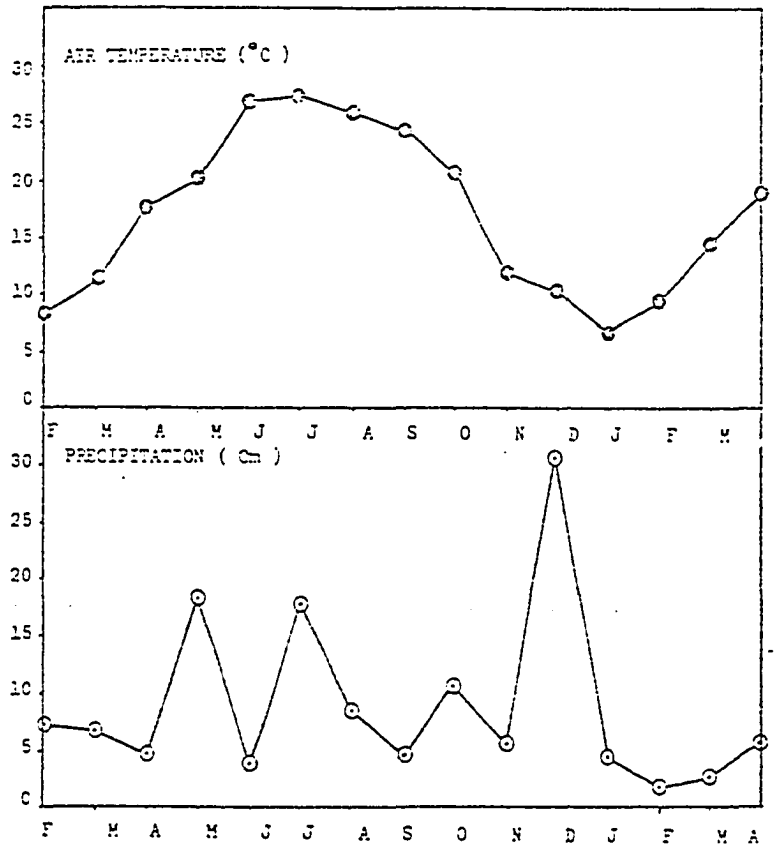
AIR TEMPERATURE (°C)
30
25
20
15
10
5
0
F M A M J J A S O N D J F M
PRECIPITATION (Cm)
30
25
20
15
10
5
0
F M A M J J A S O N D J F M A

Figure 2

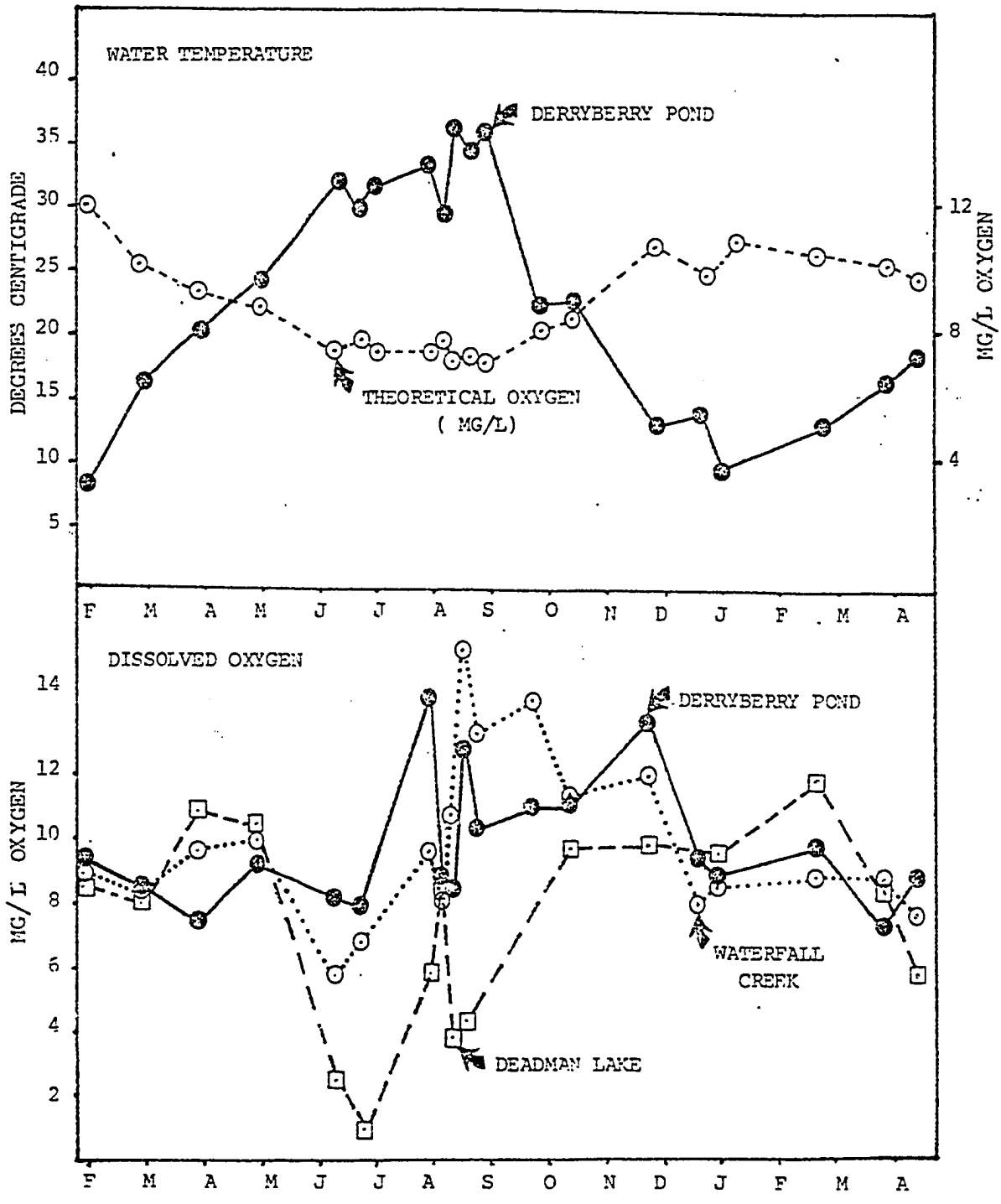
WATER TEMPERATURE
DERRYBERRY POND
THEORETICAL OXYGEN
(MG/L)
DEGREES CENTIGRADE
MG/L OXYGEN
40
35
30
25
20
15
10
5
12
8
4
F M A M J J A S O N D J F M A
DISSOLVED OXYGEN
DERRYBERRY POND
WATERFALL
CREEK
DEADMAN LAKE
MG/L OXYGEN
14
12
10
8
6
4
2
F M A M J J A S O N D J F M A

Figure 3.

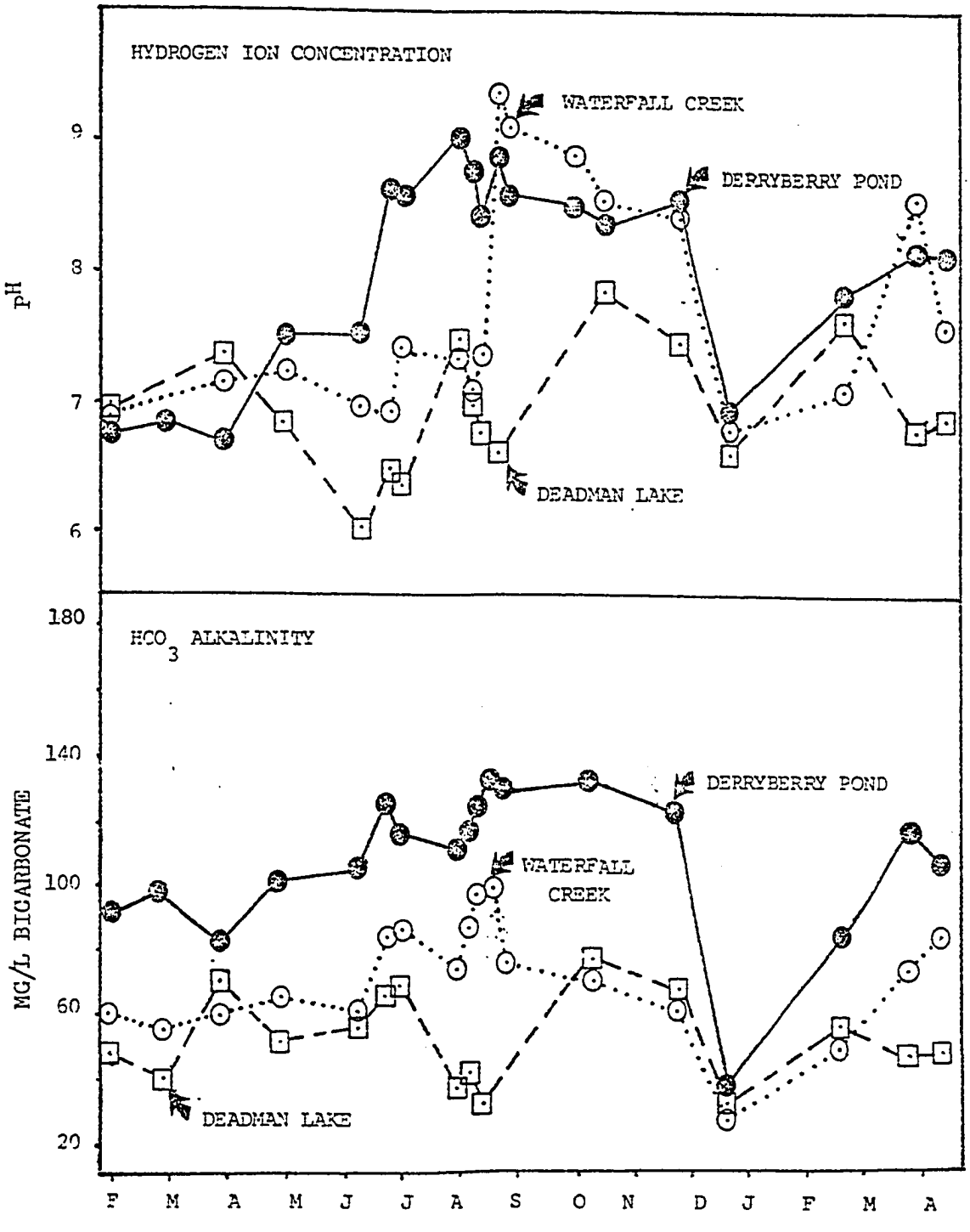
HYDROGEN ION CONCENTRATION
WATERFALL CREEK
DERRYBERRY POND
DEADMAN LAKE
pH
9
8
7
6
HCO3 ALKALINITY
MG/L BICARBONATE
180
140
100
60
20
DERRYBERRY POND
WATERFALL CREEK
DEADMAN LAKE
F M A M J J A S O N D J F M A

Figure 4

CHAPTER III

GILL AND BLOOD CHARACTERISTICS OF THE BOWFIN, *AMIA CALVA*

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ABSTRACT

1. Bowfin gill morphology is unique among most fish because of an interlamellar septum which fuses the tips of opposing lamellae into a fixed position. Such an arrangement reduces respiratory surface area 10 to 25 percent but, produces a series of sieve-like channels that restrict water flow and reduce diffusion dead space. The blood-water diffusion distance ranged from 1.7 μ to 2.9 μ.

2. Opercular rate, gill stroke volume, total ventilation volume and oxygen consumption increased only slightly as temperature was raised from 15° to 25°C. Oxygen extraction efficiency was variable at both 15° and 25°C. At times, efficiency was high (58.9%) then low (5.7%). Some fish did not show this irregular gill activity and, in these fish, oxygen uptake efficiency and ventilation volume increased significantly with a change from 15° to 25°C water. The gill dynamics of the fish were related to the transition to aerial breathing which begins in the 15° to 25°C temperature range.

3. Hematological characteristics were temperature related. Red cell cell number, hematocrit and hemoglobin concentration were all higher at 25°C than at 15°C. Red cell size was unchanged.

4. Oxygen capacity and P_{50} values were higher at 25°C than 15°C. Blood pH decreased nearly 0.2 of a unit as temperature was increased 10°C. The Bohr effect (-0.42) was unchanged by temperature. Temperature coefficients were as high as -0.05. These data indicate types of respiratory adaptation that would enable the bowfin to survive more readily in high temperature, hypoxic water.

INTRODUCTION

The bowfin, *Amia calva*, is a monotypic Holostean found in the major drainage systems of eastern North America. This fish is an efficient predator and codominates with basses, pikes, and gars as a top order consumer. *Amia* shows a preference for weedy backwater areas of streams and lakes that have good water quality but can survive in hypoxic habitats (Dence, 1933; Gowanlock, 1933) due to several well developed respiratory mechanisms: (1) an air sac that provides an alternative gas exchange route when water quality conditions reduce gill function (Johansen *et al.*, 1970), (2) a cardiovascular shunting mechanism that facilitates gas exchange in air by shifting blood from the gills during periods of hypoxia (Johansen, 1972), (3) efficient gills with interlocked lamellae (Bevelander, 1934), (4) a type of hemoglobin that can shift oxygen affinity as water and acid-base changes occur (Black, 1940; Johansen *et al.*, 1970; Weber *et al.*, 1976) and a (5) proficient aerial breathing behavior (Horn and Riggs, 1973).

Previous studies on *Amia* have answered several questions relating to the respiratory anatomy and physiology of this primitive fish but have neglected certain aspects of gill morphology, ventilation dynamics and blood respiratory properties. Some of the respiratory data are based on observations from only one, two or three fish. This study provides additional information on respiratory

function in Amia relative to gill filament dimensions, diffusion distance, oxygen extraction and ventilation dynamics, and blood oxygen dissociation characteristics. These data were collected to further explain the adaptive roles played by the bowfin gill and blood system when this fish encounters variable temperatures, oxygen and pH conditions in its habitat.

MATERIALS AND METHODS

Experimental Animals. Fish were obtained from Jenkins-Reilly slough in McCurtain County, Oklahoma, transported to the University of Oklahoma Department of Zoology, Norman, and held individually in 50 gallon aquaria at 15° and 25°C. The animals were fed crayfish and minnows and remained in apparent good condition throughout confinement. General fish condition was assessed through periodic weighings and measurements of blood pH and serum glucose. Prior to experimentation, food was withheld 2-3 weeks in order to induce a fasted state.

Respirometry. In measuring gill function, an operculum of each fish was fitted with a PE 90 catheter for water sampling. The animal was then placed in a continuous flow respirometer (Figure 1) modified after the open respirometry system of Saunders (1962). Water from a reservoir (a) was pumped to the respirometer (b) through a regulated Tri-Flat fluid flowmeter (c) (Fisher-Porter Co., Warminster, PA) with 1-703 ml/min capacity. Circulation tubes (d) and magnetic stirring bars (e) were used to insure equal distribution of water within the chamber. Water leaving the respirometer was routed through an aerating chamber (f) and filter (g) then returned to the reservoir for temperature equilibration through the use of air stones (h) and heating or cooling coils (i). Gill function was measured at 15° and 25° C which are temperatures commonly

experienced by the bowfin in southeastern Oklahoma. Following a 5-6 hour adjustment period in the respirometer, gill function measurements were commenced.

Opercular Activity. Opercular rate was measured with a Model P23BB Statham pressure transducer (Statham Laboratories, Inc. Hato Rey, Puerto Rico) and recorded on a Model 5DWC1 Grass polygraph (Grass, Inc., Quincy, Mass.) coupled to the opercular catheter.

Oxygen Extraction. Oxygen extraction efficiency of the gill was determined from the P_{O_2} of inspired and expired water samples. The P_{O_2} of the samples was measured with a Model E 5046 oxygen electrode (Radiometer, Inc., Copenhagen, DK). The oxygen extraction efficiency was then estimated as follows:

$$\text{Percent Oxygen Extraction} = \frac{P_{I_{O_2}} - P_{E_{O_2}}}{P_{I_{O_2}}} \times 100$$

where $P_{I_{O_2}}$ and $P_{E_{O_2}}$ are oxygen partial pressures of inspired and expired water, respectively.

Oxygen Uptake. Oxygen consumption ($\dot{V}_{O_2}$) was estimated according to the procedure of Garey (1970). The respirometer was filled with water and sealed at time zero. The rate of oxygen partial pressure decline thereafter was used as a measure of oxygen uptake. Oxygen consumption was then calculated using:

$$\dot{V}_{O_2} = \Delta P_{O_2} \cdot \alpha_{O_2} \cdot V/t$$

where $\dot{V}_{O_2}$ is oxygen consumption in ml O_2 (STPD)/min, ΔP_{O_2} is the change in oxygen tension (torr), α_{O_2} is the solubility coefficient of O_2 (Rahn, 1966), V is the effective volume of the respirometer in liters and t is time in minutes. The P_{O_2} was not allowed to

drop below 50 torr during the experiment in order to reduce fish restlessness and their efforts to aerial breathe.

Ventilation Volume. The rate of water movement over the gill apparatus ($\dot{V}g$) was estimated as follows:

$$\dot{V}_g \text{ (ml/min)} = \frac{\dot{V}_{O_2}}{\alpha P_{I_{O_2}} - \alpha P_{E_{O_2}}}$$

Gill Morphology. The bowfin gill system was also examined at the gross and histological level. Histological material was prepared from excised gill filaments which were fixed in Zenkers' fluid, embedded in paraffin, sectioned at 8 microns then triple stained with Mallory's trichrome.

Erythrocyte, Hematocrit and Hemoglobin Determinations. Erythrocytes were counted and sized with a Coulter Model B particle counter and Model J plotter. Hematocrits were determined after centrifugation of samples in standard hematocrit tubes. Hemoglobin was estimated indirectly with the cyanomethemoglobin method (Wintrobe, 1965).

Blood Respiratory Properties. Blood P_{O_2} and pH was measured with a Clark oxygen electrode (Radiometer Model E 5046) and pH microelectrode (Radiometer Model E 5036) coupled to a gas monitor (Radiometer Model PHA 9276) and pH meter (Radiometer Model 27). Blood oxygen and carbon dioxide concentration (Vol %) was determined with a Natelson microgasometer.

Hemoglobin oxygenation properties were determined under different temperature and pH conditions with a modification of the mixing technique discussed by Edwards and Martin (1966).

A blood sample (5-6 ml) was divided equally between two Model K 125 ml Kontes tonometers that were arranged to rotate in a constant temperature bath (Hall, 1965). Both tonometers were initially immersed in a 15°C temperature bath. The blood in on tonometer was equilibrated with nitrogen gas to produce deoxyhemoglobin (Hb) while the blood in the second tonometer was equilibrated with air to provide saturated oxyhemoglobin (HbO_2). The equilibration process was complete in approximately 1 hour. Portions of blood then were removed from each tonometer and mixed under mineral oil to provide the following fractions of oxyhemoglobin: 25, 50, 65, 75 and 90 per cent. Samples of each fraction were then transferred with a gas free microsyringe to oxygen and pH electrode chambers. The remainder of the blood in each tonometer was then adjusted to 25°C and analyzed with the same procedures.

In the second set of measurements, blood samples were equilibrated at 15°and 25°C according to the above procedure except the gases with high CO_2 content were used to produce deoxyhemoglobin (5.44% CO_2, balance N_2) and oxyhemoglobin (21.0% O_2, 5.25% CO_2, balance N_2). Blood disscciation curves were constructed and the Bohr effect was estimated according to Lenfant et al., (1966). The temperature shift of the oxygen dissociation curves was calculated from the equation of Vokac et al., (1972).

RESULTS

Gill Morphology

The bowfin gill arch (Figure 2) has short rakers and moderately long filaments with dorsal and ventral lamellae. The tips of these lamellae are embedded in an interlamellar septum (Figure 3). Because of this anatomical feature, the lamellae are held in a fixed position to create a seive-like arrangement. Thus, when water moves through the gill, it is descretely channeled through narrow passageways that are rectangular shaped.

Each lamella consists of two layers of thin epithelium held together internally by pillar cells. Capillary-like vessels pass through this gill unit in parallel fashion (Figure 3). These vessels number from 10 to 16 per lamella and have a mean diameter of 4.1 μ. Blood-water diffusion distance averaged 2.1 μ. Approximately 10 to 25 percent of each lamella tip is enclosed by the interlamellar septum. Consequently a portion of blood flow through the lamella moves within the interlamellar septum and is not exposed to water for gas exchange. Dimensions of the filament-lamella system are summarized in Table 1.

Gill Respiration

Animal condition. Weight declined from time of capture to time of use. The average weight loss over a period of 4.5 months was 7.9 percent and related to the confinement in relatively small aquaria which restricted movement during feeding. Despite weight loss, the animals

were in apparent good condition at the time of experimentation and actively resisted capture.

During respirometer confined experimentation, serum glucose slowly increased (unpublished data) and blood pH decreased (Table 5). The weight loss and serum glucose change indicated a fasted condtion. Therefore, the respiratory data were obtained from fish at a reduced level of metabolism.

Gill Function at 15°C. Mean opercular rate was variable between fishes and ranged from 7 to 27 bpm (Table 2). The percent extraction of oxygen was also variable and ranged from an average of 8.3% to 42.7% (Table 2). The single highest value was 58.9% (Fish 1) while the lowest was 5.7% (Fish 6). Individual fish had the ability to extract oxygen at some given level for a time then later change the extraction process several percent (Figure 4). Percent oxygen extraction varied inversly with opercular rate.

Oxygen consumption ($\dot{V}_{O_2}$) ranged from 19.7 to 50.3 $ml \cdot hr^{-1} \cdot kg^{-1}$ (Table 2) and varied directly with fish body size. Ventilation volume ($\dot{V}_g$) ranged from 196 to 645 $ml \cdot min^{-1} \cdot kg^{-1}$ and the amount of water moved by each opercular stroke was between 18-45 $ml \cdot beat^{-1} \cdot kg^{-1}$ (Table 2).

Gill Function at 25°C. Average opercular rate ranged from 10 to 24 bpm while percent oxygen extraction varied from 8.9 to 53.4% (Table 3). The P_{O_2} of the inspired water was lower at this temperature which led to increased gill breathing. The opercular breathing rate then influenced the oxygen extraction process in an inverse manner. For example, Fish 3 (Table 3) had the lowest rate

of operculation but the highest oxygen extraction efficiency.

Mean oxygen consumption increased slightly over the 15°C data and ranged from 19.8 to 56.7 $ml \cdot hr^{-1} \cdot kg^{-1}$ (Table 4). Again, the smaller fish had higher $\dot{V}O_2$ values. Ventilation volume also increased (Table 3). In some fish, the volume nearly doubled with the 10° C increase in water temperature. $\dot{V}g$ ranged from 253 to 1018 $ml \cdot min^{-1} \cdot kg^{-1}$ and gill stroke volume extended from 15 to 50 $ml \cdot beat^{-1} \cdot kg^{-1}$.

Hematology

At 15°C, the mean erythrocyte (RBC) count was 1.01 x $10^6/mm^3$ (Table 4). The RBC number at 25°C showed much less variability and averaged 1.21 x $10^6/mm^3$. Mean hematocrits (Hct) at these respective temperatures were 22.6 and 29.5%. Erythrocyte mean cell volume (MCV) was variable but was unchanged by temperature (Table 4). Average hemoglobin concentration was different at each temperature and averaged 6.7 g% at 15°C and 7.9 g% at 25°C (Table 4).

Blood Respiratory Characteristics

The calculated blood oxygen capacity for 15°C acclimated fish was 8.9 Vol % and was 10.6 Vol % for fish at 25°C (Table 4). These estimates were close to actual determinations of 6.9 and 9.4 Vol % at 15°and 25°C, respectively. Oxygen capacity changed little when measured at different P_{CO_2}'s. At 15°C, the capacities were 6.8 and 6.9 Vol % for the respective C_{O_2} percentages of 0.035% and 5.35%. At 25°C, the respective capacities were 9.2 and 9.5 Vol %.

Prior to experimentation, bowfin blood had a mean arterial pH of 7.75 and an average venous blood pH of 7.56 at 15°C. At 25°C, the blood pH values were 7.45 for mean arterial and 7.34 for mean venous blood. During experimentation, blood pH decreased (Table 5).

At 15°C, the oxygen dissociation curves for whole Amia blood were hyperbolic at low P_{CO_2} and slightly sigmoid at high P_{CO_2} (Figure 5). At a P_{CO_2} of 1 torr, the P_{50} ranged from 3.1 to 5.3 torr. This range increased approximately 3 fold when P_{CO_2} was elevated to 37 torr and gave a Bohr factor of -0.421 (Table 5).

The oxygen dissociation curves at 25°C were sigmoid (Figure 6). The temperature change from 15° to 25°C produced no significant alteration in the Bohr factor (-0.42) but P_{50} values approximately trebled (Table 5). Temperature coefficient values fell within a -0.04 to -0.05 range.

DISCUSSION

Gill Morphology

The most unusual feature of the bowfin gill is the interlamellar septum which serves as a membranous interface between opposing lamella. Bevelander (1934) first described this structure but did not deal extensively with its structure or function. The tarpon, swordfish, sailfish, marlin and the tuna are apparently the only other fish that have this type of gill structure (Bevelander, 1934; Hughes, 1963; Muir and Kendall, 1968). These fish are strong, rapid swimmers and have rapid water flow through the gills. The interlamellar septum holds the lamella securely in a fixed position during rapid swimming which maximizes gas exchange surface area. This gill arrangement would provide the bowfin with the same advantage if it were a rapid swimmer and would also prevent collapse of the lamella during aerial breathing or estivation on land (Bevelander, 1934).

Since the interlamellar septum is sandwiched between opposing lamella, a series of sieve-like channels are created between adjacent gill filaments. These channels are rectangle shaped and contain a space capable of holding water by capillary action. These water reservoirs would offer several advantages to the fish. For example, if the bowfin were out of water, the

trapped water would help offset desiccation and prevent sudden changes in the gas exchange process at the lamellar sites. Of major importance would be the provision of a carbon dioxide "sink". The retention of water in the sieve channels would allow carbon dioxide exchange to continue between gill blood and gill water during aerial exposure. During normal gill function, however, this type of structural design would cause increased resistance to water flow. But, the subsequent reduction of water movement through the channel would increase time for diffusion of gases (Randall, 1970). Thus, efficiency would be increased (Hughes, 1972) provided the openings of the channels were not blocked by gill mucus or solid particles from incoming water.

The interlamellar septum encloses 10 to 25 percent of the distal edge of the lamellae and prevents a large portion of the lamella surface from making contact with water. Therefore, any advantage gained by the sieve channel arrangement in reducing functional dead space appears to be offset by the loss of gas exchange surface area.

No other air breathing fish have the sieve channel feature. The South American lungfish (Lepidosiren paradoxa) has reduced the gill filament length and lamellar number (Johansen and Lenfant, 1967). The lamellae are thickened which results in a greater diffusion distance (Fullarton, 1931). The Australian lungfish (Neoceratodus forsteri) has the most highly developed gills of the Dipnoi. Their lungs play only a small accessory role in the total gas exchange process; accordingly, the gill efficiency is much

higher than in Lepidosiren (Lenfant et al., 1966). The African lungfish (Protopterus aethiopica) is intermediate in respiratory structure and function compared to the other Dipnoans (Lenfant et al., 1970). The gars, in the primitive Holostean group, also lack the sieve channel arrangement and have different gill dimension features (Landolt, 1970).

Several authors (Steen and Berg, 1966; Hughes, 1966) have summarized gill dimensions for a variety of fishes and found that active fish tend to have thinner gill lamellae, more lamellae per mm of filament and small blood-water diffusion distance. The opposite was generally observed for sluggish fishes. According to this classification scheme, the bowfin would fit in the active fish category. However, the bowfin spends most of its time resting on bottom substrate or in tangled vegetation. The bowfin also feeds by either quietly stalking prey or by lying in wait until prey comes near. The quiet resting behavior and controlled feeding activity somewhat contradicts the active fish characteristic revealed by the gill morphology study until the following is considered. When alarmed, the bowfin will swim about rapidly often for several minutes. Furthermore, the bowfin has the habit of migrating from one pond or slough to another by when flooding permits movement through interconnecting passageways. This type of movement is difficult because the temporary water passages are often shallow, grass or weed filled and meandering. The bowfin gill would be well suited to meet the physical and metabolic demands of such types of activity, since it is well protected and arranged in an efficient manner.

Gill Respiratory Dynamics

Interpretation of gill respiration in fish is complex because of the influence of water temperature, gas solubility, blood perfusion, body weight, age, sex, reproductive condition, nutritional state and stress induced metabolic change (Steen and Kruysee, 1964; Hughes, 1964; Rahn, 1966; Hoar and Randall, 1970; Chavin and Young, 1970; Hughes, 1972). In air breathing fish, aerial gas exchange further complicates evaluation of gill function. Efficient bimodal breathers such as Lepidosiren, Protopterus, Polypterus, Lepisosteus, Neoceratodus and Amia have gas exchange divided between the gill and lung (or lung-like air sac)(Johansen, 1972; Rahn et al., 1971). Mechanisms exist to route blood flow to one or the other (or both) respiratory compartments depending on water temperature and P_{O_2} (Lenfant et al., 1970). A shift to exclusive aquatic or aerial breathing requires a period of transition in ventilation-perfusion patterns. During this transition, patterns of gas exchange are not uniform (Lenfant et al., 1966; Johansen et al., 1970).

In this study, Amia gill respiration was investigated in a temperature range where a shift toward aerial breathing occurs (Horn and Riggs, 1973). At 10°C, Amia is a strict aquatic breather but at 28°C, aerial gas exchange predominates (Johansen et al., 1970; Horn and Riggs, 1973). At 25°C, the air sac contributes over half the total oxygen uptake which is almost three times the amount supplied at 15°C (Johansen et al., 1970).

The gill oxygen uptake results of the present study were lower than the data published by Johansen, et al., 1970. Their

data show an oxygen uptake of approximately 48 and 54 ml·hr^{-1}·kg^{-1} at the respective temperatures of 15° and 25°C. This higher rate of oxygen uptake could be due to a less fasted condition and a greater activity state in the animals. Some difference in activity was revealed by different aerial breathing rates. This same report indicated that the bowfin breathes air at a frequency of 5, 11 and 42 breaths per hour at the respective temperatures of 10°, 20°and 30°C and at 140 torr. These data were higher than the aerial breathing rates of 0.5, 4 and 16 breaths per hour, at the respective temperatures above, published by Horn and Riggs (1973). Aerial breathing rates in my study were 1-3 breaths per hour at 15°C and 4-8 breaths per hour at 25°C (Unpublished data). It is presumed that the lower aerial breathing rates were due to reduced activity induced by fasting conditions. This is further supported by the observation that the bowfin in this respiratory study had lower serum glucose values than fish that were actively feeding (Unpblished data).

The gill respiratory performance of Amia is similar to other air breathing fish. The spotted gar (Lepisosteus occulatus) in the size range of 290 to 600 g and at 12°C, had gill breathing rates and gill efficiency ranges of 11 to 30 bpm and 5.1 to 7.9 %. Stroke volume ranged from 0.55 to 30.7 ml/beat . $\dot{V}_{O_2}$ ranged from 5.1 to 54.6. Except for $\dot{V}_{O_2}$, these variables did not increase appreciably when measured at 20°C (Landolt, 1970). The longnose gar (Lepisosteus osseus) had a $\dot{V}_{O_2}$ range of 10.2 to 26.8 ml/hour at 22°C (Rahn et al., 1971). Neoceratodus had a similar oxygen extraction efficiency of between 10 and 74%, an opercular rate range of 22 to 42 bpm, a

ventilation range of 83 to 747 ml/min and a $\dot{V}_{O_2}$ range of 11.4 to 104 ml/hr at 18°C (Lenfant et al., 1966). Fasting Protopterus gave an oxygen consumption range of 12 to 31 ml/hr/kg at 25°C (Delaney et al., 1974). However, Lenfant and Johansen (1968) found that fasting Protopterus had a mean oxygen uptake of only 1.3 ml/hr/kg at 20°C. Johansen and Lenfant (1967) found that juvenile Lepidosiren had virtually no gill respiratory behavior. Oxygen extraction efficiency was low and attempts to measure gill ventilation failed. These results were not unusual since this lungfish has degenerate gills and is an obligate aerial breather.

Hematology

The hematology of the bowfin (Table 4) shows characteristics that would be adaptive in habitats with variation in oxygen content and temperature. For example, from 15° to 25°C erythrocytes and hematocrit increased 20.2 and 29.5 % respectively. Hemoglobin had a 18 % increase. Such changes would permit an increase in oxygen carrying capacity of the blood and allow greater oxygen uptake when oxygen solubility is decreased because of water temperature increases.

The mean erythrocyte volume (MCV) of the bowfin blood was unchanged over the 15° to 25°C range. The mean volume range (282 to 306 μm^3) is intermediate among the Dipnoans, Teleosts, Elasmobranchs and Chondrosteans (Satchell, 1971; Wintrobe, 1934; Vokac et al., 1972; Swan and Hall, 1966).

The hematology feature of the bowfin were similar to the blood data collected for other air breathing fish (Table 6). However, the spotted gar (Lepisosteus occulatus) had hematology values much

in excess of the bowfin (Smith, 1968). The hematology of the bowfin was most like the African bichir (Polypterus senegalis) and the Australian lungfish (Neoceratodus forsteri) (Table 6). These latter two fish live in habitats that are similar to those described for the bowfin.

Blood Respiratory Characteristics

The increase in oxygen capacity from 15° to 25°C was related to increased hematocrit and represented a functional change that would support the more active metabolic conditions that the bowfin experiences at 25°C.

The bowfin had a mean arterial blood pH that was about 0.2 of a unit higher at 15°C than at 25°C (Table 5). The pH values at each temperature were variable which is not uncommon for fishes (Rahn and Baumgardner, 1972). The pH change with respect to temperature fits the acceptable model for blood pH - temperature relationships which predicts that, at 15°C, fish will have normal blood pH values close to 7.9, whereas, at 25°C, pH values will be near 7.7 (Rahn and Baumgardner, 1972). The inverse relationship between ambient temperature and blood pH has also been observed for a number of other ectotherms (Howell et al., 1970). The bowfin followed this model but, pH values were somewhat below prediction. Since the time between capture of the fish and actual blood sampling was often several minutes, it is likely that the lower pH values of this study were due to accumulations of acid metabolites (Black et al., 1962).

Normal blood pH data for air breathing fish are sparce (Table7). Smith (1968) found that the spotted gar had a venous pH range of 7.08 to 7.73 over a temperature range of 18° to 26.5°C. Swan and Hall (1966)

gave values of 7.42 and 7.4 for two African lungfish at 25°C. Lenfant et al., (1966) reported that the Australian lungfish had a mean arterial pH of 7.64 and an average venous pH of 7.57 at 18°C. Amia blood pH values fell within the range of observations reported above and showed no unusual pattern.

The oxygen dissociation curves showed that Amia blood responded in logically adaptable ways. The high affinity hemoglobin (Table 7) of this species insures that the blood will be saturated with oxygen, even in hypoxic water, during aquatic respiration. Inspection of available data (Table 7) reveals that the high oxygen affinity of Amia blood is equivalent to that of the carp which also has the ability to survive in hypoxic water. However, the African and South American lungfishes and the African Bichir had hemoglobin oxygen affinity values lower than Amia (Table 7).

The sensitivity of Amia hemoglobin to temperature and pH insures adequate transport of oxygen in the warm, hypercapnic conditions asssociated with aerial breathing. The temperature coefficient range (-0.04 to -0.05) for the hemoglobin is one of the highest for fish. Most fish have temperature coefficients in the range of -0.01 to -0.03 which is considerably below the value estminated in this study (Prosser, 1973). A second temperature coefficient of -0.045 for Amia hemoglobin was estimated from the data of Johansen et al., (1970). The high temperature sensitivity measured here would allow the hemoglobin to unload oxygen more easily at higher water temperatures which would elevate tissue oxygen tension in support of a more active metabolism. The Bohr

coefficient for Amia blood was intermediate compared to other fish (Table 8). However, there was sufficient pH sensitivity to allow adaptive shifts in hemoglobin oxygen affinity when acidic water conditions prevail. At 25°C, the P_{50} increased about 2.6 times as pH declined one unit. This would produce a tissue oxygen tension change comparable to the temperature effect data.

Whole blood findings generally agree with molecular studies on Amia hemoglobin. Weber et al., (1976) found that the hemoglobin system of Amia was of the undifferentiated Class I type which represents a primitive stage in the evolution of Teleost fish hemoglobins. Class I hemoglobin has high oxygen affinity, temperature and pH sensitivity. It may be argued that these features of Class I hemoglobin, especially the type 1 component, served to preadapt this fish for aerial breathing.

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Tables

Table 1. Gill dimensions of the bowfin Amia calva L. *

Desc. Stat.	Distance between filaments (mm)	Filamental Lamellae Per mm	Lamellae thickness (μ)	Distance between Lamellae (μ)	Capillary diameter (μ)	Gill diffusion distance (μ)
$\bar{x}$	0.29	16.0	8.2	33	4.1	2.1
σ	0.01	1.3	1.2	8.9	0.8	0.4
Range	0.26-0.31	14-18	6.4-9.8	21-50	3.0-5.1	1.7-2.9
N	10	10	10	10	10	10

*Taken from the outer hemibranch of right first gill arch of 543-686 g fish.

TABLE 2. Anta gill respiratory variables at 15C.

Lab No.	Weight (g)	$P_{I_{O_2}}$	$P_{I_{O_2}}$	O_2 Extraction[1] (%)	$\dot{V}_{O_2}$ (ml · hr^{-1} · kg^{-1})	$\dot{V}_R$ (ml · min^{-1} · kg^{-1})	Opercular rate[1] (BPM)	Gill stroke volume (ml · beat^{-1} · kg^{-1})
1	466	149	100	31.7(16.5-58.9) N=12	50.3	365	17(10-27) N=21	22
2	764	146	125	14.5(11.2-15.9) N=5	36.1	620	14(12-15) N=6	44
3	906	141	80	42.7(31.6-50.0) N=7	32.4	196	7(5-8) N=7	28
4	1036	177	106	12.9(9.4-19.7) N=5	22.8	523	27(24-32) N=7	18
5	1051	140	107	9.6(7.4-11.9) N=5	23.0	624	14(13-16) N=5	45
6	1318	136	125	8.3(5.7-10.4) N=5	19.7	645	15(13-17) N=5	42
7	2147	137	110	19.9(19.7-20.9) N=5	26.9	364	19(18-20) N=5	19

(1) Results expressed as $\frac{\text{Mean (Range)}}{\text{Number of observations}}$ The number of observations for $P_{I_{O_2}}$ and $P_{E_{O_2}}$ are the same as O_2 extraction. $\dot{V}_{O_2}$ values are averages of two experimental runs. $\dot{V}_R$ and stroke volumes are derived data.

TABLE 3. *Amia* gill respiratory variables at 25C.

Fish No.	Weight (g)	$P_{I_{O_2}}$	$P_{E_{O_2}}$	O_2 Extraction[1] (%)	$\dot{V}_{O_2}$ (ml · kg^{-1} · kg^{-1})	$\dot{V}_B$ (ml·min^{-1}·kg^{-1})	Opercular Rate[1] (BPM)	Gill Stroke Volume (ml·beat^{-1}· kg^{-1})
1	466	145	75	48.7(39.9-57.1) N=9	56.7	355	21(16-24) N=7	17
2	764	123	104	15.8(12.7-23.5) N=6	43.9	1018	21(19-24) N=6	49
3	906	139	65	53.4(42.5-57.6) N=6	42.0	253	10(9-11) N=6	25
4	1035	124	100	19.1(17.4-22) N=5	19.8	369	24(21-26) N=14	15
5	1051	137	120	12.7(9.8-16) N=5	21.7	561	15(11-18) N=5	37
6	1318	125	115	8.9(8.4-9.2) N=5	24.2	897	18(17-20) N=6	50

(1) Results expressed as $\frac{\text{Mean (Range)}}{\text{(number of observations}}$. The number of observations for $P_{I_{O_2}}$ and $P_{E_{O_2}}$ are the same as O_2 extraction. $\dot{V}_{O_2}$ values are averages of two experimental runs. $\dot{V}_B$ and stroke volume are derived data.

Table 4. Hematology of the bowfin, Amia calva L. at 15 and 25 C.

	Descriptive Statistic	RBC No ($\#/mm^3 \times 10^6$)	HCT (%)	RBC MCV (μ^3)	Hb (g%)	Oxygen Capacity* (vol. %)
	X	1.01	22.6	306	6.7	6.9
	S.E.	0.51	0.9	36	--	--
15 C	N	4	5	3	--	1
	Range	0.62-1.2	20.1-25.5	239-361	--	6.4-7.3
	X	1.21	29.5 %	282	7.9	9.4
	S.E.	0.13	1.4	40	--	--
25 C	N	5	9	5	--	1
	Range	0.86-1.81	21.5-40.7	189-407	--	8.7-9.7

N = number of fish used; * = blood equilibrated with 20.9% O_2, 5.25% CO_2, bal N_2

Table 5. *Amia calva* blood respiratory characteristics at 15 C and 25 C.

Temp (C)	Desc. Stat.	Normal Blood pH (arterial)	(venous)	Expt'l Blood pH (arterial)	P_{50} at: * (1% CO_2)	(5.25% CO_2)	Bohr Factor
	X	7.75	7.60	--	--	--	
	S.E.	0.04	0.03	--	--	--	
15 C	N	5	5	2	3	3	-0.42
	Range	7.68-	7.54-	7.44-	3.1-5.3	9.6-15.4	
		7.88	7.69	7.51	(8.01-7.92)	(6.89-6.81)	
	X	7.50	7.34	--	--	--	
	S.E.	.02	0.02	--	--	--	
25 C	N	10	10	2	4	4	-0.42
	Range	7.31-	7.22-	7.24-	11.8-15.6	34.2-41.0	
		7.51	7.43	7.18	(7.96-7.78)	(6.81-6.75)	

*P_{50} expressed as a range only due to variation in pH. pH range follows P_{50} range as (pH).

Table 6. Comparison of the hematology of air breathing fish.

Species	RBC ($N \times 10^6$)	Hct (%)	MCV (μ^3)	Hb (g%)	Oxygen Capacity (Vol %)	Temp (C)	Author
Amia calva	1.01	22.6	306	6.7	6.9	15	Present study
	1.21	29.3	282	7.9	9.4	25	Present study
	-	27.1	-	-	11.8	15	Black, 1940
	-	22.8	-	5.8	7.8	18-20	Johansen et al., 1970
	-	26.4	-	7.9	-	23	Weber et al., 1976
Lepisosteus occulatus	3.08	32	-	9.9	15.7	18	Smith, 1968
Protopterus aethiopicus	-	21.9-28.7	-	6.9,7.1	-	25	Swan and Hall, 1966
Polypterus senegalis	0.33-1.15	17-34	296-636	4.3-14.0	-	-	Vokac et al., 1972
Lepidosiren paradoxa	-	14-19	-	-	4.9 6.8	18	Johansen and Lenfant, 1967
Neoceratodus forsteri	-	31	-	-	7.7	18	Lenfant et al., 1967

RBC (erythrocyte No.), Hct (hematocrit), MCV (mean RBC volume), Hb (hemoglobin)

Table 7. Comparison of blood respiratory properties for air breathing fish and common carp.

Species	Temp. (C)	P_{CO_2}[1] (torr)	pH	Bohr Factor $\frac{\Delta \text{Log } P_{50}}{\Delta \text{pH}}$	P_{50} (torr)	Author
Amia calva	15	0-1	--	--	4	Black, 1940
	15	23	--	--	11	Black, 1940
	15	--	7.6	-0.57	9	Johansen et al.,1970
	27	--	7.6	-0.54	24	Johansen et al., 1970
	15	0-1	7.9-8.0	-0.42	3-5	Present study
	15	37	6.8-6.9	-0.41	10-15	Present study
	25	0-1	7.8-8.0	--	12-16	Present study
	25	37	6.7-6.8	-0.42	34-41	Present study
Heoceratodus sp.	18	--	7.6	-0.62	13	Lenfant et al., 1966
Protopterus sp.	23	--	7.6	-0.34	27	Swan and Hall, 1966
Lepidosiren sp.	--	--	--	-0.30	--	Johansen, 1970
Polypterus senegalus	30	--	7.7	-0.43	24	Vokac et al., 1972
Cyprinus carpio	15	1-2	--	--	5	Black, 1940
	15	14-16	--	--	14	Black, 1940

(1) These variables represent blood tonometer conditions

Figure Legends

Figure 1. Continuous flow respirometer showing water reservior (a), respirometer (b), flowmeter (c), circulation tubes (d), stirring rod (e), aerating chamber (f), filter (g), air stones (h), and heating-cooling coil (i).

Figure 2. Histological view of *Amia* gill apparatus. A is a diagram of entire section of the *Amia* gill showing gill arch (ga), gill raker (gr), cartilage support rod (csr), efferent branchial artery (eba), afferent branchial artery (aba), gill filament (gf), lamellae (l), and interlamellar septum (is).

Figure 3. An oblique view of gill filament that shows cartilage rod (cr), efferent filamental artery (efa), gill filament (gf), lamellae (l), and interlamelar septum (is).

Figure 4. Representative opercular rate and oxygen extraction efficiency for an *Amia calva* (466 g) at 15° and 25° C.

Figure 5. Oxygen dissociation curves for the bowfin Amia calva L. at 15°C and at 0.034% and 5.35% carbon dioxide conditions.

Figure 6. Oxygen dissociation curves for the bowfin Amia calva L. at 25°C and at 0.034% and 5.35% carbon dioxide.

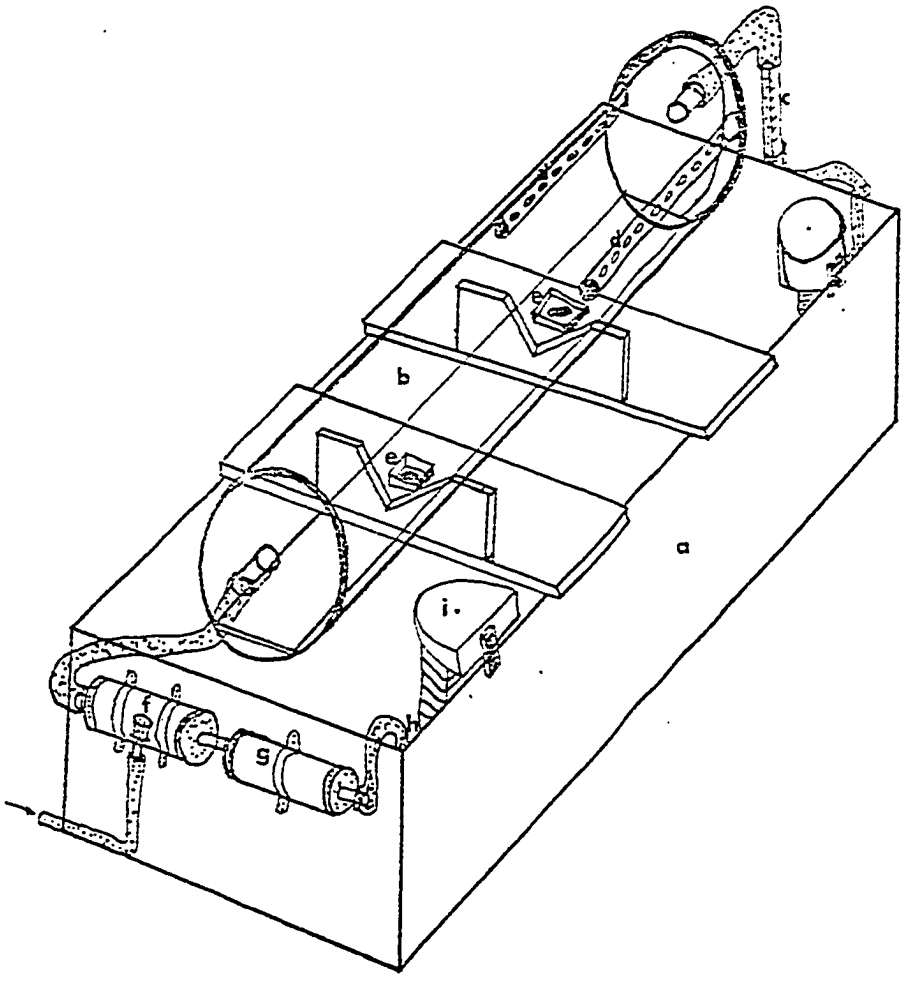

Figure 1

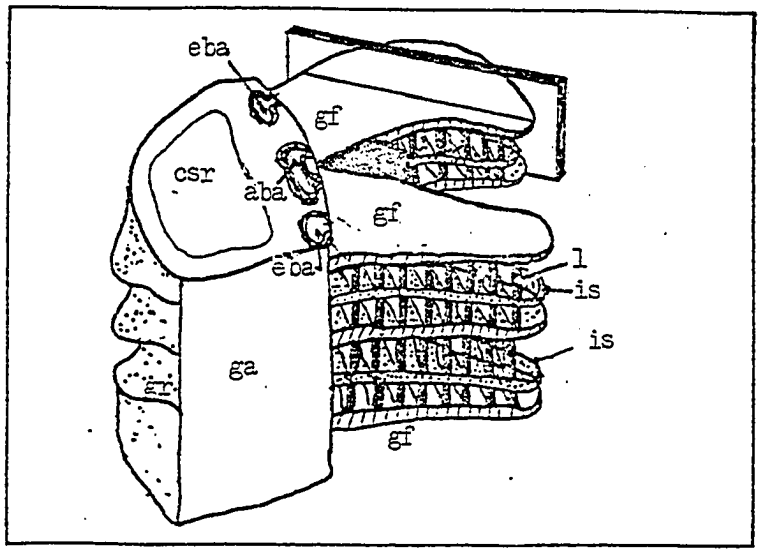

Figure 2

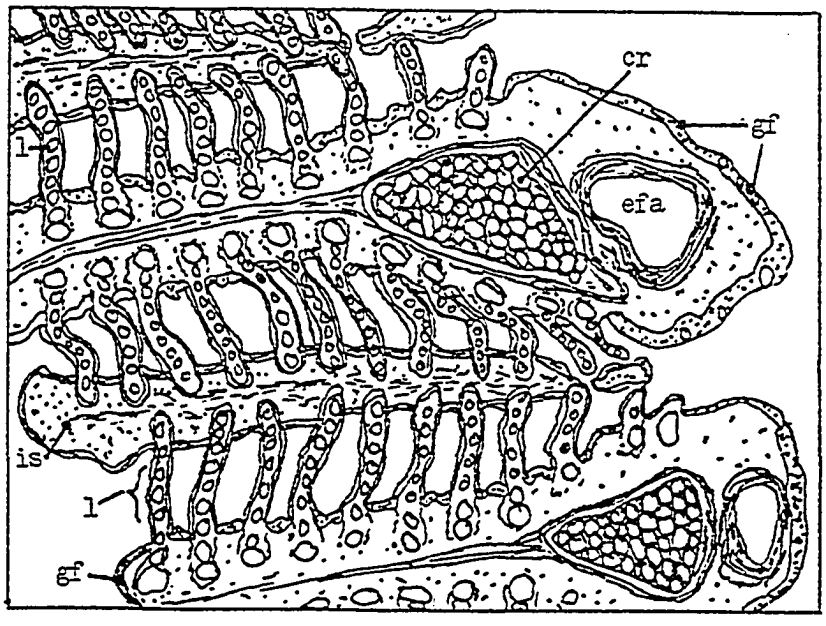

Figure 3

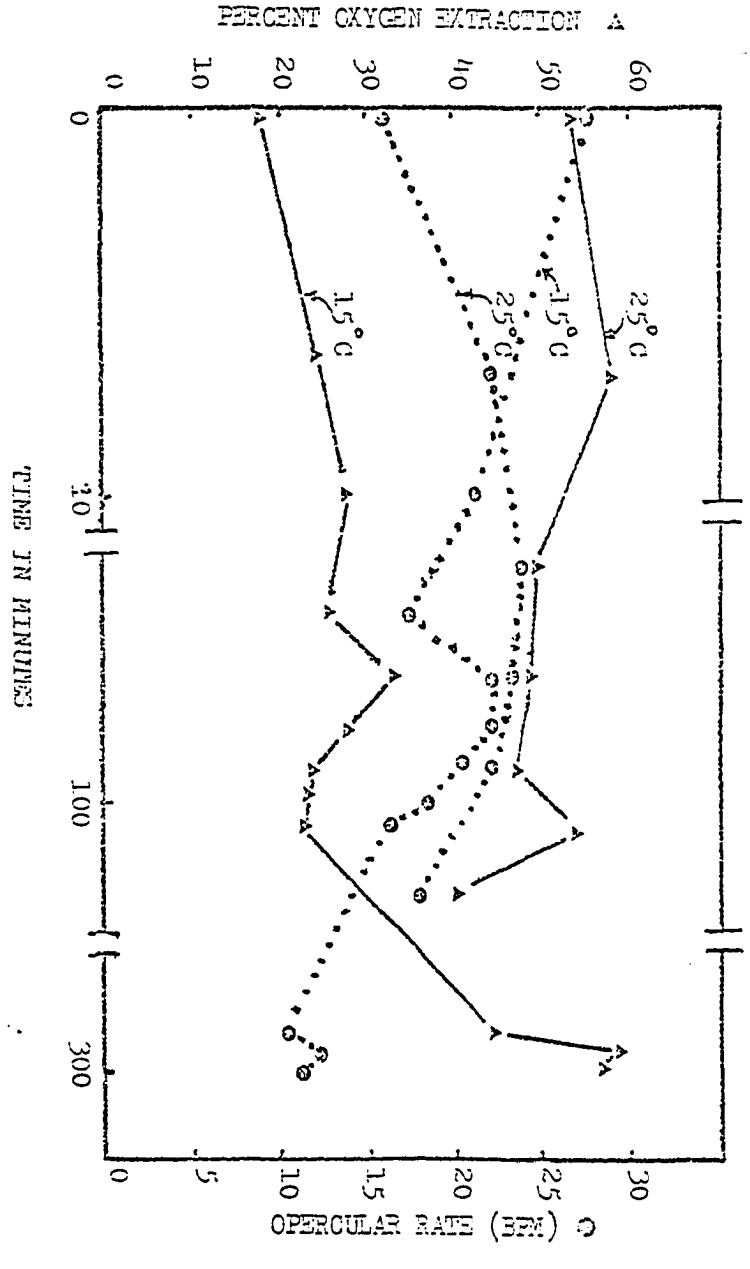
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Figure 4

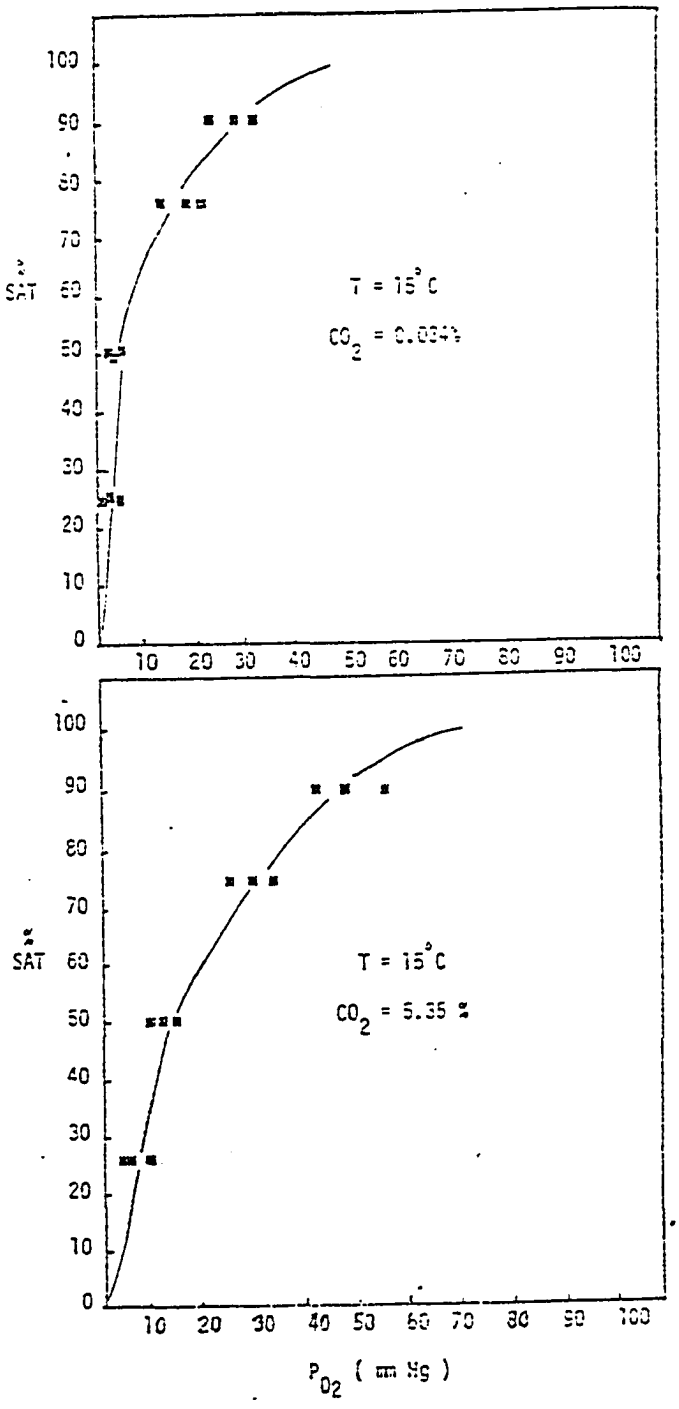

Figure 5

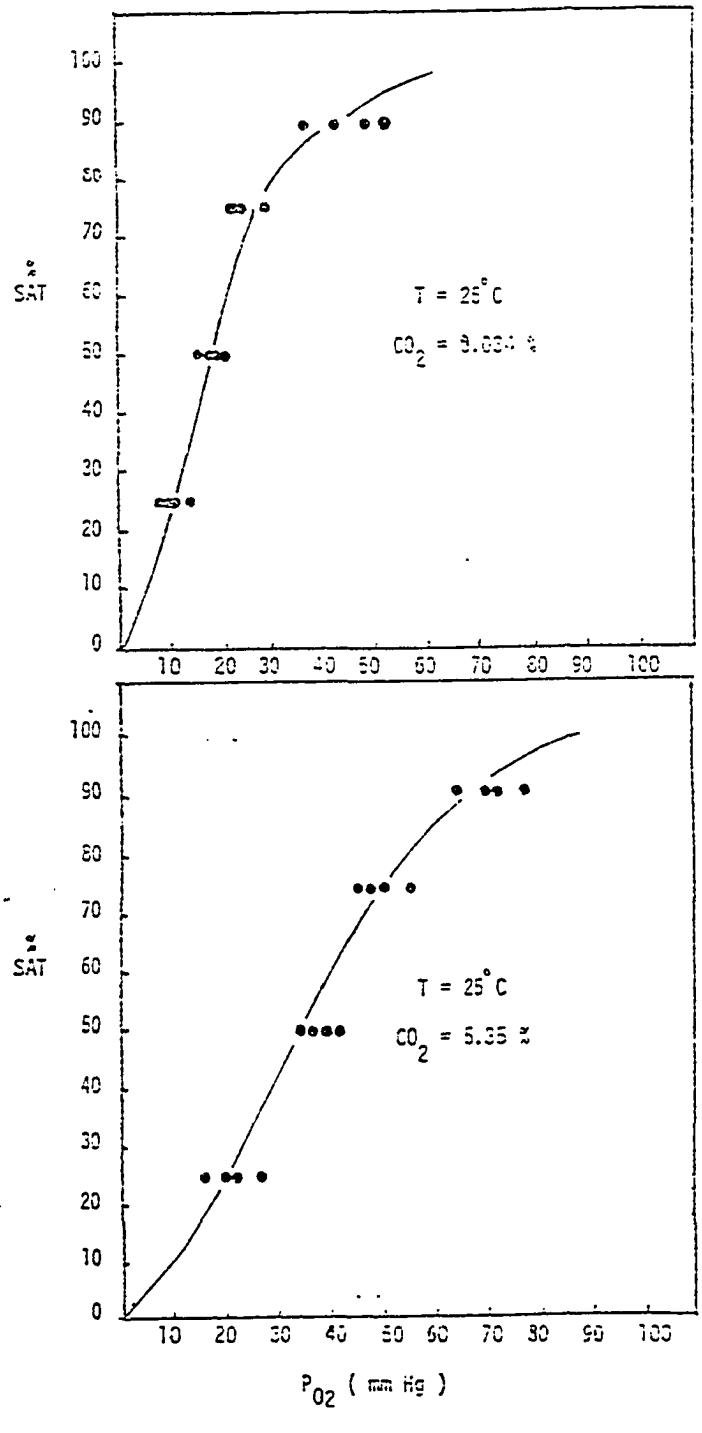

Figure 6

CHAPTER IV

MORPHOMETRIC AND PHARMACOLOGICAL ASPECTS OF THE AIR SAC OF THE BOWFIN, *AMIA CALVA*.

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ABSTRACT. The air sac of the bowfin, *Amia calva* has a complex anatomical organization. The interior consists of a central airway with lateral compartments that are partitioned by thin septa. The air chambers created by the septal partitions are irregular in shape and constitute the respiratory gas exchange unit for the air sac. This functional area is analogous to the alveoli of lungs but is several orders of magnitude larger in size. Each septum has epithelium on the air surface which covers blood vessels and connective tissue. The entire air sac is surrounded by a striated muscle sheath and muscle bundles of this structure are organized into circular and longitudinal layers. The interior of the air sac has a noticeable lack of smooth muscle which was unlike the condition found in lungfish and gars. Since the air sac is partitioned into several air compartments with little supporting structure, it is a flacid and compliant structure.

An allometric analysis of 20 air sacs revealed the extent this gas exchange structure follows the morphometry of vertebrate lungs. Inspection of allometric power functions for air sac variable body weight comparisons indicated that many features (lung weight, length, volume, diameter) were similar to lungs of amphibians and reptiles. Dissimilarity was noted in Amia air chamber-lung alveolar diameter comparisons and the ratios of the central air space diameter to the total air sac diameter.

The sum of the air sac features studied here indicates that the bowfin air sac probably uses a different strategy in the gas exchange process compared to other air breathing fish. Briefly, the relatively large air compartments of the air sac are very compliant and lack smooth muscle trabeculae (as in gars) and strands (as in lungfish). Therefore, when the bowfin surfaces to breathe, the air sac can be readily evacuated and then quickly filled with fresh air.

The striated muscle sheath of the air sac had moderate sensitivity to ACh (ED_{50} range of 3.6 to 8.4 µg/ml) and could be blocked by tubocurarine which suggested a nicotinic receptivity. The internal septal tissue of the air sac was less responsive to ACh and had an ED_{50} range of 22 to 45 µg/ml. This response was blocked by atropine suggesting the presence of a muscarinic receptor. Whole air sac preparations consisting of striated muscle and septal tissue gave a bimodal ACh response consisting of a rapid and slow contraction pattern. The rapid phase was characteristic of the striated sheath preparation response, whereas, the slow phase paralleled the response of the separate septal tissue preparation.

The relatively slow, tonic-like response of the striated sheath to ACh suggests the presence of red type striated muscle fibers.

The striated air sac preparations responded poorly to adrenergic agents but the septal tissue strips were relaxed with isoproterenol. However, relaxation responses developed slowly with low magnitude.

The moderate responses of the air sac tissue preparations to ACh and adrenergic agents suggests that the bowfin air sac is incompletely developed with respect to neuromuscular control of the ventilation process. This may explain why the compliant nature of the air sac is useful. The development of a flacid air sac would allow inflation and deflation to occur more rapidly without much assistance or antagonism from air sac muscle. Therefore, it appears that the main functional role played by the bowfin air sac muscle is that of air sac volume adjustment in the hydrostatic control process.

INTRODUCTION

The respiratory system of Amia calva consists of an air sac and gills. The air sac and aerial breathing habit is of special interest since it is reminiscent of structure and behavior found in higher vertebrates (Lenfant et al., 1970). Cuvier (1829) was the first to state that the air sac of Amia was highly subdivided and not unlike that of a reptilian lung. Wilder (1875) later described natural history observations of the aerial breathing process and provided a brief functional account of the air sac. Wilder also indicated that the bowfin air sac was noticeably lung-like. Despite the early presumptions regarding bowfin air sac function, gas exchange data for thisstructure were not presented until the published accounts of Lenfant et al., (1970) and Johansen et al., (1970). These workers showed this air sac capable of augmenting aquatic respiration to a significant degree in high temperature water. From 20°to 30°C, the air sac of this animal almost tripled its oxygen uptake. This shift in respiratory activity was indirectly substantiated by Horn and Riggs (1973) in their description of aerial breathing patterns. The functional capacity of the air sac and the well developed gas exchange capacity of the gills (Bevelander, 1934; Johansen et al., 1970) indicate a high degree of respiratory adaptation for the bowfin.

There are several additional features of the bowfin air sac that require investigation. One of these is quantification of air sac structure by allometric techniques which will provide standardized structural data that can be compared with similar data from other vertebrates. Another one concerns the cholinergic and adrenergic response pattern. Investigation of these two areas should reveal whether the bowfin air sac is similar to the lungs of other vertebrates or whether it employs a unique approach to the process of aerial breathing.

MATERIALS AND METHODS

Experimental Animals

Juvenile and adult *Amia* were seined from Jenkins-Reilly slough in McCurtain County, Oklahoma, transported to the University of Oklahoma Department of Zoology, Norman and held individually in 50 gallon aquaria at 25°C. Following observation of respiratory behavior, the animals were sacrificed and prepared for air sac experimentation.

Morphometric Methods

Air sacs with glottis and external muscle sheath were dissected intact from 20 *Amia* 258 to 2394 g in size. The preparations were washed clear of blood and mucus, attached to an inflation device (Figure 1) and then dry fixed according to the procedure of Tenney and Tenney (1970). A constant inflation pressure of 40 mm H_2O was used which was sufficient to inflate the air sac fully but not over stretch it (Figure 2). After fixation, the air sacs were weighed and measured for length, volume displacement, diameter (total and central air space) and for air chamber size and number. The allometric power function, $Y = k\ BW^m$, was used to describe the dimensional relationships between the air sac variables and body weight (Y = any air sac variable, BW = body weight in grams, and k and m are empirically derived constants). The constants k and m were obtained by least squares regression

analysis of Log_{10} - Log_{10} transformed data. This analytical model has been used successfully by Lasiewski and Dawson (1967) and Lasiewski and Calder (1971).

Histological Technique

A brief histological study of the preparation was also made. Strips containing both external and internal layers of the air sac were fixed in buffered formalin, sectioned at 8-10 μ and triple stained with Mallory's Trichrome.

Survey of Tissue Response to Cholinergic and Adrenergic Agents

To complete this part of the study, samples of the bowfin air sac were excised from the midsection region and suspended in a 10 ml modified Metro-Ware tissue bath (Farmingdale, N. Y.) containing modified Kreb's solution (Holmes and Stott, 1960). The saline solution used in this study has been evaluated for acceptability in fish studies by Lockwood (1961) and contains: NaCl, 7.41; KCl, 0.36; $CaCl_2 \cdot 2H_2O$, 0.23; $NaHCO_3$, 0.31; $Na_2HPO_4 \cdot 2H_2O$, 0.2; NaH_2PO_4, 0.4; $MgSO_4$, 0.15; and glucose, 1.0 g/liter. Temperature was held at 25°C and aeration was provided with compressed air. Tissues were allowed to equilibrate in the saline bath for approximately 45 minutes before drug testing commenced.

Tissue strip preparations of intact air sac were used in part of the study. Later in the work, the striated covering of the preparation was removed so that the effector properties of this layer could be studied independent of the internal septal tissue.

Tension changes in the tissue strips were monitored with a

Grass strain gauge transducer (Model FT03) coupled to a Grass polygraph (Model 5 D). After stable baseline conditions were reached, the tissue was assayed for response to acetylcholine chloride, pilocarpine sulfate, histamine dihydrochloride, phenylephrine hydrochloride, epinephrine hydrochloride and isoproterenol hydrochloride (Sigma Co., St. Louis, Mo.). Each stock solution was prepared just prior to use by dissolving the appropriate drug in Kreb's saline, and then injected into the bath as needed for a wide dose range (expressed as μg/ml). The blocking characteristics of atropine sulfate, tubocurarine dihydrochloride and dichloroisoproterenol chloride were also evaluated.

All tissue strips were assayed under isotonic conditions with a weight tension of 1.0 gram. This tension was determined by comparing response against a series of applied weights (0.05-2.0 grams).

RESULTS

Air Sac Anatomy

Figure 3 shows a cut-away view of the lateral aspect of Amia. The air sac (as) opens dorsally from the pharyngeal-esophageal region by way of a glottis (g) and runs the entire length of the visceral cavity along the dorso-posterior aspect of the intestine (i). The air sac (Figure 4) has an external striated muscle sheath (sms) that provides support and a mechanism for controlling volume and pressure. Air sac circulation is supplied by a pair of pulmonary arteries that receive blood directly from the 3rd and 4th epi-branchial arteries. Venous drainage occurs via two pulmonary veins which emerge ventro-anteriorly from the air sac and then join the left duct of Cuvier (Goodrich, 1930). The interior of the air sac is organized into two series of lateral compartments (lc) that open from a central air space (cas). Each series contains 8-11 lateral compartments making up a total of 16-22 for each air sac. Along the dorsal midline of the air sac, an additional number of smaller dorsal compartments are found. Most lateral compartments resemble a cone, which is partitioned into honey comb-like air chambers (ac)(Figures 4 and 5) by both large and small septal (s) partitions. Each septum facing the air space is thin and contains two epithelial layers (epi), blood vessels (bv), capillaries and

connective tissue (c). Each epithelial layer is made up of squamous to cuboidal epithelial cells and some cells that produce mucus. The exposed surfaces of the septa provide extensive surface area for gas exchange.

With respect to compartment size and septation, the bowfin air sac is more complex than the gar Lepisosteus osseus air sac (Potter, 1927), the lungs of the lungfish Neoceratodus (Grigg, 1965) and Protopterus (Johansen, 1970), and certain reptiles (Burnstock and Wood, 1967). However, the bowfin air sac lacks the smooth muscle development found in the air sacs and lungs of the above vertebrates.

Quantitative Aspects

Table 1 summarizes the allometric comparisons made in this study and includes coefficients of correlation (r) and F ratios for the regression analyses. The ranges reported in each of the following sections represented measurements from the smallest (256.1 g) and the largest 2394.2 g) fish used in this study.

Air Sac Weight (A_W)

The wet weight of freshly dissected air-sacs gave inconsistent values thus only dry weight was reported. Air sacs weights ranged from 0.24 to 2.86 g and increased at a rate that was directly proportional to the rate of body weight increase ($\text{Log } A_W \text{ dry} = -3.115 + 1.03 \text{ Log BW}$). The variance of this comparison was low and the degree of correlation high (0.95). The exponent (m) was 1.03 and indicated that air sac weight was proportional to $BW^{1.03}$.

Air Sac Length (A_L)

Air sac lengths ranged from 9.51 to 20.84 cm. Regression of A_L on BW gave an exponent of 0.31 (Log A_L = 0.229 + 0.31 Log BW), an r value of 0.96 and F (p) of (0.001). When A_L was regressed against A_W a nearly identical relationship was revealed (Log A_L = 1.162 + 0.28 Log A_W) (Table 2). A similar relationship was also found for standard body length-body weight comparisons (Log BL = 0.695 + 0.30 Log BW). These comparisons were highly correlated (Table 1) and approached the geometric shape law, $L = k\ W^{0.33}$, to a remarkable degree.

Air Sac Diameter ($A_{dia.}$)

Air sac diameter ranged from 2.4 to 6.1 cm over a body weight range of 258.1 to 2394.2 g. The change in this dimension with increased body size was proportional to 0.477 cm $BW^{0.30}$ (Log $A_{dia.}$ = -0.321 + 0.30 Log BW). The diameter of the lateral compartment ($ALC_{dia.}$) opening ranged from 0.84 to 1.734 cm. Mean air chamber diameters ($A_{ac_{dia}}$)(based on widest diameter of every fifth air chamber that fell on the dorsal mid line) ranged from 0.49 to 1.08 cm. The respective regression equations for these latter two variables were: Log $A_{LC_{dia.}}$ = -0.789 + 0.29 Log BW and Log $A_{ac_{dia.}}$ = 1.098 + 0.31 Log BW. When $A_{dia.}$ was regressed on air sac weight (A_W) (Table 2), the relationship Log A_{dia} = 0.595 + 0.29 Log A_W was revealed. The exponents of all regressions for the various air sac diameters ($A_{dia.}$, $ALC_{dia.}$, $A_{ac_{dia}}$) approached the ideal geometric relationship (dia = $k\ W^{0.33}$). These three air sac dimensions were

more variable from one air sac to the next than the other air sac variables. However coefficients of correlation for each of the above regressions were only slightly less (Table 1).

Air Sac Volume (A_V)

The volume of the air sacs increased in proportion to 0.138 cc $BW^{0.93}$ (Log A_V = 0.861 + 0.93 Log BW) (Table 1) and ranged from 21 cc to 230 cc. The change in Av in relation to BW was another variable air sac feature measured in this study and was no doubt related to the displacement method used to measure volume. Despite air sac rigidity, some compression occurred in the more pliable regions. The A_V vs BW comparison was almost identical with A_V vs A_W (Log A_V = 1.929 + 0.89 Log A_W) (Table 2). Both comparisons were highly correlated.

Cholinergic and Adrenergic Responses of Air Sac Tissues

Response of Complete Air Sac Strip Preparations. This preparation contained both the external striated sheath and the internal septal tissue and was cut from an arc that connected the mid-dorsal and mid-ventral line of the air sac. Thus, only the circular striated fibers were intact for the test. The strip was trimmed to approximately 0.25 cm wide and 0.75 cm long and then maintained in cold (1 to 3°C) Kreb's saline (Wolfe, 1963) until time of use (1-2 hours).

Acetylcholine (ACh) treatment produced strong dose-dependent contractions over the concentration range of 0.1-1000 µg/ml (Figure 6 E,F). Threshold responses were detected in the 0.1-1.0

µg/ml range. However, maximum activity was not evident until 500-1000 µg/ml concentration were used. The ED_{50} for this preparation ranged from 19 to 26 µg/ml and averaged 22.5 µg/ml.

The response curves (a-c in Figs. 6 E and F) at lower ACh concentrations (1-50 µg/ml) had logarithmic form and required 20-80 seconds before reaching maximum amplitude. At higher concentrations (100-1000 µg/ml ACh), the curves (d-h in Fig. 6 E and F) had a bimodal shape consisting of an early rapid phase and late slow phase. The maximum response time of both phases was variable and decreased as ACh concentration increased (2-30 seconds for the rapid phase; 80-160 seconds for the slow phase). A remarkable feature of each curve was the time for which maximum muscle tension could be maintained. As long as ACh was kept in the tissue bath, contractions could be sustained for several minutes (2-5).

The reaction of this preparation to pilocarpine paralleled the ACh treatment data. There was no noticeable bimodal response but initial and maximum response times were greater. The response to catecholamines was minimal. Despite concentrated applications (25-100 µg/ml) of phenylephrine, epinephrine and isoproterenol, tissue reaction was virtually absent. Some relaxation of the preparation was noted when treated with isoproterenol, but tissue activity was low (Fig. 7).

Response of Striated Muscle Sheath Strip Preparation.

Figures 6 B and D show representative muscle sheath responses to ACh.

The general pattern demonstrated by each curve was logarithmic and similar to the early rapid phase shown by the complete air sac (Fig. 6 E and F). The contraction velocity and maximum response time for each curve developed quickly but varied as a function of ACh concentration. This preparation displayed an overall greater sensitivity than the whole air sac strips used in the previous section. ED_{50} values ranged from 3.6 to 8.4 µg/ml and averaged 6 µg/ml.

The ACh responses of this preparation were incompletely blocked by atropine. However, tubocurarine dihydrochloride had a noticeable blocking action (Fig. 8). Hexamethonium was not tested. Curve A shows a control test response to 5 µg/ml ACh. This response was reduced approximately 86 % following treatment with 10 µg/ml tubocurarine dihydrochloride (curve B). This experiment was quickly followed with a 10 µg/ml ACh treatment which gave a response 59 % below the control level (curve C). Data from two additional experiments confirmed that tubocurarine dihydrochloride was an effective ACh inhibitory agent for this preparation.

This preparation was also treated with various catecholamines (epinephrine, norepinephrine, isoproterenol). Response to these agents was not perceptable.

Response of the Internal Septal Tissue Preparation. In contrast to the striated muscle sheath preparation, this preparation reacted more slowly (curves a - f in Figure 6 A, C). The time required for each curve to reach maximum amplitude was dose dependent and similar to the time intervals associated with the development of the slow component in the complete air sac response curves (Fig. 6 E, F). The ED_{50} values for ACh

data were 45 and 22 µg/ml and represented the lowest sensitivity of the three preparations used in this study.

Unlike the striated sheath data, atropine was found to be an effective ACh blocking agent (Fig. 9). Curve A shows pretreatment with 10 µg/ml tubocurarine dihydrochloride and curve B demonstrates the response to 50 µg/ml ACh following this treatment. This response was no different than the previous control test with 50 µg/ml of ACh. When this preparation was treated with 10 µg/ml of atropine (curve C), response to 50 µg/ml of ACh was abolished. In order to produce a response comparable to the control data, 1500 µg/ml of ACh was required (curve D). The effect of atropine on this preparation was consistent and was demonstrated in three other experiments. The response of this preparation to epinephrine and isoproterenol was similar to that observed in the complete air sac preparation (Fig. 7).

DISCUSSION

Anatomic and Morphometric Considerations

Anatomically, the bowfin air sac lacks abundant smooth muscle but has an elaborate external striated muscle sheath which was initially described by Wilder (1875). The blood vessel circuit of this structure is unlike the amphibian or reptilian condition and has no separation from the gill circulatory system. The extent of septation is more complex than the lungs of many amphibians (Whitford and Hutchison, 1967) and is as complex as the lungs of certain reptiles (Tenney and Tenney, 1970).

While the bowfin air sac has certain similarities with lungs, it nonetheless remains a dorsal air exchange compartment with large air chambers. These occur in the lateral compartments and are analogous to higher vertebrate alveoli. The nearest analog would be the lung of the snakefish (Calmoichthyes calabaricus). The garfish (Lepisosteus osseus)(Potter, 1927) is a close homolog. Both of these fish are competent air breathers. Allometrically, the bowfin air sac has several dimensional features in common with higher vertebrate lungs in a similar weight range and ecophysiological category (Tenney and Tenney, 1970). Air sac weight and volume is directly proportional to body weight ($k_{A_W} BW^{1.03}$ and $k_{A_V} BW^{0.93}$). The exponents 1.03 and 0.93 are not significantly different from

1.0 and indicates that air sac and body weight variables increase in the same proportion (Gould, 1966). These data reveal that bowfin air sac density is nearly constant over the weight range studied. This is quite similar to the amphibian data described by Tenney and Tenny (1970).

All linear regressions obtained in this study (Table 1) gave allometric exponents that follow the predictions of Günther (1972). Air sac diameter is nearly proportional to both k $BW^{0.33}$ and $A_v^{0.33}$. When the ratio of central air space diameter (D_1) to total diameter (D_2) is plotted as a function of total air sac diameter, a constant function is revealed ($\frac{D1}{D_2}$ = 0.38 cm D_2). The k constant (0.38) indicates that compartmentalization is higher than for several amphibians and comparable to many reptiles (Tenney and Tenney, 1970).

The diameters of the lateral compartments and the air chambers -alveoli like units contained inside also conform to the predictions of Günther's (1972) similarity analysis; Dia = k $BW^{0.29-0.31}$. This result is interesting from a comparative standpoint. Tenney and Tenney (1970) found that amphibians and reptiles in the size range of this study had lung alveolar diameters that were proportional to k $BW^{0.2}$. Although amphibians and reptiles showed variability with respect to this parameter, their alveoli chambers were relatively smaller and more constant in size than the bowfin air chamber ("alveoli"). Thus, while the bowfin air sac is as compartmentalized as that of some reptiles, the air chambers are large and therefore would be fewer in number per unit of volume.

Since there are no comparable data for other air breathing fish, additional comparisons cannot be made.

The function of the bowfin air sac is linked to water temperature and oxygen conditions. At high water temperatures, the bowfin makes almost exclusive use of the air sac and the gills are only marginally operational (Johansen et al., 1970). At high temperatures, general metabolism is high and oxygen must be efficiently supplied. Thus, if the air sac is to be an effective supplier of oxygen, the ratio of its surface area to body weight should be similar to the $\dot{V}_{O_2}/BW$ ratio. However, field observations indicate that bowfin air ventilation may be insufficient in some cases to meet total metabolic needs. I have observed dead and dying bowfin in warm and hypoxic waters ($\angle$ 2 ppm oxygen). Therefore, it may be that the air sac does not have the necessary surface area required to meet total metabolic needs, especially under long term conditions of severe dissolved oxygen deprivation. This is supported by the observation that the number of air compartments (the gas exchange unit) per unit volume is small compared to those in the higher vertebrate lungs. Consequently, internal spaces are comparatively large which reduce the over all gas exchange surface area inside the structure.

The pressure-volume curves obtained from inflation experiments suggest that compliance in the bowfin air sac was high(Fig. 2) compared tc higher vertebrate lungs. This was probably due to the thin septal partition's lack of internal smooth muscle trabeculae or strands. Perhaps the increased compliance offsets the lack of

respiratory surface area up a point of acute hypoxia and then fails as a compensatory mechanism thereafter.

Pharmacological Considerations

All tissue preparations of the bowfin air sac responded to acetylcholine, though at a moderate level. The striated muscle sheath preparation had greater sensitivity to ACh than did the internal septal tissue preparation, which probably related to the more extensive muscle organization of the striated sheath. There was no striated muscle located internally and smooth muscle appeared in association with blood vessels. In higher vertebrates, cholinergic nerve supply is much reduced in the blood vessels (Goodman and Gilman, 1971). This feature apparently exists in the bowfin air sac septal tissue which would explain the reduced cholinergic response.

The cholinergic blocking response in the air sac preparations suggests a dual cholinergic receptor system. Atropine sulfate had little effect on the striated sheath response to ACh, whereas, tubocurarine dihydrochloride produced a blocking action. The reverse of this was true in the septal tissue preparation. This was not unusual since, nerve-muscle junctions are generally nicotinic in striated muscle (Eccles and Macfarlane, 1949; Burns and Paton, 1951, Burnstock and Holman, 1961) and muscarinic in the smooth muscle of blood vessels (Fange, 1962).

The general model of the bowfin air sac has few counterparts in the animal kingdom. One exception is the lung of the African snake fish (Calamoichthyes calabaricus). This primitive Chondrostean air breather (related to the air breathing African bichir, Polypterus senegalis) has a true lung enclosed by striated muscle fibers (Hildebrand, 1974).

The air sac of the closely related gars (Lepisosteus sp) has anatomical organization that is opposite Amia. The effector system of this structure consists of a series of trabecular strands that have a core of striated fibers surrounded by thick layers of smooth muscle (Potter, 1927). Response to ACh was not bimodal and tubocurarine had no effective ACh blocking action (Perry and Haines, 1973). The primitive lung of the African lung fish (Protopterus aethiopicus) has abundant smooth muscle in the internal compartments, but lacks striated muscle. Unlike Amia, this structure contracted strongly in response to ACh in the 0.1-2.0 μg/ml range (no ED_{50} values were given) and was blocked by atropine (Johansen and Reite, 1967). The Protopterus lung was also more sensitive to catecholamines.

The nearest neuromuscular fish analogue to the bowfin air sac appears to be the tench (Tinca vulgaris) gut (Mehes and Wolsky, 1932). This structure has an internal layer of smooth muscle (with muscarinic receptors) surrounded by striated muscle (with nicotinic receptors). Both muscle layers are innervated by the vagus (Frey, 1928). The biphasic ACh and cholinergic blocking responses observed in the Amia air sac were similar to the tench (Tinca vulgaris) gut preparation.

Studies of amphibian (Dykstra and Nyons, 1939; Wood and Burnstock, 1967) and reptilian (Luckhardt and Carlson, 1921; Burnstock and Wood, 1967) lungs demonstrated that smooth muscle was the only effector present. Pharmacological studies of these organ tissuesindicated somewhat greater sensitivity to adrenergic

and cholinergic drugs. Mammalian tracheo-bronchial smooth muscle preparations displayed greater sensitivity to adrenergic and cholinergic agents than was the case for the bowfin air sac (Widdicombe, 1963).

The response of the bowfin air sac preparations to adrenergic agents was poor when compared to that of higher vertebrate lungs. The poorly developed response of the striated sheath was not unusual since fish muscle systems often contain red muscle fibers with less extensive motor innervation (Fange, 1962). The failure of the septal tissue to respond extensively to adrenergic drugs was not unexpected in view of the lack of smooth muscle strands in this preparation. An alternate experiment involving perfusion of the air sac vasculature with adrenergic drugs could possibly reveal a more definitive response pattern. Furthermore, additional histochemical flourescent studies (methods of Carlsson et al., 1962; Falck, 1962) would show the presence or absence of adrenergic nerve fibers.

In summary, the bowfin air sac showed no unusual allometric growth pattern compared to body size. Air sac weight, length, diameter and volume increased in the same proportion as body weight. The internal air chambers occupied approximately two thirds of the total air sac volume. However, each chamber was relatively large which minimized the number of air chambers that could occupy a unit volume.

Internally, the bowfin air sac lacked an extensive smooth muscle effector system found in many other air breathing fish. A striated muscle sheath with circular and longitudinal fibers covered

the air sac exterior. The muscle fibers gave slow tonic responses to ACh which is characteristic of slow contracting red muscle. However a full confirmation of this muscle type requires an examination of myoglobin content, the respiratory enzyme system and the motor innervation pattern (Fange, 1962). Since the bowfin air sac is a compliant structure and can easily collapse and reinflate, it is suggested that the slow acting striated muscle participates more effectively in hydrostatic volume control than in active deflation during the ventilation process.

The similarity of the bowfin air sac with the lungs of the primitive African snake fish and the bichir could indicate a closer phylogenetic relationship between these fish. However, Lovtrup (1977) in a recent analysis of vertebrate phylogeny shows that Amia is more closely related to the Teleosts than the more primitive ray finned fishes. Thus it appears that the striated muscle sheath of the bowfin air sac is a plesiotypic character which arose independently and apart from any influence provided by Polypterans.

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LIST OF TABLES

Table 1. Allometric constants* from air sac dimension-body weight regression.

Air Sac Variable (Units)	$\left(\frac{\Delta \overline{\text{Log } A_n}}{\Delta \text{Log BW}}\right)$	Log K (Log Y at Log 1 g)	N	r	F	(P)
Air Sac Weight (A_w)	1.03	-3.115	20	0.95	117.2	($<$ 0.001)
Air Sac Length (A_L)	0.31	0.229	20	0.96	232.0	($<$ 0.001)
Air Sac Dia. ($A_{dia.}$)	0.30	-0.321	20	0.85	42.6	($<$ 0.001)
Lateral Compartment Dia. ($A_{LC_{dia.}}$)	0.29	-0.789	8	0.91	27.9	($<$ 0.005)
Alveolar Dia($A_{a_{dia}}$)	0.31	-1.098	8	0.85	15.9	($<$ 0.005)
Air Sac Vol. (A_v)	0.93	-0.861	18	0.95	141	($<$ 0.001)
Standard Body Length (BL)	0.30	-0.695	20	0.99	746.8	($<$ 0.001)

*m and k from $Y = k \cdot BW^m$, N = # of Fish, r = correlation coef., F = F ratio (prob.)

Table 2. Allometric regression constants for A_L, A_{dia} and A_V versus A_W.*

Comparison	$\left(\frac{\Delta \text{Log } A_X}{\Delta \text{Log BW}}\right)$	Log K (log Y at log 1 g)	N	r	F	(P)
A_L vs A_W	0.28	1.162	20	0.93	113.9	(0.001)
A_{dia} vs A_W	0.29	0.595	20	0.88	63.1	(0.001)
A_V vs A_W	0.89	1.929	18	0.95	163.3	(0.001)

*For symbol designation see Table 1. A_X equals some air sac dimension

Figure Legends

Figure 1. Dry fixing apparatus used to prepare Amia air sac showing air pump (1), pressure adjustment (2), water manometer (3), protective chamber (4) and air sac (5).

Figure 2. In vivo air sac compliance curve for 686 g Amia.

Figure 3. Cut-away view of Amia showing pharynx (p), glottis (g), air sac (as) and intestine (i).

Figure 4. Schematic diagram of Amia air sac in cross (A), longitudinal (B) and lateral section (C). Structure designation is the same as in 3 except for: esophageal tube (e), central air space (cas), lateral compartments (lc), septum (s), air chamber (ac), and striated muscle sheath (sms).

Figure 5. Perspective of Amia air sac septa showing flattened epithelium (epi), connective tissues (c), blood vessels (bv), smooth muscle (sm) and air chamber (ac).

Figure 6. Tracings of Amia air sac strip preparation in response to acetylcholine (ACh): A (internal septal tissue of Fish 3); B (striated muscle sheath of Fish 2);

C (internal septal tissue of Fish 4); D (striated muscle sheath of Fish 1); E (complete air sac of Fish 1); F (replicate of E above). Lower case letters (a-h) represent response curves at specific doses of ACh. The ACh dosage (µg/ml) that applies to each curve is shown by numbers in the following matrix:

Response Curves	Figure 6 Sections					
	A	B	C	D	E	F
a	1	5	1	1	1	5
b	5	10	5	5	10	10
c	50	50	50	10	50	25
d	100	100	100	20	100	50
e	500	500	500	40	500	100
f	1000	1000	1000	100	1000	200
g				500		500
h				1000		1000

All recordings were obtained from air sac strips approximately 0.25 x 0.75 cm under 1.0 gram isotonic conditions.

Figure 7. Representative Amia air sac strip preparation responses to 5 (curve A) and 25 (curve B) µg/ml isoproterenol. Curve C shows response to preliminary treatment of 25 µg/ml ACh.

Figure 8. ACh blocking action by tubocurare in Amia striated muscle sheath strip preparation. Curves are representative of three experiments and show response to control ACh dosage (A), tubocurarine (B) and ACh test dose (C).

Figure 9. Response of Amia internal air sac matrix strip preparations to tubocurarine (curve A) and atropine (curve C). Curves B and D are representative post-treatment ACh responses following tubocurarine and atropine treatment.

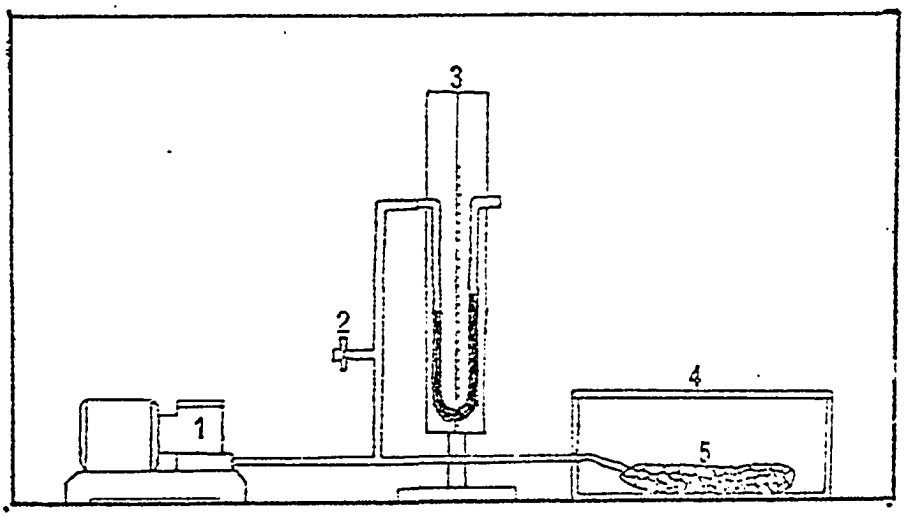


Figure 1

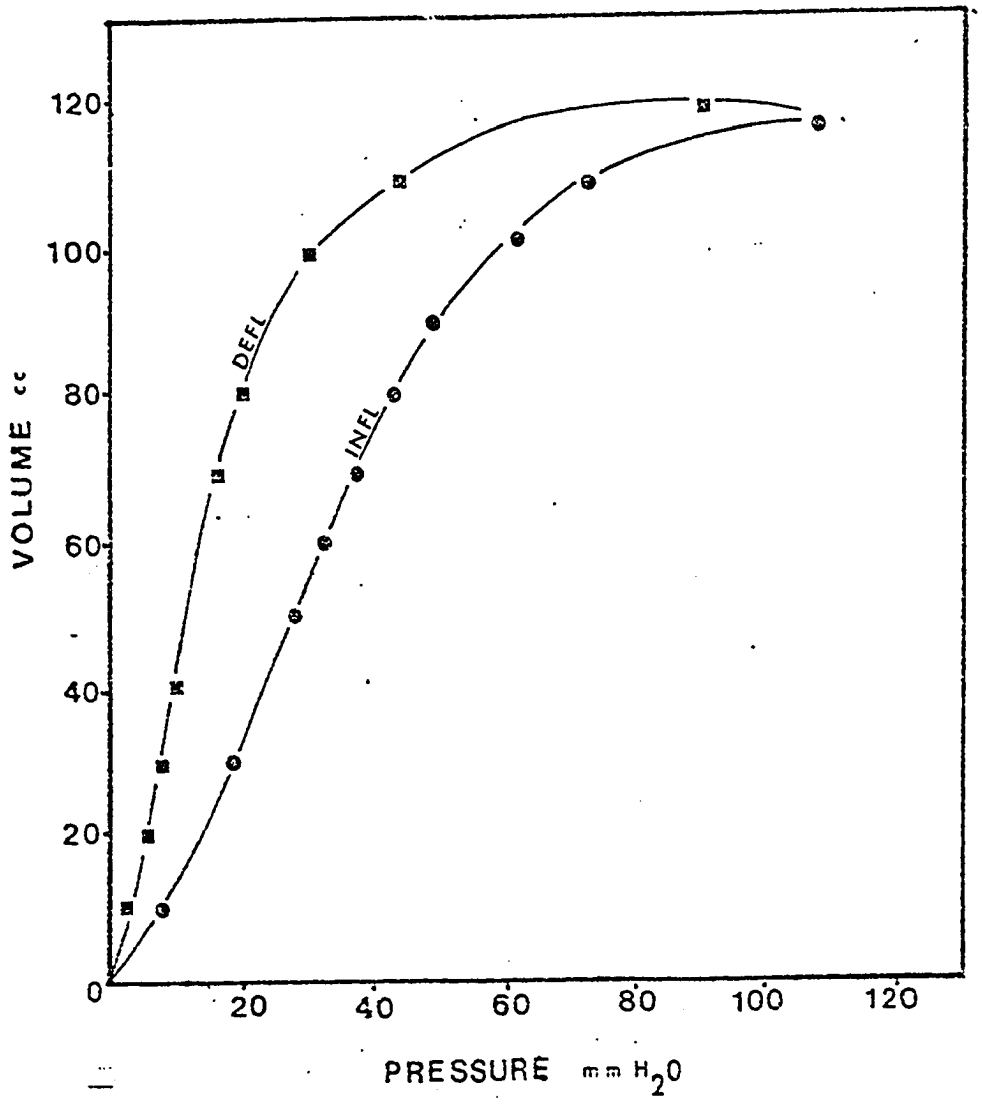
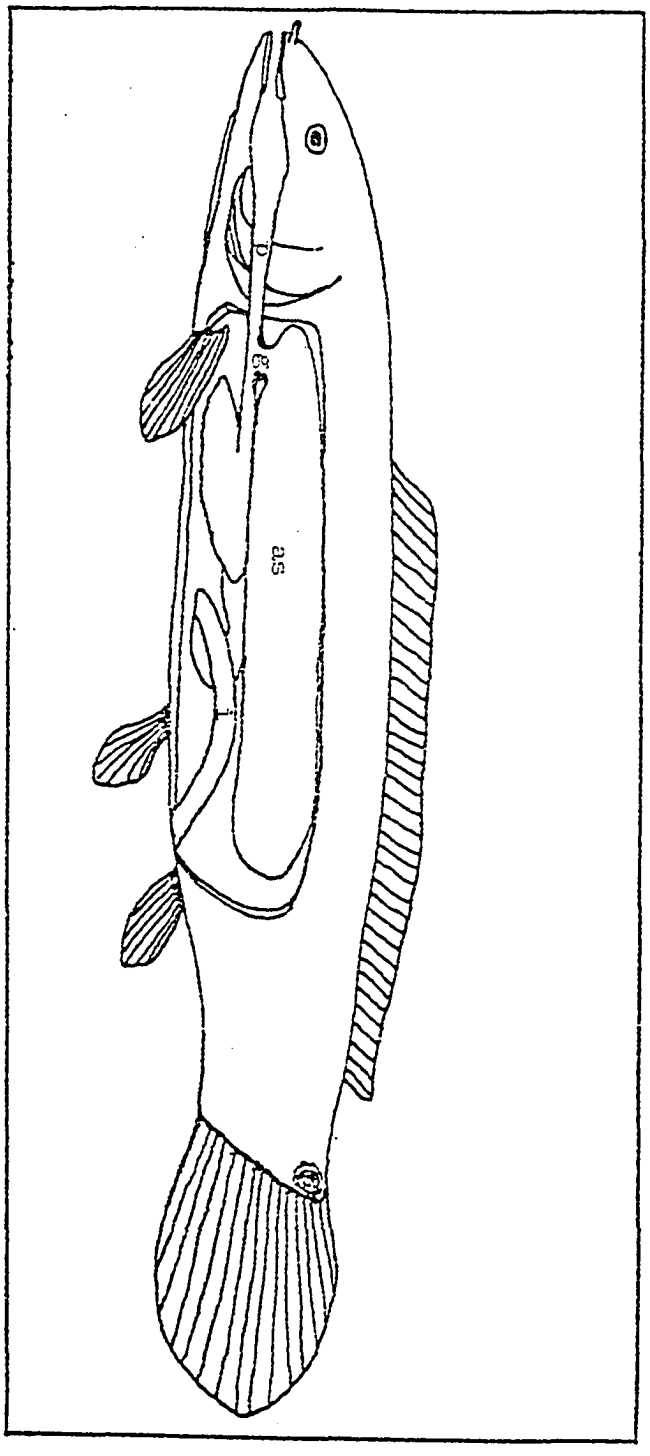

120
100
80
60
40
20
0
VOLUME cc
DEFL
INFL
20
40
60
80
100
120
PRESSURE mm H2O

Figure 2



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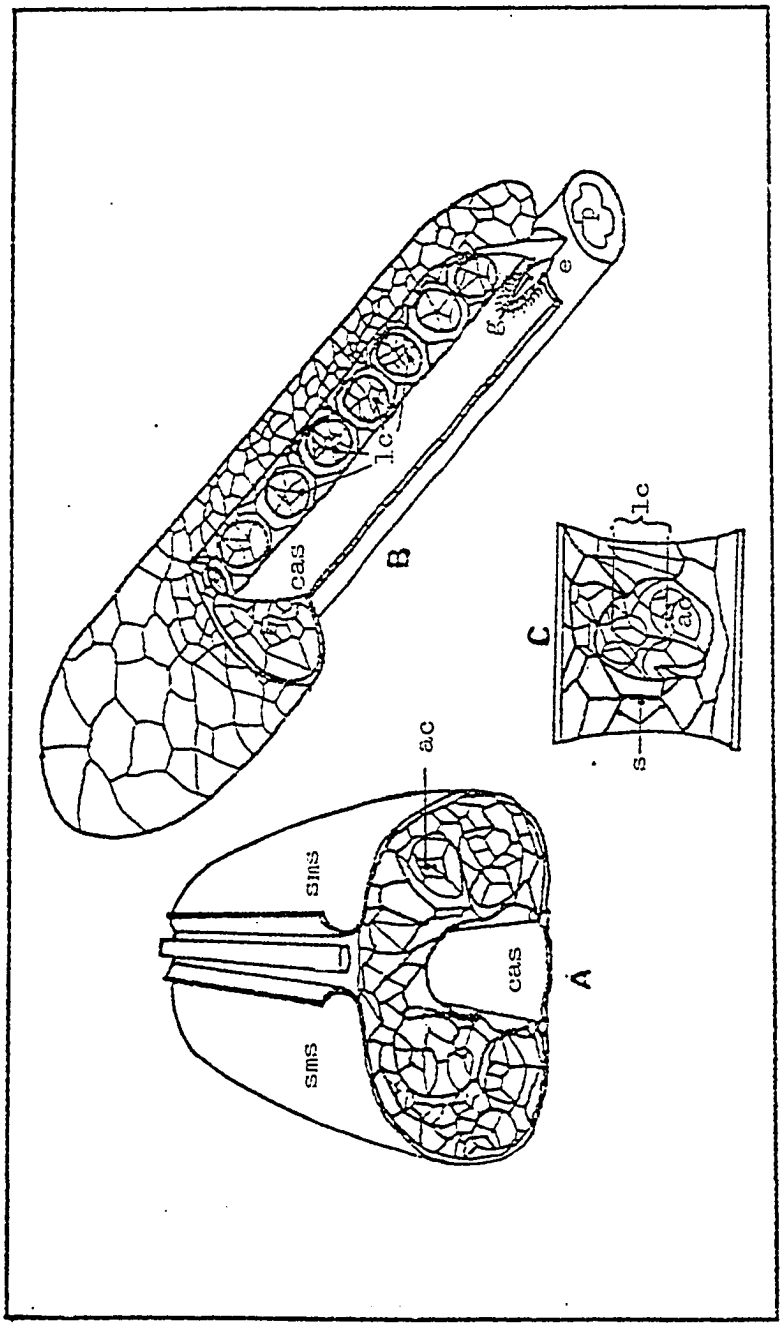

Figure 4

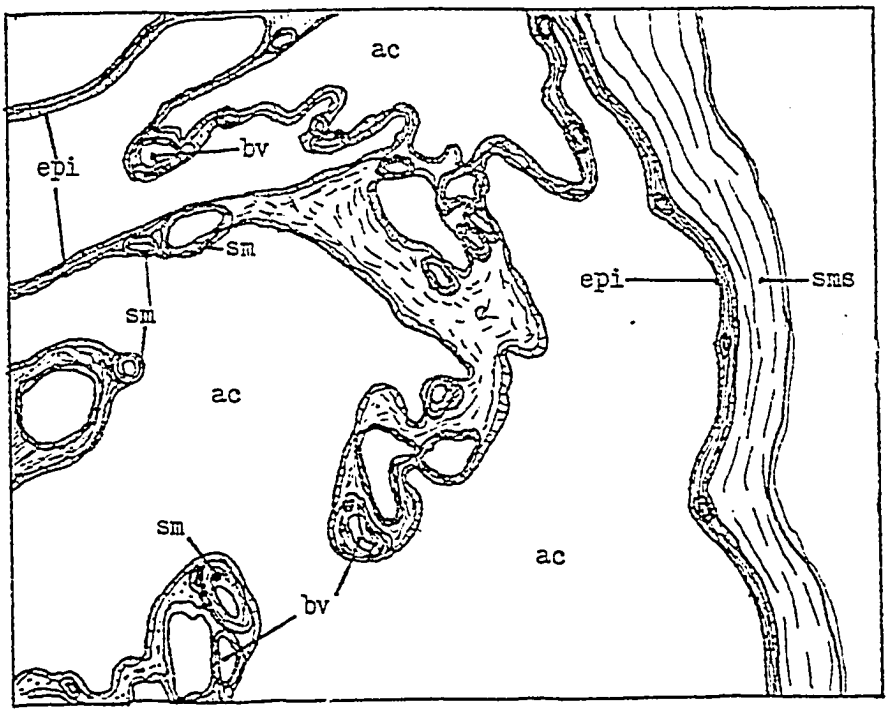

Figure 5

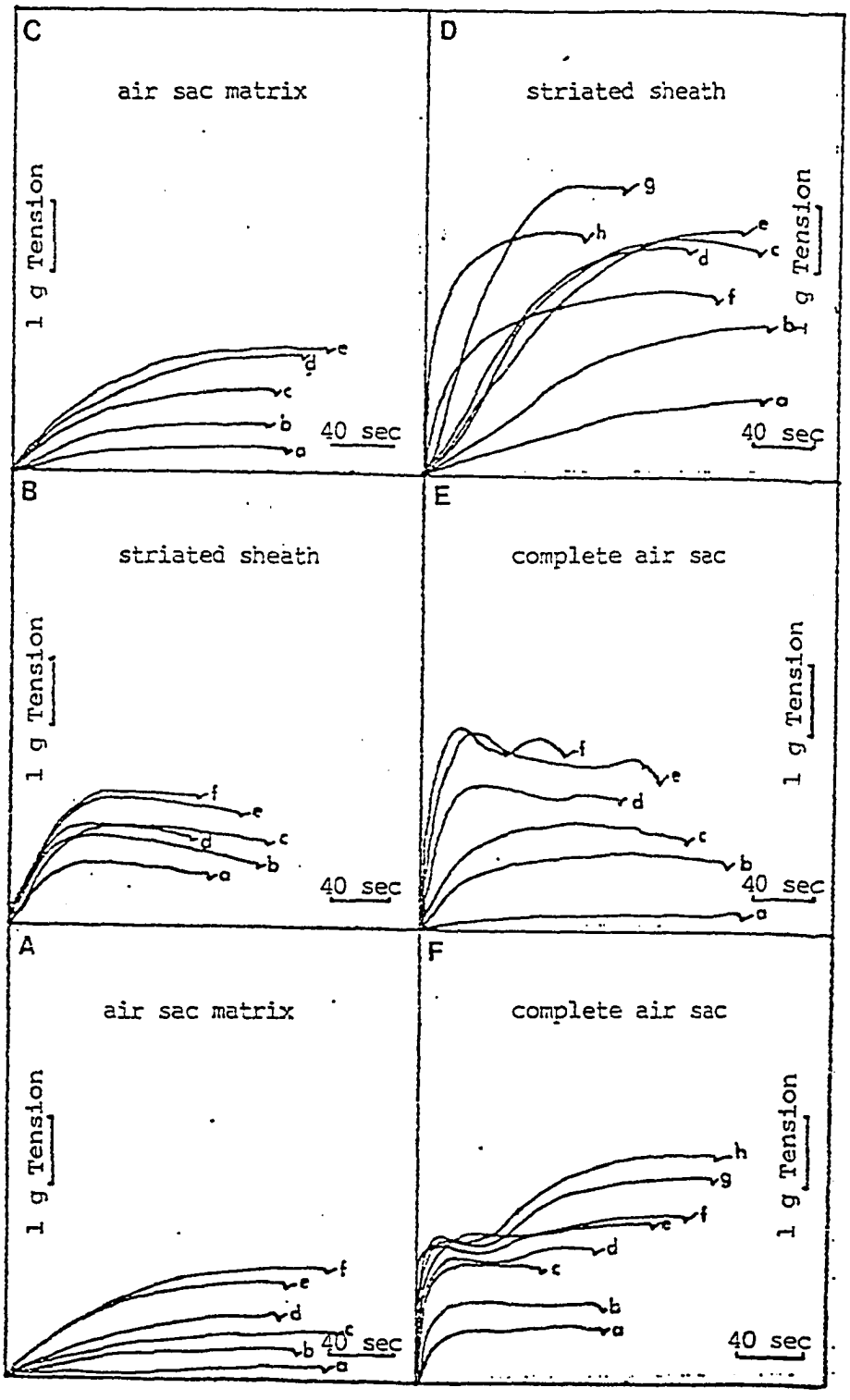
C
air sac matrix
1 g Tension
a
b
c
d
e
40 sec
D
striated sheath
1 g Tension
a
b
c
d
e
f
g
h
40 sec
B
striated sheath
1 g Tension
a
b
c
d
e
f
40 sec
E
complete air sac
1 g Tension
a
b
c
d
e
f
40 sec
A
air sac matrix
1 g Tension
a
b
c
d
e
f
40 sec
F
complete air sac
1 g Tension
a
b
c
d
e
f
g
h
40 sec

Figure 6

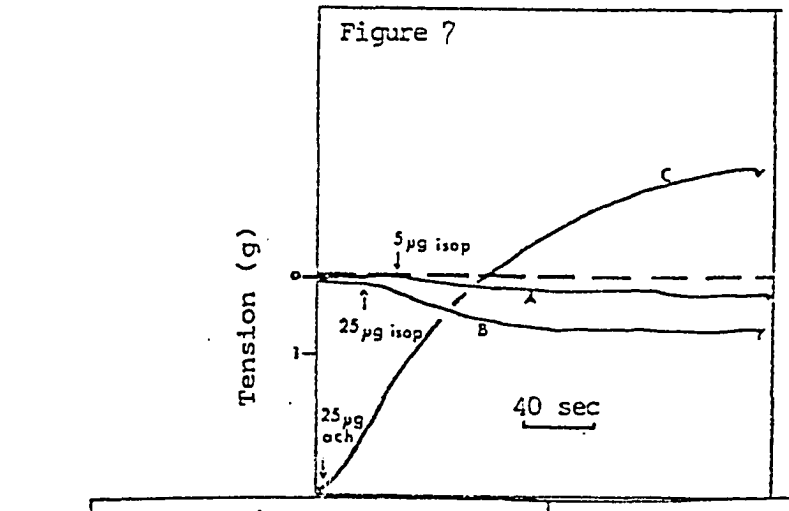
Figure 7
Tension (g)
5 µg isop
25 µg isop
25 µg och
A
B
C
40 sec

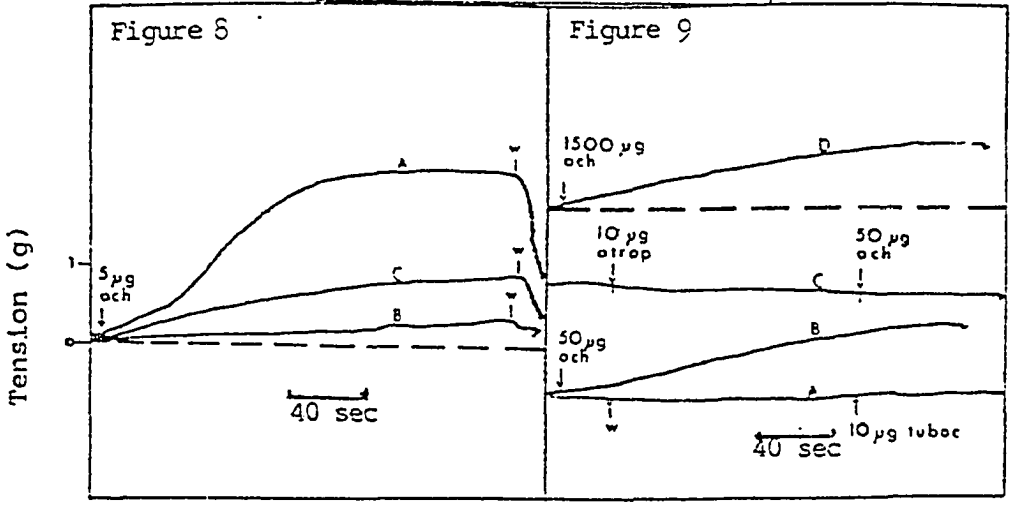
Figure 8
Figure 9
Tension (g)
5 µg och
A
B
C
40 sec
1500 µg och
D
10 µg atrop
50 µg och
50 µg och
10 µg tuboc
40 sec