BIMODAL BREATHING AND DIVING BEHAVIOR IN SOFTSHELL, STINKPOT, AND MUD TURTLES

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STINKPOT, AND MUD TURTLES

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THESIS ABSTRACT

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This thesis investigates bimodal breathing in freshwater turtles. Three species, Trionyx spiniferus asper, Sternotherus odoratus, and Kinosternon subrubrum subrubrum, were used because they represent a gradient of cutaneous surface area. Cutaneous surface areas were measured by skinning museum specimens. Simultaneous measurements of aerial and aquatic oxygen uptake and carbon dioxide excretion were obtained from freely diving turtles. Cutaneous surface area was related to the partitioning of respiratory gas exchange. Diving and ventilatory behavior were monitored by videotaping turtles in three aquatic oxygen tensions: hypoxia ($\leq$ 30 torr), normoxia (= 150 torr), and hyperoxia ($\geq$ 250 torr). Cutaneous surface area was highest in T. spiniferus, intermediate in S. odoratus, and lowest in K. subrubrum (ANCOVA, $P \leq .01$). This was also the pattern for aquatic VO_2 and VCO_2. Aquatic VO_2 accounted for 37.5 $\pm$ 4.0% of total VO_2 in T. spiniferus, 25.8 $\pm$ 2.7% in S. odoratus, and

13.6 ± 1.0% in K. subrubrum. Aquatic VCO_2 in these species accounted for 85.4 ± 1.9%, 55.6 ± 1.2%, and 45.8 ± 3.6% of total VCO_2, respectively. All three species responded differently to changes in aquatic oxygen tension. In normoxia, T. spiniferus had the lowest ventilatory rate, due to fewer breaths taken during emersion. There were no differences among the species in dive durations during normoxic exposure. The ventilatory rate of T. spiniferus increased with decreasing PO_2, due to shorter dives and not because of changes in ventilation once emmersed. In S. odoratus there was a curvilinear effect, ventilatory rate was highest in normoxia and lower at both high and low oxygen tensions. This was due to a combination of changes in dive duration and ventilation once emmersed. Aquatic PO_2 had no effect on the ventilatory rate of K. subrubrum. Dive duration in T. spiniferus is lower than was previously suspected. Trionyx spiniferus takes one breath per ventilatory burst and is the first freshwater turtle reported to do so.

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TABLE OF CONTENTS

LIST OF TABLES. ix

LIST OF FIGURES. x

I. INTRODUCTION. 1

II. CHAPTER 1: BIMODAL BREATHING IN SOFTSHELL, STINKPOT, AND MUD TURTLES. 10

- Introduction
- Materials and Methods
  - Animals
  - Cutaneous Surface Area
  - Respiratory Gas Exchange
  - Statistical Analysis
- Results
  - Cutaneous Surface Area
  - Respiratory Gas Exchange
- Discussion
  - Body Size, the Skin, and Aquatic Respiration
  - The Partitioning of Respiratory Gas Exchange
  - Ecological Implications

III. CHAPTER 2: DIVING AND VENTILATORY BEHAVIOR AS A FUNCTION OF AQUATIC PO_2 IN SOFTSHELL, STINKPOT, AND MUD TURTLES. 32

- Introduction
- Materials and Methods
  - Statistical Analysis
- Results
  - Immersion
  - Emersion
  - Ventilatory Rate
- Discussion
  - The Softshell Turtle Paradox
  - What Limits Dives?

IV. SUMMARY. 53

BIBLIOGRAPHY. 55

LIST OF TABLES

Table 1. Aquatic and Aerial O_2 Uptake and CO_2 Excretion in Freshwater Turtles. 28

LIST OF FIGURES

Chapter 1:

Figure 1. Log Cutaneous Surface Area vs Log Body Mass Regression Lines in Softshell, Snapping, Stinkpot, and Mud Turtles. 18

Figure 2. O_2 Uptake in Softshell, Stinkpot, and Mud Turtles. 20

Figure 3. CO_2 Excretion in Softshell, Stinkpot, and Mud Turtles. 21

Figure 4. Mean Aerial and Aquatic O_2 Uptake in Softshell, Stinkpot, and Mud Turtles. 22

Figure 5. Mean Aerial and Aquatic CO_2 Excretion in Softshell, Stinkpot, and Mud Turtles. 23

Figure 6. Aquatic CO_2 Excretion vs Aquatic NH_4 Excretion in Stinkpot and Mud Turtles. 25

Chapter 2:

Figure 1. Mean Cumulative Percentage of Individual Dives vs. Time. 37

Figure 2. Mean Dive Durations of Softshell, Stinkpot, and Mud Turtles in Hypoxia, Normoxia, and Hyperoxia. 39

Figure 3. Ventilatory Patterns of Softshell, Stinkpot, and Mud Turtles in Hypoxia, Normoxia, and Hyperoxia. 41

Figure 4. Mean Apneic Periods of Softshell, Stinkpot, and Mud Turtles in Hypoxia, Normoxia, and Hyperoxia. 43

Figure 5. Mean Ventilatory Rate of Softshell, Stinkpot, and Mud Turtles in Hypoxia, Normoxia, and Hyperoxia. 45

INTRODUCTION

All amniotes except some aquatic reptiles are strictly lung breathers. Certain aquatic turtles and snakes, in addition to pulmonary respiration, are capable of aquatic respiratory gas exchange. Such animals are classified as bimodal breathers. Three principle sites have been implicated as surfaces for aquatic gas exchange; the pharynx, the cloaca, and the skin. If a high level of aquatic respiration is to be achieved, these regions must be well ventilated, well supplied with capillary beds, and have high surface areas. It is not surprising therefore that animals that utilize pharyngeal respiration have well developed, highly vascularized, buccopharyngeal processes. Likewise, animals that undergo cloacal respiration have well-developed, highly vascularized cloacal bursae. Finally, animals proficient at cutaneous respiration have skin that is well supplied with blood vessels and display adaptations that facilitate increased skin surface area, including the loss or reduction in bony scutes and/or small body size (increased mass-specific surface area).

Aquatic Gas Exchange

Softshell turtles (Trionyx*) provide an excellent example of these adaptations. They lack bony scutes and are dorsoventrally flattened. Both factors result in increased

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* North American species of softshells have recently been placed in the genus Apalone (Meylan 1987).

cutaneous surface area. Agassiz (1857) noted the extremely thin and highly vascularized nature of softshell turtle skin. These features also increase the potential for aquatic gas exchange. Agassiz (1857) also noted that, "in Trionyx, the whole pharynx is beautifully fringed with fine, tree-like, branching papillae," and that "these fringes may be similar to the internal gills of tadpoles, not only in their shape, but also in their function." His measurements of lung volumes of various terrestrial and aquatic turtles showed that highly aquatic turtles such as softshells had smaller lung volumes than more terrestrial species. These observations led Agassiz to hypothesize that softshells could exchange respiratory gases with the water.

Gage and Gage (1886) confirmed this hypothesis, demonstrating that forcibly submerged softshells (T. spinifer and T. muticus) could exchange O_2 and CO_2 with the water. By covering the plastron with vaseline, they showed that the principal site of aquatic gas exchange was the pharynx; oxygen removed while the skin was blocked "was nearly as great as when the skin was unvaselined." They also showed that aquatic CO_2 excretion (VCO_2) exceeded aquatic O_2 uptake (VO_2).

Since this pioneering work, a number of studies have focused on aquatic oxygen uptake in various species of softshells (Dunson 1960; Girgis 1961; Zhao-Xian et al. 1989). These studies all involved forcibly submerged turtles and have shown that cloacal respiration is of minor significance in softshells, whereas both pharyngeal and cutaneous respiration can be of considerable magnitude. The actual magnitude of aquatic VO_2 is debatable; reported values have differed by nearly seven-fold. Likewise, whether the buccopharynx or the skin is the principle site of aquatic gas exchange is arguable. Gage and Gage (1886), Dunson (1960), and Zhao-Xian (1989) all found pharyngeal respiration to be the major contributor, while Girgis (1961) found the skin to be the

main aquatic exchanger. Finally, it is not known how the magnitude of aquatic VO_2 compares to the magnitude of aerial VO_2 in softshells. This information is a critical prerequisite to any assessment of the importance of aquatic respiration in an ecological context.

Musk turtles (Sternotherus), like softshells, are highly aquatic freshwater turtles. The principle adaptations for aquatic respiration shown by this genus is small body size and the reduction of the bony elements of the plastron. Like softshells, musk turtles lack cloacal bursae. Musk turtles also lack any specialized buccopharyngeal structures.

Root (1949) measured aquatic and aerial VO_2 in forcibly submerged stinkpots, Sternotherus (= Kinosternon) odoratus. He found that cutaneous and pharyngeal VO_2 were about 58% and 29% of aquatic VO_2, respectively. Aquatic VO_2 accounted for about 12% of total VO_2, leading Root to conclude that aquatic respiration in stinkpots was an insignificant factor in their ability to remain submerged for long periods.

Belkin (1968) measured aquatic and aerial VO_2 in S. minor and Pseudemys (= Trachemys) scripta. He varied aquatic PO_2 in these experiments, measuring aquatic VO_2 at 0.0, 154, and 738 torr. He found that 4% of total VO_2 in normoxia was due to aquatic respiration in P. scripta. In water saturated with O_2, this species could increase aquatic VO_2 by a factor of five. In S. minor, aquatic VO_2 was 24% of total VO_2, and saturating the water with oxygen led to a threefold increase in aquatic VO_2. The percent aquatic VO_2 reported by Belkin is twice that reported by Root (1949) for the congeneric S. odoratus, despite the fact that the two estimates of aquatic VO_2 are similar. The discrepancy lies in the unusually high aerial VO_2 reported for S. odoratus by Root

(1949), and may more accurately reflect his methodology rather than any true differences between the species.

Gatten (1984) performed the first experiment involving aquatic respiration in freely diving must turtles, using S. minor. He found that aquatic VO_2 was about 1/3 the values reported by Root (1949) and Belkin (1968) for forcibly submerged turtles. Aquatic VO_2 was 10% of total VO_2. These data led Gatten to conclude that under more natural conditions (free diving as opposed to forcible submersion), aquatic respiration may play a less important role in the genus than Belkin (1968) suspected. Thus, Gatten (1984) agreed with Root, both suggesting, for different reasons, that aquatic respiration was relatively unimportant to the ecology of musk turtles.

Gatten (1980) also measured oxygen uptake in freely diving common snapping turtles, Chelydra serpentina. He found that the aquatic contribution to total VO_2 was about 5%. Snapping turtles have a highly reduced plastron and thus increased cutaneous surface area. However , they are large animals, without the suite of adaptations found in softshells. The low aquatic VO_2 reported by Gatten (1980) reflects their size and points to the importance of small body size as an adaptation favoring aquatic respiration.

A recently described species of Australian chelid, Rheodytes leukops, possesses well developed cloacal bursae that are actively ventilated at rates of 15-80 times per minute (Seymour 1982). Freely diving individuals of this species obtain 17-23% of their total O_2 from the water (Gatten, personal communication).

Curiously, after the experiments of Gage and Gage (1886) demonstrated that aquatic CO_2 excretion may be of a greater magnitude than aquatic O_2 uptake, 90 years passed before another study was conducted on aquatic CO_2 excretion in turtles. Jackson

et al. (1976) measured aquatic and aerial VCO_2 in six species of turtles. These species occurred in a variety of habitats ranging from wholly terrestrial to wholly aquatic. Their data show that aquatic VCO_2 decreases as the degree of terrestriality increases. Two of the species in the study were S. minor and T. muticus. These turtles excreted 30.9% and 64.5%, respectively, of total VCO_2, via non-pulmonary surfaces. These values are substantially higher than estimates of aquatic VO_2 reported for these species by other researchers, reaffirming the findings of Gage and Gage (1886).

The paucity of scrutiny that aquatic CO_2 excretion has received is especially unfortunate in light of the evidence provided by studies involving bimodally breathing fish (Singh 1976; Smatresk and Cameron 1982a, 1982b, 1982c; Graham 1983; Graham and Baird 1982, 1984). Such studies have revealed that, largely due to the high solubility of CO_2 in water, aquatic VCO_2 always exceeds aquatic VO_2 in bimodal breathers. Most bimodally breathing fish excrete very little CO_2 into the air. Thus, a turtle that is relatively poor at aquatic O_2 uptake might still rely to a large extent on the water as a means of CO_2 excretion.

Dive Duration and Underwater Survival

One of the obvious advantages a turtle capable of a high level of aquatic respiration might have over a strict lung breather is an increased ability to remain submerged for long periods. There are reports in the literature of softshells undergoing dives of extreme duration. Girgis (1961) reported that captive T. triunquis sometimes remained submerged voluntarily for at least six hours. Gage and Gage (1886) reported voluntary dives lasting "from two to ten consecutive hours." Similarly, Seymour

(1982) stated that R. leukops rarely breathes air.

In contrast, turtles known to be or suspected of being poor at aquatic respiration typically undergo much shorter dives. Hulse (1974) reported that foraging Kinosternon terrapin sonoriense surface every five to ten minutes. Belkin (1964) reported a mean dive duration for Pseudemys concinna of 62.8 minutes. These data were collected at night and the subject animals were asleep (Belkin 1964). Because it has been shown that night dives in the related Chrysemys dorbignyi are longer than day dives (Santos et al. 1990), these estimates perhaps are liberal. Santos et al. (1990) reported dive durations for C. dorbignyi to range from 4.1 minutes in the daytime in the summer to 18.7 minutes at night in the winter. McCutcheon (1943) found that most dives by Malaclemys centrata are between one and two minutes in duration, with occasional dives as long as 10 minutes. Lenfant et al. (1970) reported that the duration of the average dive in Chelys fimbriata was 35.1 minutes. Burggren et al. (1989) reported highly variable dives of up to one hour in duration for Chelodina longicollis.

Belkin (1968) measured survival time in P. scripta and S. minor when forcibly submerged in anoxic, normoxic, and hyperoxic water. Mean survival time for P. scripta was 20 hours in anoxic water, 23 hours in normoxic water, and 28 hours in hyperoxic water. For S. minor, these times were 13, 59, and >5000 hours, respectively. Because every S. minor tested seemingly was in perfect health six months after being submerged in hyperoxic water, Belkin suggested that turtles of this species could survive indefinitely under these conditions. These experiments demonstrate that surviving forced submergence is directly related to the ability to exchange respiratory gases with the water.

Ultsch et al. (1984) also found aquatic respiration to be correlated with survival during forced submergence in softshells, stinkpots, snapping turtles, and

painted turtles (Chrysemys picta). The former two species show a high degree of aquatic respiration, and both survived for over 100 days in 10 C water. The latter two species are poor at aquatic respiration and survived for only 14.2 and 29.3 days, respectively, at 10 C. Interestingly, softshells and stinkpots had the shortest survival times (2.6 and 5.2 days, respectively) when submersed in anoxic water at 10 C. This illustrates the importance of aquatic respiration to softshells and stinkpots.

Control of Ventilation

Because of the high aquatic VCO_2 of bimodal breathing fish, CO_2 buildup in the blood during a dive is not a powerful stimulus under natural conditions for the initiation of aerial ventilation. Instead, the main stimulus for air breathing in fish is hypoxia (Smatresk and Cameron 1982a, 1982b, 1982c; Graham and Baird 1982, 1984). In contrast, CO_2 levels in the blood probably are the main stimulus initiating lung ventilation in terrestrial vertebrates (Gesell 1939). Because turtles differ interspecifically in their ability to exchange respiratory gases with the water, it seems reasonable to predict that some genera (Trionyx, Rheodytes) would, like fish, be on a hypoxic drive, while others (Pseudemys, Chelydra) would, like terrestrial vertebrates, be on a hypercapnic drive.

In salamanders, there is evidence that the respiratory gas controlling ventilation depends on the habitat a given species occupies and its ability to exchange respiratory gases with the water. Aquatic species with a high capacity for aquatic gas exchange are less sensitive to high CO_2 levels than are more terrestrial species with reduced aquatic gas exchange capacities (Wakeman and Ultsch 1975).

Whether reptiles also differ in their sensitivity to respiratory gases with their degree of bimodal breathing is less clear. Direct evidence for the existence of a hypoxic/hypercapnic drive gradient is generally lacking, especially on the bimodal breather end of the gradient. However, measurements of blood PCO_2 and PO_2 during dives in C. serpentina, a species poor at aquatic exchange, have shown that CO_2 is the gas that initiates air breathing (Smits et al. 1987).

Studies linking increases in ventilation with changes in the composition of the inspired air are abundant in species that are poor at aquatic respiration. Such studies have shown that breathing either hypercapnic or hypoxic air will lead to hyperventilation in C. picta (Randall et al. 1944; Funk and Milsom 1987; Wasser and Jackson 1988), P. scripta (Frankel et al. 1969; Jackson et al. 1974), Terrapene carolina, Gopherus polyphemus (Ultsch and Anderson 1988), Testudo pardalis, and Pelomedusa subrufa (Burggren et al. 1977; Glass et al. 1978). Similarly, in C. serpentina, breathing hypoxic air leads to an increase in ventilatory rate (Boyer 1963, 1966).

There also is indirect evidence that reptiles capable of high levels of aquatic gas exchange are similar to fish in that the stimulus for air breathing is hypoxia and not hypercapnia. The highly aquatic elephant trunk snake, Acrochordus javanicus, responds to breathing 8% CO_2 by a depression of ventilation and a nearly two-fold increase in cutaneous CO_2 excretion (Glass and Johansen 1976). Also, normal blood PCO_2 in softshells, stinkpots, painted turtles, and common snapping turtles is inversely related to aquatic respiration in each species (Ultsch et al. 1984). Furthermore, during forced submergence in normoxic water , blood PCO_2 in softshells and stinkpots remained near control levels, while blood PCO_2 in the less aquatic painted and snapping turtles rose sharply (Ultsch et al. 1984). Low blood PCO_2, attributed to high levels of CO_2

excretion, suggest that it is O_2 and not CO_2 that limits dive duration in highly bimodally breathing reptiles.

The first chapter of this thesis quantifies aerial and aquatic oxygen uptake and carbon dioxide excretion in three species of freshwater turtles; the Gulf Coast spiny softshell (Trionyx spinifer asper), the stinkpot (Sternotherus odoratus), and the eastern mud turtle (Kinosternon subrubrum subrubrum). These species have high, intermediate, and low amounts of cutaneous surface area, respectively. The partitioning of VO_2 and VCO_2 were correlated with cutaneous surface area in the three species. Ecological ramifications due to observed differences among the species are discussed.

The second chapter of this thesis investigates the effect of aquatic PO_2 on ventilatory and diving behavior of these three species. Individuals of each species were exposed to hypoxic ($PO_2 \leq 30$ torr), normoxic ($PO_2 = 150$ torr), and hyperoxic ($PO_2 \geq 250$ torr) waters. Subsequent behavior was monitored, and responses were related to the ability of each species to exchange respiratory gases with the water. Implications concerning the control of ventilation are discussed with respect to the results of these experiments.

CHAPTER 1: BIMODAL BREATHING IN SOFTSHELL, STINKPOT, AND MUD TURTLES

INTRODUCTION

Bimodal breathing in freshwater turtles first was demonstrated in softshell turtles (Trionyx) over 100 years ago (Gage and Gage 1886). Since then, a chelid (Rheodytes leukops), musk turtles (Sternotherus odoratus and S. minor), the common snapping turtle (Chelydra serpentina), and the pond slider (Pseudemys scripta) have all been reported to take up oxygen and excrete carbon dioxide in both air and water (Root 1949; Belkin 1968; Jackson et al. 1976; Gatten 1980, 1984, personal communication).

Species that are well adapted to exchange respiratory gases with the water typically have reduced lung volumes (Agassiz 1857) and increased non-pulmonary respiratory surface area. Surface area expansion can be achieved by alterations in body shape, reduction or loss of epidermal scutes, possession of accessory respiratory epithelium in the cloaca or buccopharynx, or a combination of these factors (Agassiz 1857; Smith and James 1958; Feder and Burggren 1985). Species that lack such modifications (Pseudemys) often are capable of aquatic respiration, although the magnitude, and thus the importance of the pathway, is diminished (Belkin 1968; Jackson et al. 1976).

Despite the amount of information available on bimodal breathing in turtles, a number of questions remain unresolved. Reported values for aquatic VO_2 differ by three-

fold in Sternotherus (Root 1949; Gatten 1984), and by seven-fold in Trionyx (Gage and Gage 1886; Dunson 1960). These discrepancies may result from forced submergence in early studies; more recent studies have indicated that forced submergence leads to altered physiological and behavioral responses in turtles (Gatten 1984), as well as in other diving reptiles and mammals (Graham 1974; Seymour 1982; Zapol 1987).

Furthermore, the partitioning of aerial vs. aquatic VO_2 and VCO_2 has received little attention. In only two species, C. serpentina and S. minor, have simultaneous aerial and aquatic VO_2 been measured (Gatten 1980, 1984). In only one study, involving six species, have researchers taken simultaneous aerial and aquatic measurements of VCO_2 (Jackson et al. 1976). Simultaneous measurements of both aerial and aquatic VO_2 and VCO_2 have not been reported in a bimodally breathing turtle.

In this study I investigated the relationship between cutaneous surface area and aquatic vs. aerial respiratory gas exchange in freely-diving Gulf Coast spiny softshells (Trionyx spiniferus asper), stinkpots (Sternotherus odoratus), and eastern mud turtles (Kinosternon subrubrum subrubrum). I hypothesized that species with relatively high amounts of cutaneous surface area will rely more on aquatic and less on aerial gas exchange than species with relatively low amounts of cutaneous surface area.

MATERIALS AND METHODS

Animals

Ten K. subrubrum (5m, 5f; 104-206g, x = 134.4g), nine S. odoratus (3m, 6f; 44-155g, x = 90.4g), and nine T. spiniferus (6m, 3f; 211-4900g, x = 2117.7g) were used. All animals were adults except two juvenile male T. spiniferus. Preliminary

videotaping revealed that turtles habituated to the laboratory setting exhibited calmer and more consistent behavior than freshly captured turtles. Therefore, all experimental animals were housed in aquaria for at least two months prior to being used in experiments. Turtles were fed ad libitum; diets consisted of chicken livers, worms, fresh fish, and Reptomin^{R} reptile food. Prior to experiments, turtles were starved for 3 to 7 days. Temperature varied between 23 and 25 C, and photoperiod was 14L: 10D. All experiments were conducted during daylight hours.

Cutaneous Surface Area

Five K. subrubrum and five S. odoratus were skinned. Cutaneous portions were outlined on graph paper. The outlined areas were cut out, and surface areas were estimated using a LI-COR model 3100 area meter. For Trionyx (T. spiniferus and T. muticus) data obtained by Dunson (1986 and unpublished) were used. Surface areas for both S. odoratus and K. subrubrum were obtained from preserved specimens. In such specimens, length is a more reliable estimate than mass (Parker 1963). In order to compare mass-specific surface areas of the three species, data from an unpublished population study were used to estimate mass on the basis of carapace length. In both K. subrubrum and S. odoratus, length was a significant predictor of mass (r^2 = .95, F = 5494, K. subrubrum; r^2 = .95, F = 1384, S. odoratus).

Unlike stinkpots and mud turtles, softshells lack epidermal scutes. Dunson (1986) separated the skin from the "skin-like" material that covers the shell of softshell turtles. He found that the "skin-like" material was about half as permeable to water influx as the skin, yet 2.9 and 9.4 times more permeable than stinkpot and mud turtle skin, respectively (13.5 and 18.4 times more permeable than scutes,

respectively). Because of these differences in permeability, and because this "skin-like" material is highly vascularized (Agassiz 1857), both the skin and the "skin-like" material were considered to be important sites of aquatic gas exchange in softshells, and both were considered in the analysis. For stinkpots and mud turtles, only the skin was considered in the analysis.

Respiratory Gas Exchange

Single animals were placed in Plexiglas chambers composed of two compartments. The larger compartment contained water, and the smaller compartment, constructed into the lid of the larger, contained air. Each compartment was fitted with two ports for collecting samples and equilibration of gases within a given medium. To accommodate the size range of the animals used in the experiments, three chambers of varying sizes were constructed. The chambers were designed so that a turtle could move freely in the water and could breathe air without lifting its plastron from the floor of the aqueous compartment.

Each turtle was placed in a chamber at least 12 hours prior to an experiment. Air-equilibrated water (PO_2 = 150 torr) and fresh air were supplied throughout this period by a gas equilibration column and an air pump, respectively. This flow-through system was converted into a closed system prior to an experiment by closing off all inflow and outflow ports. During each experiment, the water was stirred continuously with a magnetic stir bar to prevent the establishment of O_2 or CO_2 gradients in the water. Aquatic and aerial PO_2 were maintained above 100 torr throughout each experiment by periodically refreshing the water and air in the chamber. The aqueous and aerial phases were separated by a thin layer of mineral oil to prevent diffusion of O_2

into or out of either phase. Mineral oil is not an effective barrier to CO_2 diffusion. However, the small area of the air/water interface tended to minimize the movement of CO_2 between phases. Additionally, the CO_2 gradient between the aquatic and aerial phases consistently favored unidirectional diffusion from water to air, leading to conservative estimates of aquatic VCO_2.

Respiratory gas exchange was monitored for periods ranging from 3.8 to 14.0 hours. Samples were taken at one- and two-hour intervals, depending on the size of the turtle compared to the chamber and the variable involved. Turtles that were small relative to chamber volume took longer to cause measurable changes in O_2 and CO_2 levels, especially in the water. In such cases, a two-hour sampling interval was used. Conversely, turtles that were large relative to the chamber volume took in O_2 so rapidly that a two-hour sampling interval resulted in aerial hypoxia. Therefore, a one-hour interval was used. For aquatic CO_2 excretion, small errors in sample measurement were amplified much more than in any other variable. As a result, a two-hour interval was used regardless of the sampling interval used for the other variables. Six water samples were taken at each sampling period, three for CO_2 and three for O_2. The mean of the three samples was used in data analysis to reduce measurement error. Air samples were less variable; therefore, one sample for each gas was deemed sufficient.

Oxygen tensions were monitored with a Radiometer PHM73 Blood/Gas Monitor equipped with an E5046 PO_2 electrode. Carbon dioxide concentrations were monitored with a Capni-con 3 Total CO_2 Analyzer (Cameron Instrument Company). Aerial and aquatic VO_2 were determined by measuring the decrease in O_2 tensions over time, and aerial and aquatic VCO_2 were determined by following the increase in CO_2 concentrations

over the same time period. Oxygen tensions were converted into volumes using solubility coefficients from Dejours (1975).

Mean aquatic, aerial, and total VO_2 and VCO_2 were calculated for each individual based on from two to eight sampling periods per variable. The percentage of total VO_2 and VCO_2, aquatic vs. aerial, and the respiratory quotient (RQ) were calculated from these means. These values were then used to determine the mean ± standard error of each variable for a given species. Data analyses were carried out on the means of individual subject animals, not on individual observations; thus, the within animal variation was not a factor in the analyses.

Because no attempt was made to seal the cloacae of turtles during experiments, the possibility of urinary contamination existed. The presence of bicarbonate in the urine could have biased the aquatic VCO_2 estimates. To control for this I determined the ammonia content of the water at the beginning and end of each experiment using the phenolhypochlorite method (Solorzano 1969). High levels of ammonia coupled with high estimates of VCO_2 would provide evidence that such a bias was occurring. In addition, the cloaca of one K. subrubrum was sealed with a rubber band ligature, and ammonia excretion was monitored in order to determine the extent of cutaneous ammonia excretion.

Statistical Analysis

A log-log regression (cutaneous surface area vs. mass) was calculated for stinkpots and eastern mud turtles. For softshells, Dunson's (1986) regression equation for total surface area (skin + "skin-like" combined) was used. Regression equations were compared using analysis of covariance (ANCOVA). From these regression

equations, cutaneous surface area for each turtle used in this study was estimated. From these data, analysis of variance (ANOVA) was used to detect interspecific differences in mass-specific cutaneous surface areas.

Differences in cutaneous surface area (see Results), coupled with the literature on aquatic respiration in the three species (see Table 1), led to an *a priori* prediction that I. spiniferus would rely most heavily on aquatic respiration, followed by S. odoratus, then by K. subrubrum. This prediction is the basis of the one-tailed tests that are used throughout the respiratory gas exchange section (except RQ analysis).

As a result of this hypothesis, we used S. odoratus as a pivot to test for differences among species, testing S. odoratus vs. K. subrubrum and S. odoratus vs. I. spiniferus for each variable. If S. odoratus differed from either, we assumed that I. spiniferus differed from K. subrubrum for that variable. This approach is more attractive than the alternative, a multiple comparisons procedure, since it minimizes the chance of making Type II errors. For the analysis of RQ, no *a priori* prediction was made. For total CO_2 and O_2 exchange, we predicted that I. spiniferus would have the lowest values because of its larger body size.

All means are reported ± standard error. Variances were tested for equality among species for a given variable using an F'-test and a .99 level of significance. If no differences were revealed, species means were compared using a 3-way multivariate ANOVA. Cochran's approximation was used to compare means among species when the variances were different, and when appropriate, a 2-way multivariate ANOVA was used to test the remaining species (in cases when one species differed from the other two, but the other two did not differ from each other).

For ratio and percentage data (RQ, % aquatic), arcsine transformations were performed. However, in no case did the transformed data differ from the untransformed

data in statistical significance. Therefore, for simplicity in data interpretation, the untransformed data are reported.

Variance tests were performed for CO_2 versus O_2 exchange within each species in each medium. If differences were revealed, Cochran's approximation was used to compare means. If no differences were revealed, t-tests were used.

Regression analyses were used to investigate the effects of ammonia excretion on aquatic VCO_2 excretion in K. subrubrum and S. odoratus. Unfortunately, only one data point was obtained for I. spiniferus, so regression analysis was impossible.

All statistical analyses were performed using SAS.

RESULTS

Cutaneous Surface Area

The regression equations for log cutaneous surface area vs. log body mass in the three species did not differ in slope (ANCOVA, $F = 2.01$, $b_c = .501$), but did differ in elevation ($F = 254.93$, $P \leq .01$, Figure 1). Thus, for individuals of similar mass, I. spiniferus had the most cutaneous surface area, S. odoratus was intermediate, and K. subrubrum had the least.

From these regression equations, we estimated the mass-specific cutaneous surface areas of the animals from which respiratory gas exchange data were obtained and subjected these values to statistical analysis. Analysis of variance revealed that the softshells and stinkpots in this study had similar mass-specific cutaneous surface areas (1.13 ± 0.40 cm^2/g softshells, 1.05 ± 0.38 cm^2/g stinkpots, $F = 0.22$), despite softshells being much larger than stinkpots. Both the stinkpots and softshells had more mass-specific cutaneous surface areas than the mud turtles used in this study ($0.62 \pm$

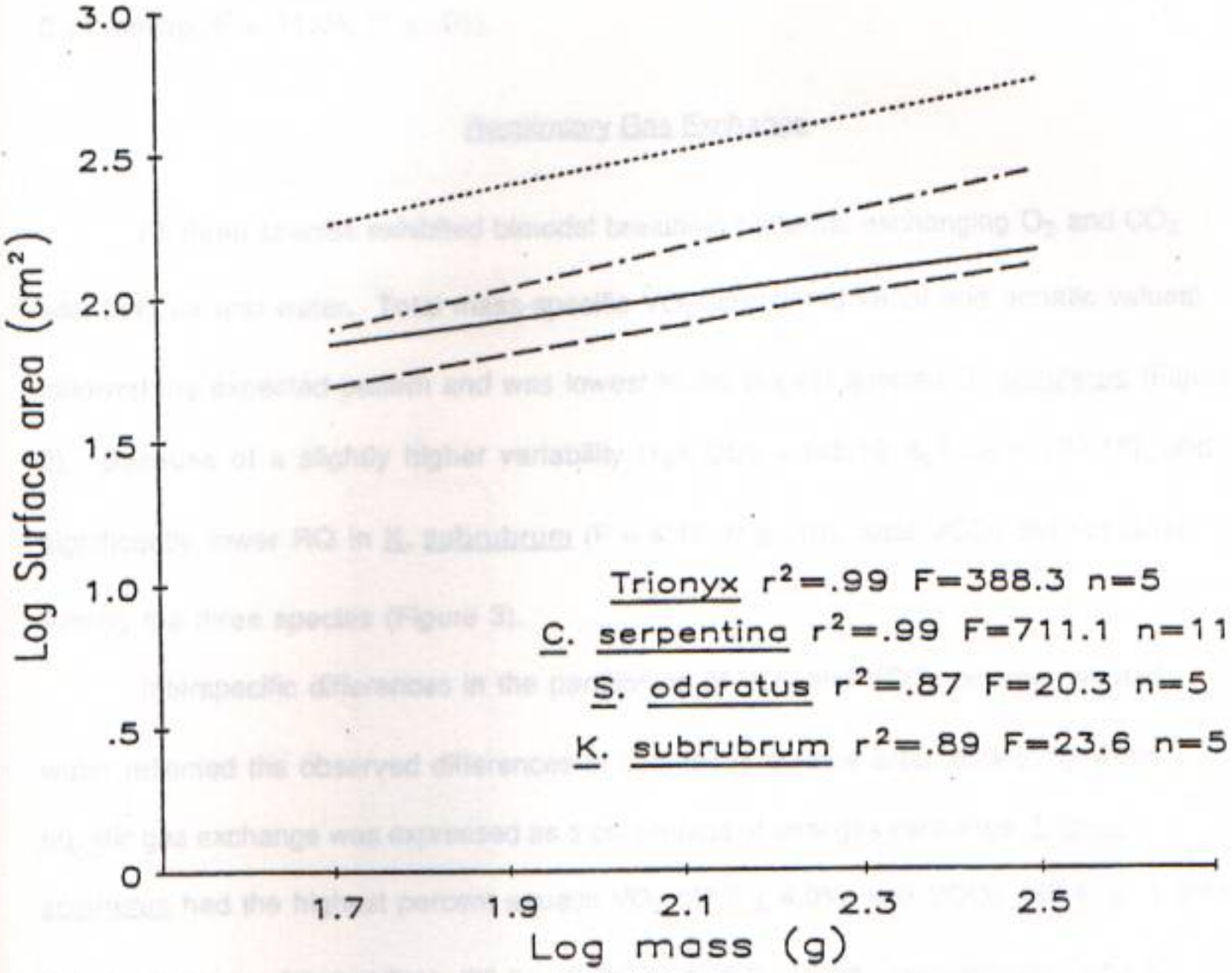

Figure 1. Log cutaneous surface area vs log body mass regression lines in Trionyx (dotted line), Chelydra serpentina (dotted/dashed line), S. odoratus (solid line), and K. subrubrum (dashed line). The data for Trionyx and C. serpentina were obtained from Dunson (1986 and personal communication). Each line is significant at the .01 level.

0.21 cm^2/g, $F = 11.04$, $P \leq .01$).

Respiratory Gas Exchange

All three species exhibited bimodal breathing patterns, exchanging O_2 and CO_2 with both air and water. Total mass-specific VO_2 (combined aerial and aquatic values) followed the expected pattern and was lowest in the largest species, T. spiniferus (Figure 2). Because of a slightly higher variability (s_p^2 CO_2 = 143.16; s_p^2 O_2 = 103.15), and significantly lower RQ in K. subrubrum ($F = 4.14$, $P \leq .10$), total VCO_2 did not differ among the three species (Figure 3).

Interspecific differences in the partitioning of VO_2 and VCO_2 between air and water reflected the observed differences in cutaneous surface area, especially when aquatic gas exchange was expressed as a percentage of total gas exchange. Trionyx spiniferus had the highest percent aquatic VO_2 (37.5 ± 4.0%) and VCO_2 (85.4 ± 1.9%), S. odoratus was intermediate (25.8 ± 2.7% and 55.6 ± 1.2%, respectively), and K. subrubrum was lowest (13.6 ± 1.0% and 45.8 ± 3.6%, respectively) (Figures 2 and 3).

While these percentages remained fairly constant from individual to individual, there was considerable variability in the actual rates, partially obscuring the pattern described above. Aquatic VO_2 was less variable ($F' = 9.39$) and lowest in K. subrubrum but did not differ between T. spiniferus and S. odoratus (Figure 4). Similarly, aquatic VCO_2 was higher in S. odoratus than in K. subrubrum, but more variable ($F' = 9.86$) and not different from softshells (Figure 5). Aerial VO_2 and VCO_2 also reflected the high variability among individuals. Aerial exchange of both O_2 and CO_2 was lowest in

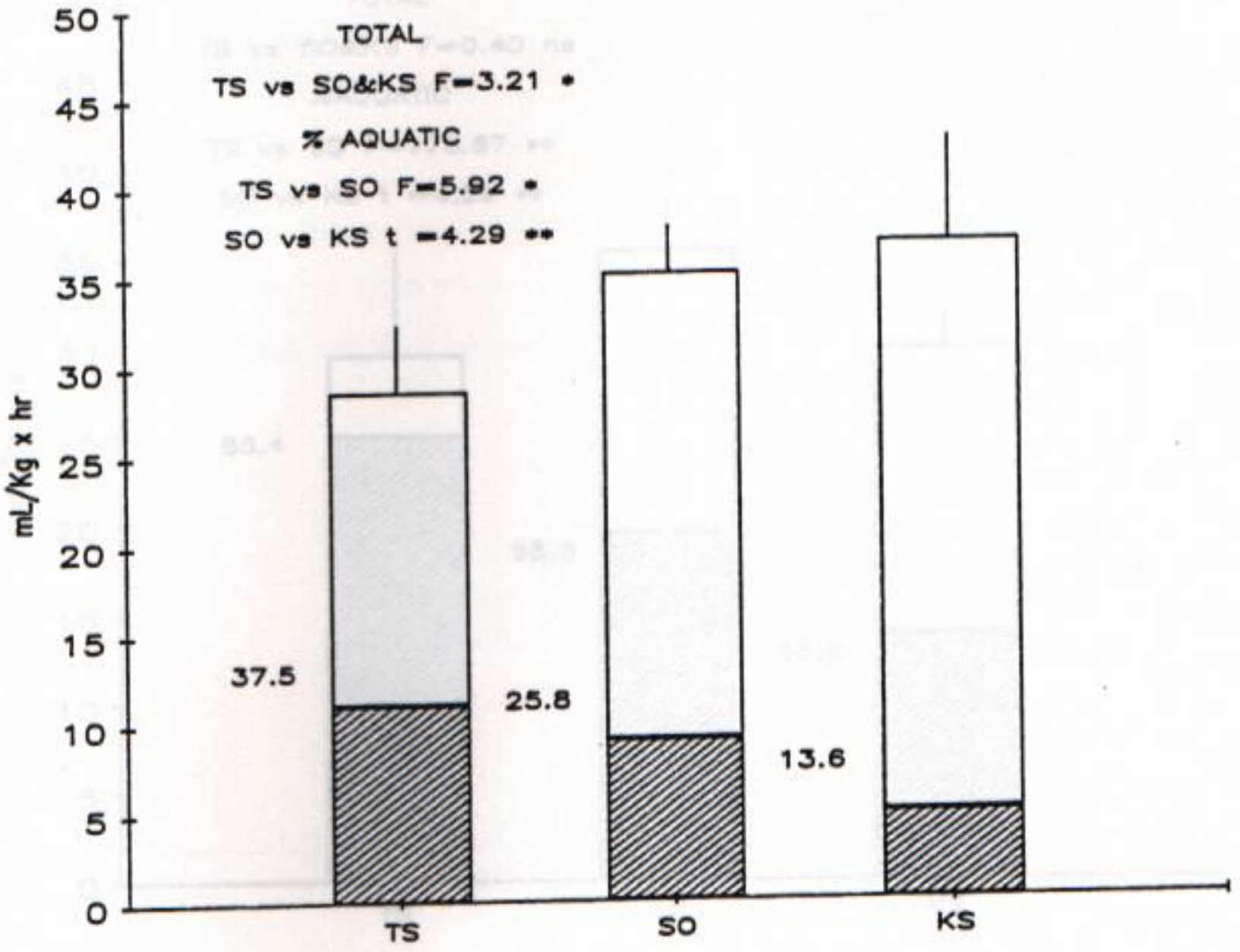

Figure 2. Oxygen uptake in T. spiniferus (TS), S. odoratus (SO), and K. subrubrum (KS). Striped portions represent mean aquatic VO_2; solid portions represent mean aerial VO_2. Mean total VO_2 is represented by the top of each stacked bar. Error bars represent standard error of total VO_2. Numbers to left of each bar are the percent total VO_2 accounted for by aquatic VO_2. F values are from analysis of variance and t' values are from Cochran's approximation. An * indicates significance at .05 level, and ** indicates significance at .01 level.

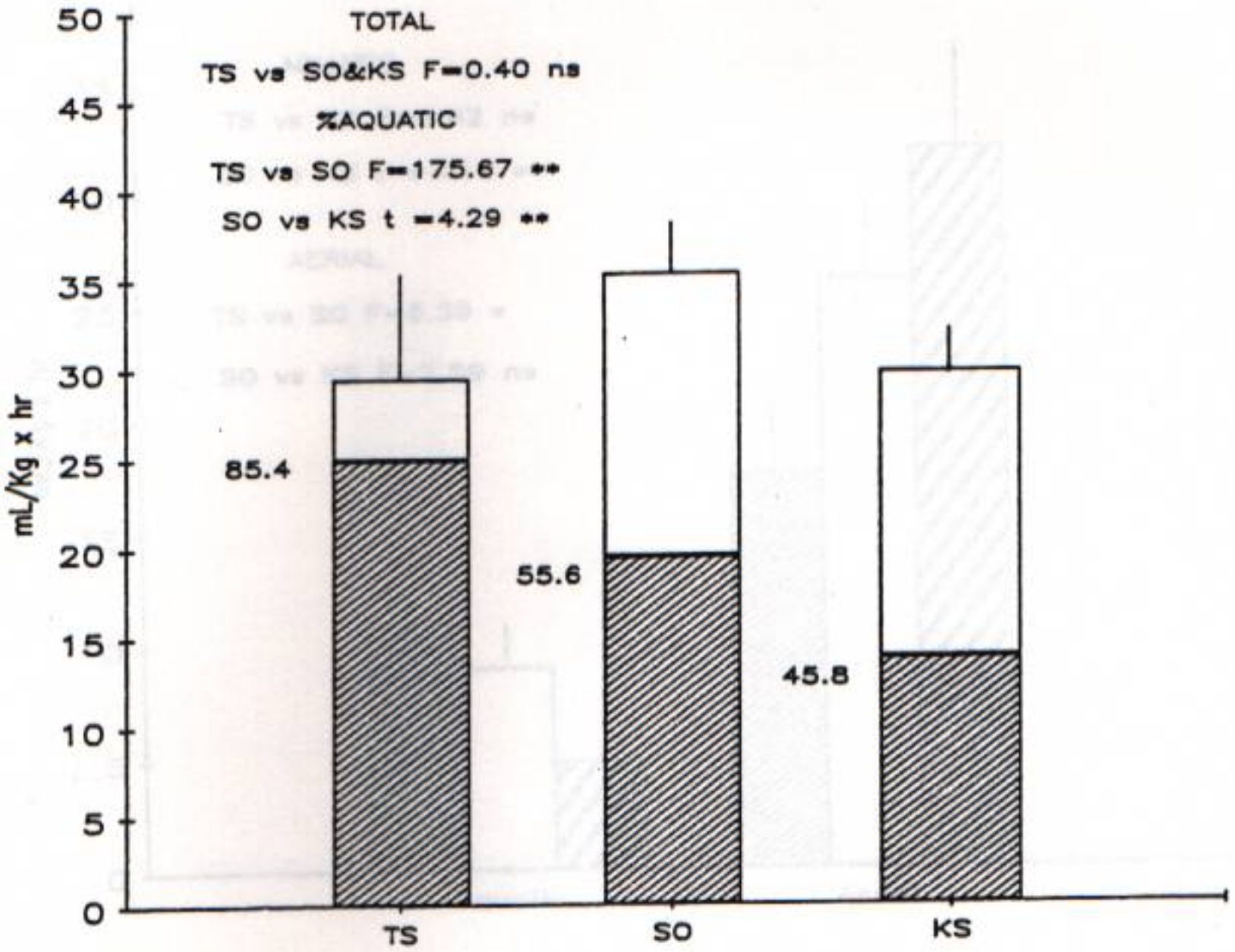

Figure 3. CO_2 excretion in T. spiniferus (TS), S. odoratus (SO), and K. subrubrum (KS). The format for Figure 3 is the same as for Figure 2 except ns in Figure 3 indicates values that are not significant at the .05 level.

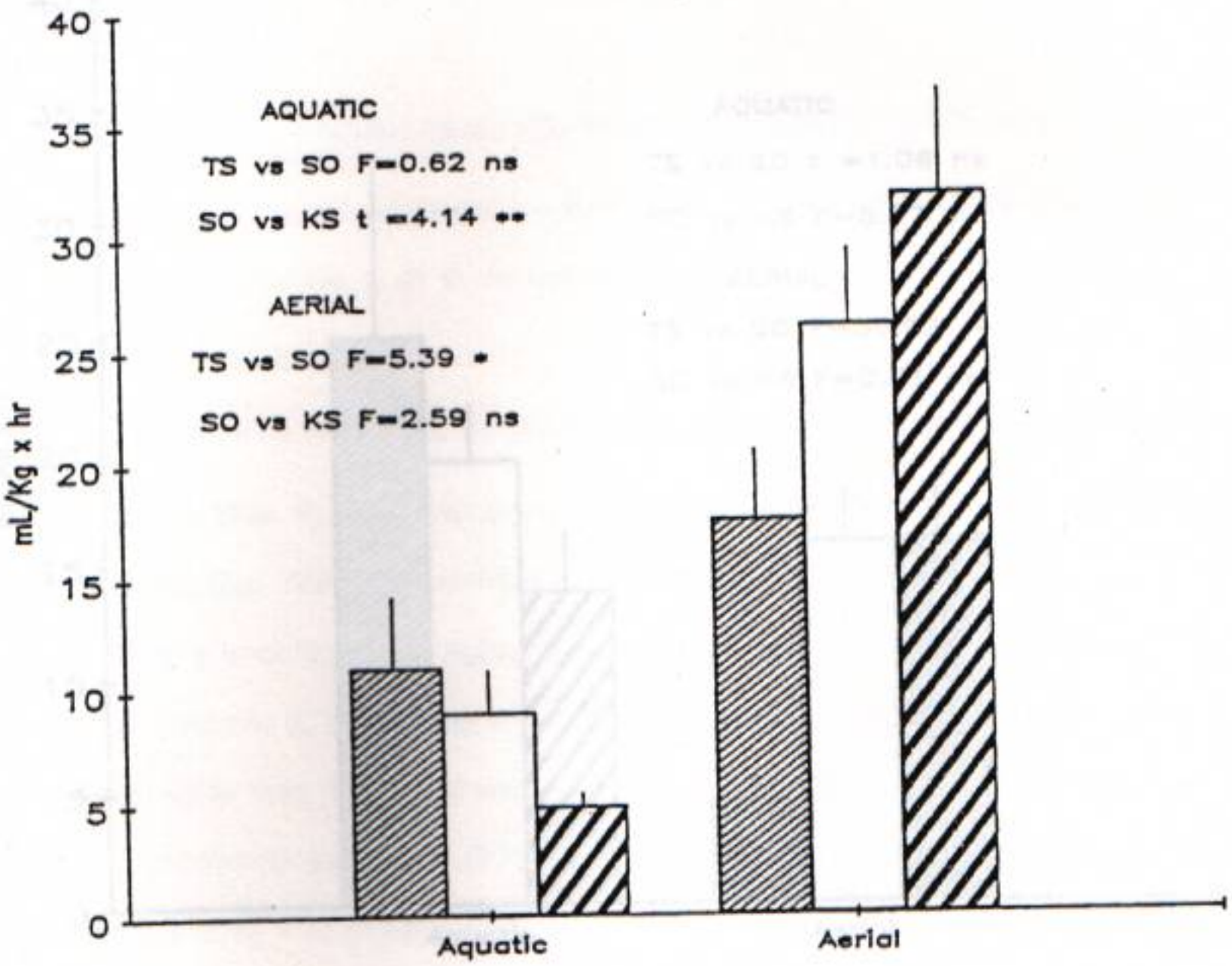

Figure 4. Mean aerial and aquatic O_2 uptake in T. spiniferus (thin striped bars), S. odoratus (solid bars), and K. subrubrum (heavy striped bars). F values are from analyses of variance, t' values are from Cochran's approximation. Error bars represent standard errors. An * indicates a .05 level of significance, ** indicates significance at .01 level, and ns indicates values not significant at the .05 level.

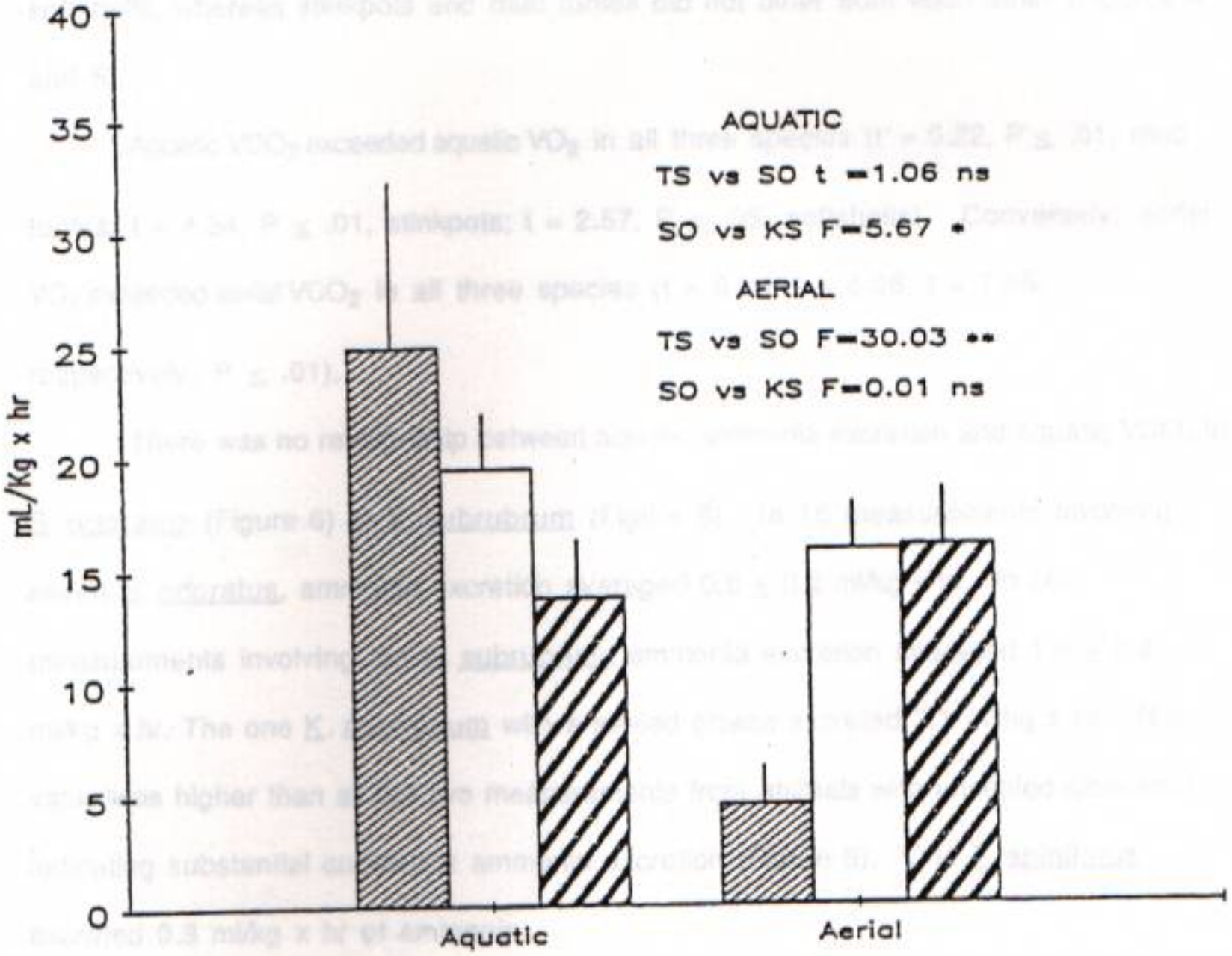

Figure 5. Mean aerial and aquatic CO_2 excretion in T. spiniferus (thin striped bars), S. odoratus (solid bars), and K. subrubrum (heavy striped bars). See Figure 4 for format.

softshells, whereas stinkpots and mud turtles did not differ from each other (Figures 4 and 5).

Aquatic VCO_2 exceeded aquatic VO_2 in all three species ($t' = 5.22$, $P \leq .01$, mud turtles; $t = 4.34$, $P \leq .01$, stinkpots; $t = 2.57$, $P \leq .05$, softshells). Conversely, aerial VO_2 exceeded aerial VCO_2 in all three species ($t = 9.36$, $t = 6.06$, $t = 7.65$, respectively, $P \leq .01$).

There was no relationship between aquatic ammonia excretion and aquatic VCO_2 in S. odoratus (Figure 6) or K. subrubrum (Figure 6). In 16 measurements involving seven S. odoratus, ammonia excretion averaged 0.8 ± 0.2 ml/kg x hr. In 26 measurements involving ten K. subrubrum, ammonia excretion averaged 1.5 ± 0.4 ml/kg x hr. The one K. subrubrum with a sealed cloaca excreted 3.1 ml/kg x hr. This value was higher than all but two measurements from animals with unsealed cloacae, indicating substantial cutaneous ammonia excretion (Figure 6). One T. spiniferus excreted 0.3 ml/kg x hr of ammonia.

DISCUSSION

Body Size, the Skin, and Aquatic Respiration

Two consequences of large body size are 1) decreased mass-specific surface area and 2) increased integument thickness (Feder and Burggren 1985; Graham 1990). According to the Fick equation, both of these factors lead to a reduction in diffusion (i.e. aquatic respiration). In this study, softshell turtles not only had the highest rates of aquatic gas exchange, but also averaged over 15 times the mass of stinkpots or mud turtles.

Softshells have circumvented the physiological limitations of large body size in

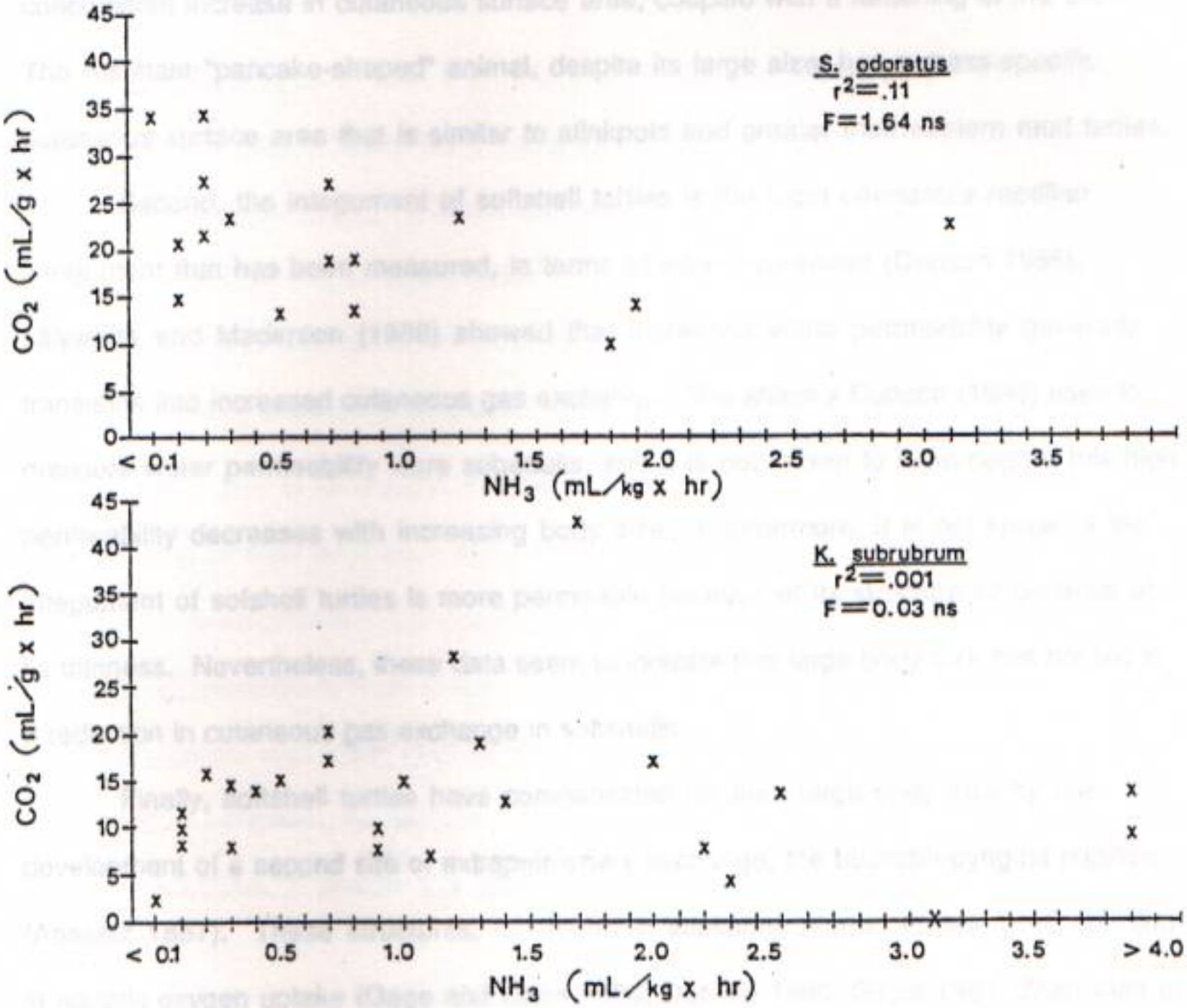

Figure 6. Aquatic CO_2 excretion vs aquatic NH_4 excretion in S. odoratus and K. subrubrum. The x on the x-axis of the lower graph indicates the NH_4 excretion of one K. subrubrum with a sealed cloaca.

three ways. First, there has been an evolutionary loss of epidermal scutes and a concomitant increase in cutaneous surface area, coupled with a flattening of the shell. The resultant "pancake-shaped" animal, despite its large size, has a mass-specific cutaneous surface area that is similar to stinkpots and greater than eastern mud turtles.

Second, the integument of softshell turtles is the most permeable reptilian integument that has been measured, in terms of water movement (Dunson 1986). Lillywhite and Maderson (1988) showed that increased water permeability generally translates into increased cutaneous gas exchange. The animals Dunson (1986) used to measure water permeability were subadults, and it is not known to what degree this high permeability decreases with increasing body size. Furthermore, it is not known if the integument of sofshell turtles is more permeable because of its structure or because of its thinness. Nevertheless, these data seem to indicate that large body size has not led to a reduction in cutaneous gas exchange in softshells.

Finally, softshell turtles have compensated for their large body size by the development of a second site of extrapulmonary exchange, the buccopharyngeal papillae (Agassiz 1857). These structures, not found in stinkpots or mud turtles, are important in aquatic oxygen uptake (Gage and Gage 1886; Dunson 1960; Girgis 1961; Zhao-Xian et al. 1989). These structures further increase respiratory surface area, so that large softshells may even exceed small stinkpots in total mass-specific extrapulmonary respiratory surface area, perhaps explaining the greater aquatic gas exchange of softshells.

The importance of the softshell adaptations discussed above is illustrated by comparing large and small bimodal breathers that otherwise possess similar suites of adaptations. Common snapping turtles (C. serpentina) are similar in size to softshell turtles yet lack most of the adaptations for aquatic respiration described above.

Snapping turtles have significantly higher cutaneous surface areas than similar sized Sternotherus (Figure 1), yet aquatic VO_2 in snapping turtles is significantly lower than that for Sternotherus ($t = 1.90$, $P \leq .10$), even if the most conservative estimate for Sternotherus is used (Gatten 1984, Table 1). Similarly, aquatic gas exchange in pond sliders (Pseudemys scripta) is small compared to the much smaller eastern mud turtle (Table 1), although similar sized individuals of both species appear similar in cutaneous surface area (personal observations).

This study shows that differences in the partitioning of VO_2 and VCO_2 among species are related to differences in cutaneous surface area. Softshell turtles have compensated for their large body mass by the extreme expansion of cutaneous surface area, by the development of extensive buccopharyngeal processes, and through an evolutionary alteration in body shape. Consequently, softshells exchange more respiratory gases with water than do the much smaller kinosternids, S. odoratus and K. subrubrum. Likewise, stinkpots and mud turtles, which are similar in body size, express differences in aquatic gas exchange that parallel differences in cutaneous surface area.

The Partitioning of Respiratory Gas Exchange

This study is the first to report aquatic VO_2 in freely diving softshell turtles. If the data from four previous studies involving forced diving (see Table 1) are pooled, the mean (10.81 ml/Kg x hr) is consistent with the mean from this study (11.05 ml/Kg x hr). Aquatic VO_2 of S. odoratus in this study agrees more closely with the studies involving forcibly submerged S. odoratus (Root 1949) and S. minor (Belkin 1968) than with the study involving freely-diving S. minor (Gatten 1984, Table 1). These

Aquatic and Aerial Oxygen Uptake and Carbon Dioxide Excretion in Freshwater Turtles (mL/Kg x hr).

	VO_2 H_2O	VO_2 Air	VO_2 Total	VCO_2 H_2O	VCO_2 Air	VCO_2 Total
<u>Trionyx</u>						
Gage and Gage (1886)	3.31			7.07		
Dunson (1960)	22					
Girgis (1961)	6.8					
Jackson et al. (1976)				10.8	5.9	16.7
Zhao-Xian et al. (1989)	11.11					
This study	11.05	17.50	28.55	29.47	4.55	24.92
<u>Sternotherus</u>						
Root (1949)	9.5	71	80.5			
Belkin (1968)	8.00	24.71	32.70			
Gatten (1984)	2.70	24.20	26.90			
Jackson et al. (1976)				11.9	26.61	38.51
This study	9.03	26.06	35.09	19.48	15.81	35.29
<u>Kinosternon</u>						
This study	4.89	31.84	36.73	13.76	15.99	29.75
<u>Chelydra</u>						
Gatten (1980)	1.95	36.17	38.12			
<u>Pseudemys</u>						
Belkin (1968)	1.19	28.20	29.39			
Jackson et al. (1976)				9.4	80.1	89.5

findings do not support Gatten's conclusion that aquatic oxygen uptake is drastically increased in forcibly submerged turtles.

There are no estimates of aerial VO_2 in softshell turtles available for comparison with this study. The aerial VO_2 of Sternotherus in this study is close to the values reported by Belkin (1968) and Gatten (1984), but not to those reported by Root (1949, Table 1). Root's measurement of aerial VO_2 involved removing turtles from water and putting them in a dry respirometer, a situation that may have produced stress on the animals. His resulting estimates of aerial VO_2 are over twice as high as any reported for Sternotherus and are perhaps biased due to his methodology.

The aquatic VO_2 reported by Gatten (1984) for S. minor is lower than any other estimate, and the aerial VO_2 reported by Root (1949) for S. odoratus is higher than any other estimate (Table 1). These observations led these two researchers to assert that aquatic respiration was relatively unimportant in Sternotherus. The data reported in this study indicate that 55.6% of all CO_2 is excreted into the water and 25.8% of all O_2 is taken up from the water in S. odoratus. Form these data, it is clear that water can be an important medium for respiratory gas exchange in S. odoratus.

In every species yet tested, aquatic VCO_2 exceeds aquatic VO_2 (Table 1). This fact is well established in bimodal breathers (Wood and Lenfant 1976) and is due primarily to the high solubility of CO_2 in water compared to O_2. In this study, aquatic VCO_2 was 2.3, 2.2, and 2.8 times higher than aquatic VO_2 in softshell, stinkpot, and mud turtles, respectively. High aquatic VCO_2 estimates, especially in softshells, suggest that the respiratory function of the lungs is mainly the absorbtion of O_2 and not the excretion of CO_2, which is primarily cutaneous. This is the pattern in bimodally breathing fish

(Singh 1976; Smatresk and Cameron 1982a; Graham and Baird 1984), in which the organ of aquatic gas exchange is primarily the gill.

Ecological Implications

The advantages a bimodally breathing turtle might have over a turtle totally reliant on pulmonary respiration include: decreased time traveling to and breathing at the surface, decreased exposure to surface predation, and increased time available for foraging, breeding, traveling, and sleeping below the surface. The disadvantages might include a thinner integument, which would be more prone to injury and more susceptible to desiccation. The bimodally breathing turtle might be more restricted to well-oxygenated, permanent bodies of water, while the strict air breather might have broader habitat tolerances.

Both I. spiniferus and S. odoratus occur frequently in shallow, well-mixed, riverine habitats (Mount 1975). Such habitats are typically devoid of aquatic vegetation and therefore are characterized by a relatively constant PO_2 approaching air-saturation. Thus, two important features that favor aquatic respiration are provided; a constant and high partial pressure gradient between the water and the blood, and ventilation of the skin with little or no cost to the animal. The latter assumes that the animal is in the water column and not buried in the substrate, as softshell turtles often are. Nevertheless, it is clear that softshells and stinkpots often live in habitats that are quite suitable for aquatic respiration.

In contrast, mud turtles typically live in stagnant or nearly stagnant bodies of water (Mount 1975) that are often choked with aquatic vegetation and are therefore prone to hypoxia and/or subject to wide fluctuations in PO_2. As a result, the partial

pressure gradient between the water and the blood is uncertain and sometimes negative. As pointed out by Feder and Burggren (1985), if cutaneous CO_2 excretion is obligatory in order to prevent acidosis, a hypoxic environment could result in the loss of oxygen to the water via diffusion from the blood to the environment. In addition, in such a habitat the skin must be ventilated actively; otherwise, a stagnant boundary layer may form and impede diffusion (Feder and Pinder 1988).

Earlier, I referred to the highly permeable integument of softshell turtles (Dunson 1986) as a factor that favors aquatic respiration. It also favors desiccation. There are differences among the three species in the extent to which terrestrial activity occurs. These differences are related to anatomical differences, which in turn have led to differences in aquatic gas exchange. Softshell turtles rarely leave the water except to lay eggs or bask. Basking time is minimized by their ability to heat themselves faster than most ectotherms (Smith et al. 1981). Eastern mud turtles, on the other hand, spend a great deal of time on land, overwintering and perhaps foraging there (Scott 1976). Stinkpots seem intermediate between softshells and mud turtles in these characteristics (Mount 1975; unpublished data).

Whether softshell turtles lost their scutes as an adaptation for aquatic respiration or to meet some other adaptive need (e.g., increased foraging efficiency; Pritchard 1984) is uncertain. It is clear, however, that the loss of epidermal scutes has limited their ability to move about on land for long periods. Softshells are not only more susceptible to desiccation but perhaps also more exposed to injury and predation than more armored turtles, such as the eastern mud turtle. It is also clear that the reduction in bony armor in softshells has led to increased aquatic respiration. The role of aquatic respiration in the evolution of softshells, and other bimodally breathing turtles, merits further investigation.

CHAPTER II: DIVING AND VENTILATORY BEHAVIOR AS A FUNCTION OF AQUATIC PO_2 IN SOFTSHELL, STINKPOT, AND MUD TURTLES

Many species of freshwater turtles possess the ability to exchange respiratory gases with either air or water, and therefore are classified as bimodal breathers (Belkin 1968; Jackson, Allen, and Strupp 1976; Gatten 1980, 1984; Chapter 1). How these species compare to species that are primarily air-breathers, both in terms of their diving ability and ventilatory behavior, is not clear. It has been documented that bimodally breathing turtles can withstand forced submergence without access to air for longer periods than can turtles that are primarily air-breathers (Belkin 1968; Ultsch et al. 1984). However, these studies may more accurately reflect what a turtle is capable of physiologically, rather than what it actually does in nature. The bulk of the evidence relating aquatic gas exchange to dive duration in free-diving turtles is anecdotal, but it does indicate that bimodally breathing turtles voluntarily undergo dives that rival any other air-breathing vertebrate in terms of duration (Gage and Gage 1886; Girgis 1961; Seymour 1982).

Likewise, the factors that limit dive length in turtles are not clear. There is evidence that aerial ventilation in turtles is driven by hypercapnia, hypoxia, or both (Lenfant et al. 1970; Smits et al. 1987; Wasser and Jackson 1988). In air-breathing fish, it is clear that the stimulus for aerial ventilation is aquatic hypoxia (Graham and Baird 1982, 1984; Smatresk and Cameron 1982a, 1982b, 1982c). It is also clear that most terrestrial vertebrates are on a hypercapnic drive (Gesell 1939). Because some species are better adapted for aquatic respiration than others, it is possible that turtles form a transitional gradient between terrestrial vertebrates and fish, with predominantly air-breathing species being more sensitive to hypercapnia, and

bimodally breathing species being more sensitive to hypoxia. It has been shown that salamanders are such a transitional group (Wakeman and Ultsch 1975).

Most of the studies on aerial ventilation in turtles have been performed on species that are poor at aquatic gas exchange (i.e. the Emydidae). In most cases, ventilatory responses to altered gas tensions of only the air phase were monitored. The single study that included alterations in aquatic oxygen tensions reported no effect on aerial ventilation, but the species examined (Chrysemys dorbignyi) is thought to be a poor bimodal breather (Santos et al. 1990). The effect of aquatic oxygen tensions on dive length and aerial ventilation in turtles proficient at bimodal breathing has never been investigated. In this study, the effects of both aquatic hypoxia and aquatic hyperoxia on diving behavior and aerial ventilation are reported on in three species of turtles that display varying degrees of bimodal breathing. The Gulf Coast spiny softshell (Trionyx spiniferus asper) is highly aquatic, exchanging a large fraction of its total gas exchange with the water; the stinkpot (Sterotherus odoratus) is an intermediate bimodal breather; the mud turtle (Kinosternon subrubrum subrubrum) is the most terrestrial and the poorest bimodal breather (see Chapter 1).

MATERIALS AND METHODS

Eight K. subrubrum, seven S. odoratus, and eight T. spiniferus were placed individually at random in chambers containing water equilibrated to one of three oxygen tensions; hypoxia ($\leq$ 30 torr), hyperoxia ($\geq$ 250 torr), and normoxic (between 100 and 150 torr). At all times, turtles had free access to normoxic air. The chambers and turtles were the same as used in previous experiments (see Chapter 1). Temperature varied between 23 and 25 C and photoperiod was 14L:10D. All experiments were conducted during daylight hours.

Normoxic water was obtained by running dechlorinated tap water through a gas equilibration column through which air was bubbled. Hyperoxic and hypoxic water were obtained by bubbling either pure oxygen or pure nitrogen, respectively, through the column. In all cases turtles were placed in chambers and exposed to continuously circulating normoxic water at least 12 hours before an experiment. After this pre-experimental phase, normoxic and hyperoxic experiments commenced immediately after the water in the chamber was equilibrated. In hypoxic experiments, after the water was equilibrated to about 50 torr with the gas equilibration column, the turtle was allowed to further reduce the PO_2 in the water via cutaneous exchange to 30 torr or below. The fact that PO_2 in the water could not be reduced below about 50 torr using the column necessitated this process, which was usually completed by the turtle within 2-3 hours, at which time the experiment commenced.

The behavior of each turtle under each condition was monitored for a four-hour period using a Panasonic AG-160 VHS movie camera. The four-hour period was long enough to insure that an adequate sample was gathered for each turtle at each condition, yet small enough to insure that the PO_2 of the water would remain within an acceptable range without reequilibration during an experiment. The aerial phase of the chamber was replenished hourly with fresh air during experiments involving stinkpots and mud turtles, and every two hours during experiments involving softshells. Based on previous findings, these intervals were adequate to insure that at no time were turtles breathing hypoxic or hypercapnic air (see Chapter 1).

Lung ventilation was detected in one of two ways. In the smaller chamber, a 1 mL pipette containing a drop of colored isopropyl alcohol was attached to a port in the aerial portion of the chamber. The displacement of this drop caused by lung ventilation was

filmed. In the two larger chambers, lung ventilation was detected by filming the displacement of water at the air/water interface. Differences in magnitude made it possible to clearly distinguish between lung ventilation and pharyngeal movement. Lung ventilations were longer in duration and displaced a much larger volume than throat movements.

Videotapes were analyzed using an Emerson VHS VCR (872HQ). Variables that were measured included the duration of each immersion and emersion period, duration of each apneic period (head above surface but not breathing), the number of breaths per ventilatory cycle, and the number of ventilatory cycles per emersion period. Also noted were the episodes of breathing immediately (< 2 sec) preceding immersion, episodes of breathing immediately (< 2 sec) following emersion, and those emersion periods not accompanied by breathing. The number of breaths taken per emersion period was calculated for each individual by multiplying the average number of breaths per ventilatory cycle by the number of ventilatory cycles per emersion period, and ventilatory rate was calculated for each individual by dividing the total number of breaths taken by the total duration of the experiment. Times were measured with a Micronta LCD quartz stopwatch.

Statistical Analysis

All means are reported ± standard error. Variances for each variable were tested for differences among species using F-tests and a .99 level of significance. A CRD multivariate ANOVA was used to test for intra- and interspecific differences among means of the variables whose variances did not differ among species. If two species had similar variances and a third had a variance different from the other two, the third species was analyzed separately. If all three species had different variances, three

separate analyses were performed. Cochran's approximations were performed to investigate interspecific differences when variances differed among species. Variance tests and Cochran's approximations were done using the TTEST procedure of SAS, and ANOVA's were done using the GLM procedure of SAS.

RESULTS

Immersion

Individual dives were typically of short duration, punctuated by frequent emersion (Figure 1). Of the 1257 total dives recorded (combining species and oxygen tensions), 1129 (89.8%) lasted under 20 minutes, while only 23 (1.8%) were longer than 50 minutes. There were no differences among or within species in the percentage of short duration dives ($F = 1.06$). Likewise, dives between 20 and 50 minutes did not differ among or within species ($F = 0.84$). In stinkpots, 15 of 425 dives (3.0%) were in excess of 50 minutes (4 hypoxia, 3 normoxia, 8 hyperoxia). There were fewer long dives by softshell turtles (7/574, 1.2% >50 min., all in hyperoxia, $F = 5.74$, $P \leq 0.05$). Mud turtles also had fewer long duration dives than stinkpots (1/258, 0.4% >50 min., hypoxia $F = 5.59$, $P \leq 0.05$), while there was no difference between softshells and mud turtles ($F = 0.71$).

The mean duration of dives was less variable in softshell turtles than in stinkpots ($F' = 2.90$, $P \leq 0.01$), while there were no other differences between species pairs. Separate ANOVA's (stinkpot and mud turtle; softshell and mud turtle) revealed that dive duration in softshells decreased linearly with decreasing aquatic PO_2 ($F = 4.42$, $P \leq 0.05$, Figure 2). This implies that dive duration may be a function of aquatic PO_2 in softshells. Aquatic PO_2 had no effect on the duration of immersion periods in either

stinkpots or mud turtles (F = 1.92, Figure 2), although the high variability in the former may have led to a Type II error (no difference).

Interspecific differences in mean dive duration could not completely explain the observed differences in the partitioning of aerial and aquatic VO_2. Aquatic respiration was highest in spiny softshells (see Chapter 1), yet dives tended to be intermediate in duration. Stinkpots were intermediate in aquatic respiration and dives were consistently longer, while in mud turtles, the least aquatic of the three species, dives were consistently shorter (Figure 2). Despite differences in aquatic respiration, analysis of variance revealed no interspecific differences in normoxic dive durations between softshells and mud turtles (F = 1.38), or between softshells and stinkpots (F = 0.13). Unlike the other two species, dive durations in softshells were directly proportional to aquatic PO_2. As a result, in hyperoxia, stinkpots and softshells had similar dive durations (Cochran's approximation, t' = 0.71), and both were longer than mud turtle dives (F = 4.91, F = 4.98, $P \leq 0.05$, respectively). In hypoxia, on the other hand, softshells and mud turtles had dives almost equal in duration (F = 0.06), while stinkpots had dives that were longer than either softshell or mud turtle dives (t' = 2.25, F = 2.96, respectively, $P \leq 0.10$, Figure 2).

Emersion

Upon surfacing, softshell turtles typically ejected water from the external nares. This behavior was observed, but with less frequency, in stinkpots and mud turtles. In all three species, breaths always began with expiration, followed by inspiration. Breaths ranged in duration from 1.7 to 5.0 s ($x = 3.0 \pm 0.19$ s) in softshells, 0.6 to 2.0 s ($x = 1.1 \pm 0.08$ s) in stinkpots, and 1.0 to 2.8 s ($x = 1.6 \pm 0.11$ s) in mud

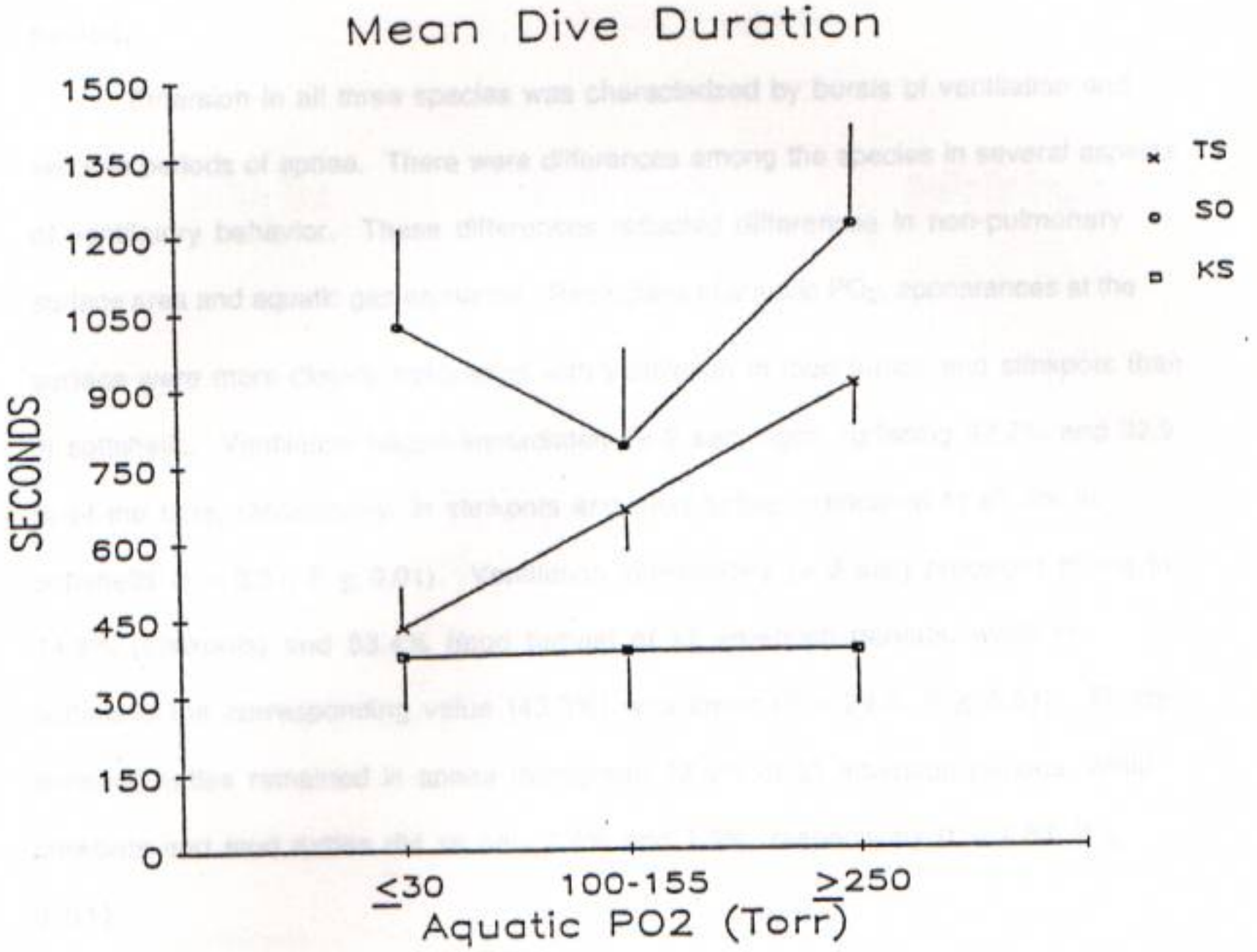

Figure 2. Mean dive durations of softshell, stinkpot, and mud turtles in hypoxia, normoxia, and hyperoxia. See text for statistical analysis.

turtles.

Emersion in all three species was characterized by bursts of ventilation and variable periods of apnea. There were differences among the species in several aspects of ventilatory behavior. These differences reflected differences in non-pulmonary surface area and aquatic gas exchange. Regardless of aquatic PO_2, appearances at the surface were more closely associated with ventilation in mud turtles and stinkpots than in softshells. Ventilation began immediately (< 2 sec) upon surfacing 92.7% and 92.9 % of the time, respectively, in stinkpots and mud turtles, compared to 67.0% in softshells ($t' = 3.91$, $P \leq 0.01$). Ventilation immediately (< 2 sec) preceded diving in 74.8% (stinkpots) and 83.4% (mud turtles) of all emersion periods, while in softshells the corresponding value (43.3%) was lower ($F = 29.8$, $P \leq 0.01$). Finally, softshell turtles remained in apnea throughout 13.6% of all emersion periods, while stinkpots and mud turtles did so only 2.6% and 1.9%, respectively ($t' = 2.84$, $P \leq 0.01$).

Other differences in ventilatory behavior helped explain why softshell turtles have a lower aerial VO_2 than stinkpots or mud turtles despite having similar normoxic dive durations. In softshells, the vast majority of emersion periods were characterized by one ventilatory cycle ($x = 1.19 \pm 0.097$). At each PO_2, softshells underwent fewer ventilatory cycles per emersion period than stinkpots (2.72 ± 0.31, $t' = 4.64$, $P \leq .01$, Figure 3) or mud turtles (8.66 ± 1.51, $t' = 4.94$, $P \leq .01$), and at each PO_2, stinkpots had lower values than mud turtles ($t' = 3.85$, $P \leq .01$). Aquatic PO_2 had no effect on this variable in softshells ($F = 1.14$) or stinkpots ($F = 0.40$), but in mud turtles, values were highest in hypoxia ($F = 6.10$, $P \leq .05$, Figure 3). In general, mud turtles spent so much time at the surface between dives that estimates of this variable are clearly biased

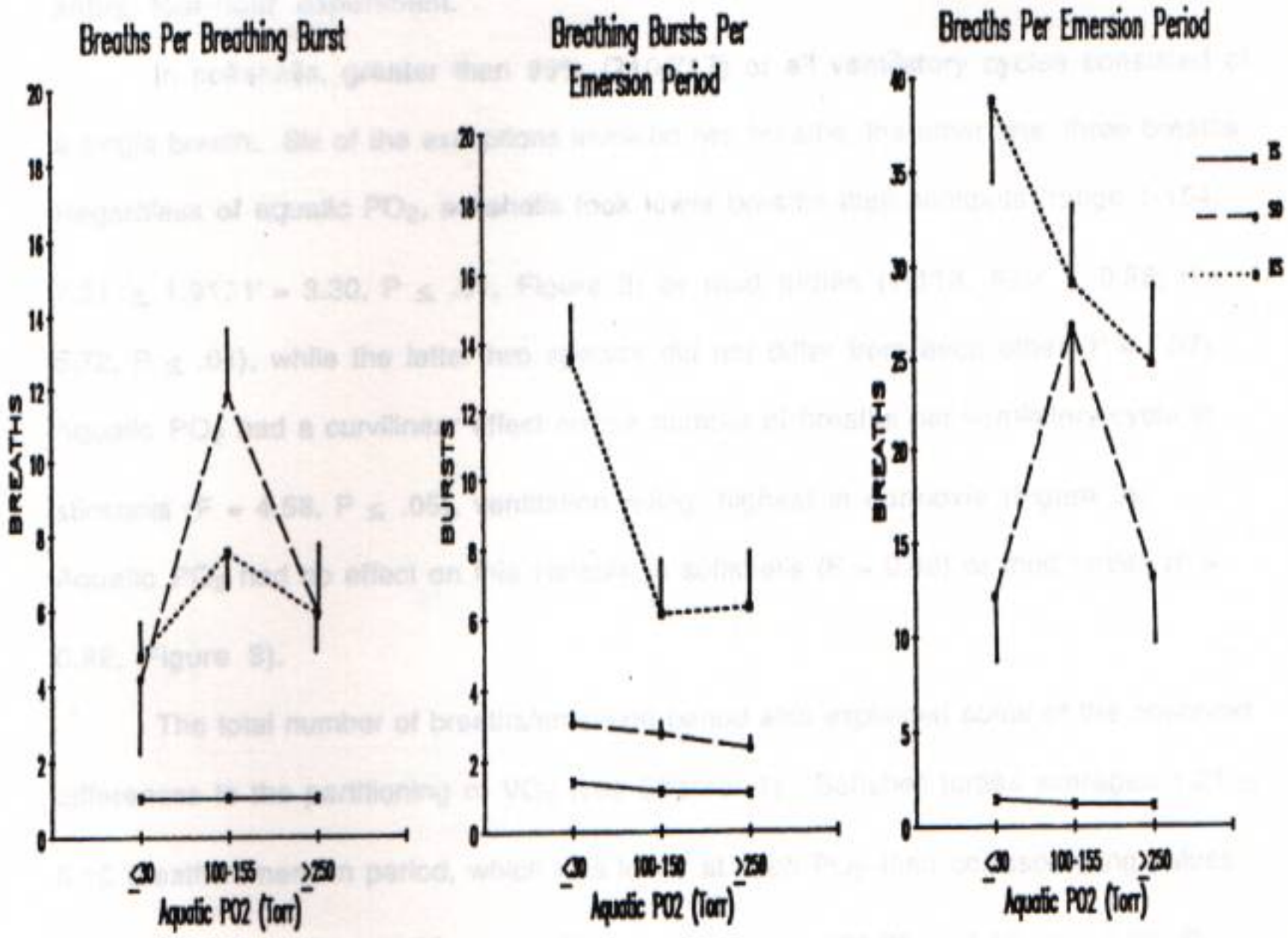

Figure 3. Ventilatory patterns of softshell, stinkpot, and mud turtles in hypoxia, normoxia, and hyperoxia. See text for statistical analysis.

downward, due to a large number of sampling periods ending in the midst of an emersion period. Four mud turtles (three in hypoxia, one in hyperoxia) never dove during an entire four-hour experiment.

In softshells, greater than 99% (710/717) of all ventilatory cycles consisted of a single breath. Six of the exceptions involved two breaths, the other one, three breaths. Regardless of aquatic PO_2, softshells took fewer breaths than stinkpots (range 1-154, 7.31 $\pm$ 1.91, $t' = 3.30$, $P \leq .01$, Figure 3) or mud turtles (1-112, 6.04 $\pm$ 0.88, $t' = 5.72$, $P \leq .01$), while the latter two species did not differ from each other ($t' = 1.37$). Aquatic PO_2 had a curvilinear effect on the number of breaths per ventilatory cycle in stinkpots ($F = 4.58$, $P \leq .05$), ventilation being highest in normoxia (Figure 3). Aquatic PO_2 had no effect on this variable in softshells ($F = 0.68$) or mud turtles ($F = 0.82$, Figure 3).

The total number of breaths/emersion period also explained some of the observed differences in the partitioning of VO_2 (see Chapter 1). Softshell turtles averaged 1.21 $\pm$ 0.10 breaths/emersion period, which was lower at each PO_2 than corresponding values for stinkpots (17.34 $\pm$ 3.38, $t' = 4.77$) or mud turtles (30.86 $\pm$ 4.18, $t' = 7.09$, $P \leq .01$). In hypoxia, stinkpots took fewer breaths/emersion period than did mud turtles ($F = 8.13$, $P \leq .01$), while there were no differences between these two species in normoxia ($F = 0.07$) or hyperoxia ($F = 1.53$). Stinkpots in normoxia took more breaths/emersion period than in hypoxia or hyperoxia ($F = 2.87$, $P \leq .10$), while aquatic PO_2 had no effect on this variable in softshells ($F = 1.17$) or mud turtles ($F = 2.43$, Figure 3).

All species underwent periodic bouts of apnea during emersion, regardless of aquatic PO_2 (Figure 4). Periods of apnea were extremely variable in all three species,

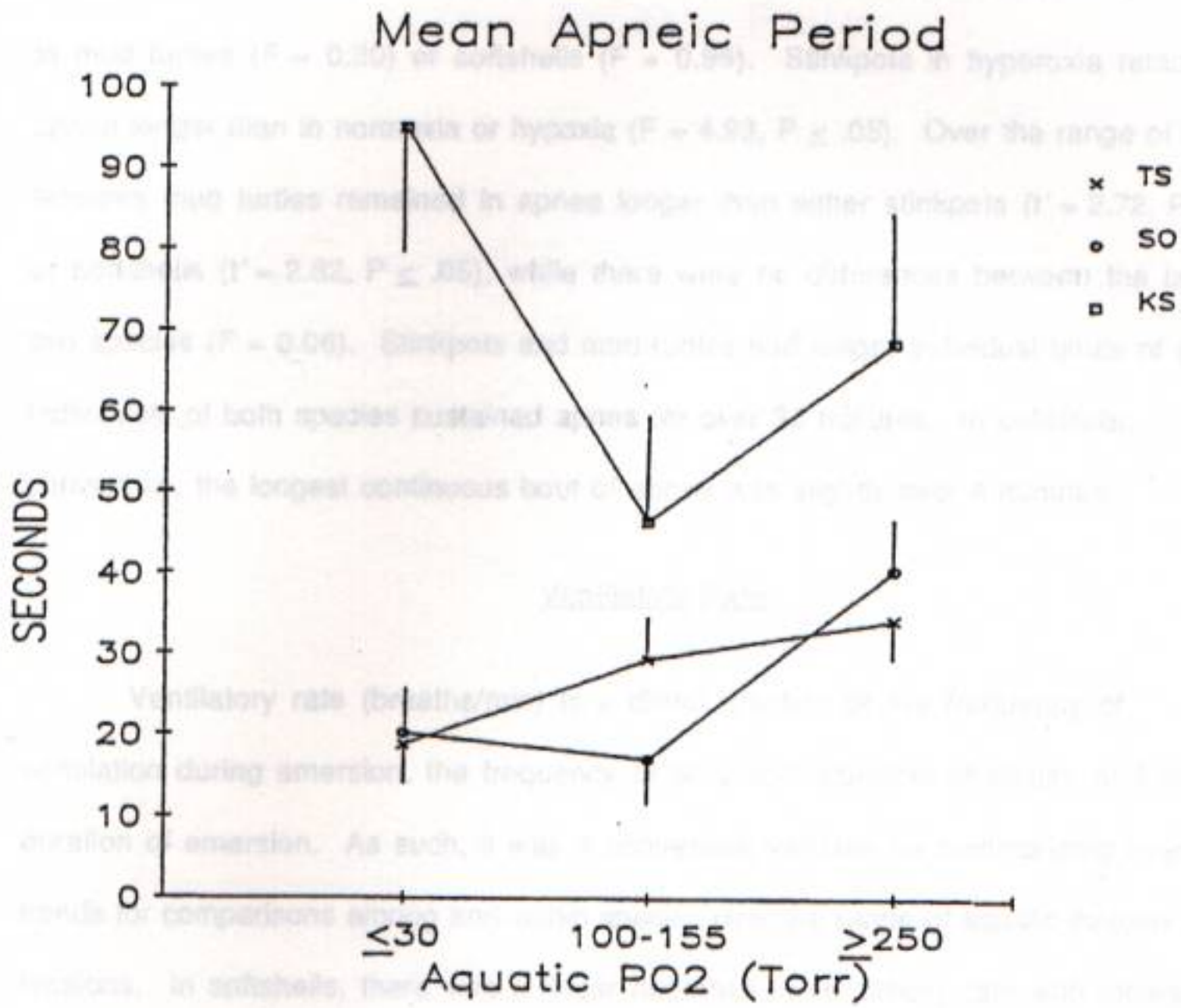

Figure 4. Mean apneic periods of softshell, stinkpot, and mud turtles in hypoxia, normoxia, and hyperoxia. See text for statistical analysis.

more so in mud turtles ($F' = 11.91$). Aquatic PO_2 had no effect on the duration of apnea in mud turtles ($F = 0.80$) or softshells ($F = 0.96$). Stinkpots in hyperoxia remained in apnea longer than in normoxia or hypoxia ($F = 4.93$, $P \leq .05$). Over the range of oxygen tensions mud turtles remained in apnea longer than either stinkpots ($t' = 2.72$, $P \leq .05$) or softshells ($t' = 2.62$, $P \leq .05$), while there were no differences between the latter two species ($F = 0.06$). Stinkpots and mud turtles had longer individual bouts of apnea; individuals of both species sustained apnea for over 30 minutes. In softshells, conversely, the longest continuous bout of apnea was slightly over 8 minutes.

Ventilatory Rate

Ventilatory rate (breaths/min) is a direct function of the frequency of ventilation during emersion, the frequency of emersion (duration of dives), and the duration of emersion. As such, it was a convenient variable for summarizing overall trends for comparisons among and within species over the range of aquatic oxygen tensions. In softshells, there was a linear decrease in ventilatory rate with increasing aquatic PO_2 ($F = 12.65$, $P \leq .01$, Figure 5), suggesting that the relative importance of aquatic respiration increases with increasing aquatic PO_2. These changes in ventilatory rate were caused by changes in dive duration (Figure 2), and not by changes in the frequency of ventilation once emersed (Figure 3). In stinkpots, the response was curvilinear; ventilatory rate was highest in normoxia and similar in hypoxia and hyperoxia ($F = 5.30$, $P \leq .05$, Figure 5). The increase was a result of increased ventilation during emersion (Figure 3) and also consistent but not significant decreases in the duration of apnea (Figure 4) and immersion (Figure 2). In mud turtles, ventilatory rate did not vary with PO_2 ($F = 0.26$, Figure 5), suggesting that, in this

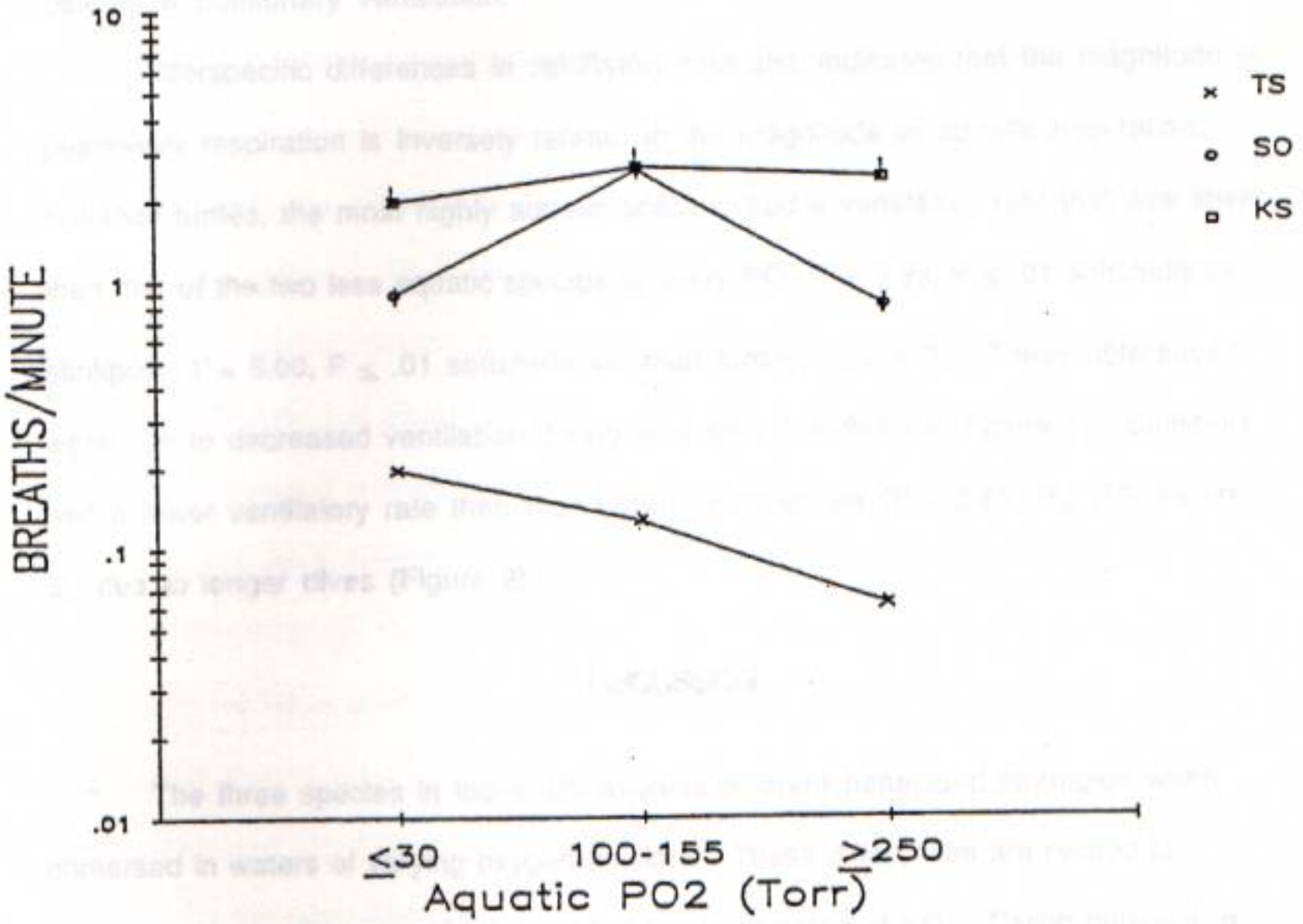

Figure 5. Mean ventilatory rate of softshell, stinkpot, and mud turtles in hypoxia, normoxia, and hyperoxia. A logarithmic scale is used on the Y-axis because of the magnitude of interspecific differences and because such a scale better illustrates intraspecific differences revealed by statistical anaylsis (see text).

species, aquatic respiration, as influenced by aquatic PO_2, is not important in the control of pulmonary ventilation.

Interspecific differences in ventilatory rate also indicated that the magnitude of pulmonary respiration is inversely related to the magnitude of aquatic respiration. Softshell turtles, the most highly aquatic species, had a ventilatory rate that was lower than that of the two less aquatic species at every PO_2 ($t' = 3.99$, $P \leq .01$ softshells vs. stinkpots; $t' = 6.00$, $P \leq .01$ softshells vs. mud turtles; Figure 5). These differences were due to decreased ventilation during emersion in softshells (Figure 3). Stinkpots had a lower ventilatory rate than mud turtles in hyperoxia ($F = 3.61$, $P \leq .10$; Figure 5), due to longer dives (Figure 2).

DISCUSSION

The three species in this study express different behavioral strategies when immersed in waters of varying oxygen tensions. These differences are related to differences in cutaneous surface area and the partitioning of VO_2. Diving behavior in spiny softshells is directly proportional to the oxygen tension of the water in which they dive; dive duration increases linearly with increasing PO_2, causing linear decreases in ventilatory rate. The stinkpot displayed a depressed ventilatory rate, relative to normoxia, at both low and high aquatic PO_2's. This was manifested through decreased breathing during emersion. Diving behavior in mud turtles appears to be independent of aquatic PO_2.

Softshells in normoxia obtain 38% of their total oxygen uptake from the water (see Chapter 1). This is significantly higher than the amount obtained by either stinkpots or mud turtles (26% and 14%, respectively). It is apparent that diving

behavior in softshells is directly affected by aquatic respiration. In hyperoxic water the need to breath air is reduced, presumably due to increased aquatic oxygen uptake, and in hypoxic water the need to breath air is increased, probably because of decreased aquatic oxygen uptake.

Stinkpots also obtain a considerable portion of their oxygen from the water (26%, see Chapter 1). Their response to hyperoxic water is similar to that of softshells, for similar reasons. In hypoxic waters stinkpots diverge from the pattern shown in softshells and display a reduced reliance on air breathing. It is possible that the cost of increased surface breathing in hypoxia exceeds the benefits in stinkpots, and therefore some other mechanism is resorted to in order to cope with aquatic hypoxia, such as depressing the metabolic rate.

The stinkpots used in this study were collected from stagnant ponds that were choked with aquatic vegetation and thus prone to nightly cycles of hypoxia. Having mechanisms that could rapidly depress metabolism would obviously be adaptive in these animals. On the other hand, the spiny softshells used in this study were obtained from a shallow, fast-flowing creek, devoid of aquatic vegetation, and therefore subject to a nearly constant PO_2 at or near air-saturation. Hypoxia is a situation these animals rarely encountered in nature.

These habitat differences are not as clear when the two species are viewed as a whole. Spiny softshells perhaps are more common in riverine habitats, but both species are common in pond habitats (Mount 1975). The two genera differ little in habitat preference, each having members that seem to favor pond habitats, members that seem to prefer riverine habitats, and members that inhabit both ponds and streams (Mount 1975). Normal diving behavior and responses to hypoxia in a stream-dwelling Sternotherus or a pond-dwelling Trionyx have not been reported. Such data would help

explain the differences observed in this study.

Mud turtles are the most dependent of the three species on aerial respiration (86% of total VO_2, see Chapter 1), and are rarely found in riverine habitats (Mount 1975). Given these facts, it is not surprising to find that their diving behavior is unaffected by alterations in the oxygen tension of the water. There has been much argument in the literature about the importance of aquatic respiration in the ecology of turtles (Root 1949; Girgis 1961; Robin et al. 1964; Belkin 1968; Gatten 1984), and whether or not it is a distinct metabolic strategy or simply a biproduct of the morphology of the animal. This study provides a simple test that qualifies the importance of aquatic respiration to a given species. If behavioral changes occur when a species is exposed to varying levels of aquatic PO_2, it seems likely that aquatic respiration is of importance to that species. Given this, we can say that aquatic respiration is important to stinkpots and spiny softshells, but perhaps not to mud turtles.

The Softshell Turtle Paradox

Assuming that the data presented above represent natural behavior and not laboratory-induced artifacts, a question arises; why do softshells surface as often as they do? Despite the metabolic and temporal costs associated with surfacing (Kramer 1988), and the potential exposure to surface predation, softshells do not take advantage of their increased aquatic gas exchange capacities by lengthening their dives. Instead, softshells breathe less while at the surface than the less aquatic stinkpots and mud turtles. Softshells take one breath per ventilatory cycle, regardless of aquatic PO_2. This phenomenon also has been reported in sea turtles (Jackson 1985) and tortoises

(Burggren 1975; Glass, Burggren, and Johansen 1978). This highly stereotyped behavior is of interest here because perhaps it limits dive duration in softshells. Softshells apparently do not have the option of increasing ventilation during emersion, a strategy which would maximize dive duration at all levels of aquatic PO_2.

The literature contains several reports that demonstrate that softshell turtles voluntarily undergo dives of extreme duration (Gage and Gage 1886; Girgis 1961; Ultsch, personal comment), especially when stressed or disturbed. Similarly, survival time during forced diving greatly exceeds survival time in less aquatic species (Ultsch et al. 1984). In this study, visual observations of softshells in the lab support the idea that softshell turtles rarely surface to breathe. However, these observations perhaps are tainted, as may be the observations cited above, by the presence of an investigator in the lab. In the absence of an investigator, videotapes reveal that softshells surface much more frequently than was previously suspected. It is possible that the reports cited above are artifactual, and reflect the inherent wariness of softshell turtles rather than their natural behavior. These long dives in the presence of an investigator perhaps are a manifestation of the behavioral and physiological changes known to occur in diverse taxa of diving vertebrates when subjected to forced or artificial submergence (Gatten1984; Zapol 1987). Softshells certainly are capable of dives of great duration; however, if left undisturbed in the lab, even in hyperoxic water, they rarely undergo them.

In nature, long dives perhaps are reserved for emergencies, foraging, or traveling. It is likely that during periods of quiescence, when softshells adopt their characteristic posture of burying themselves in the sand a few inches beneath the surface, more frequent breathing bouts are characteristic. This may explain how softshells minimize the cost of breathing; they are not far from the surface most of the time.

It has been shown that Weddell seals maximize the percentage of time spent underwater by taking relatively short, completely aerobic dives (Kooyman et al. 1980). Short dives minimize the time spent at the surface between dives, as there is no oxygen debt to repay provided that a dive is completely aerobic. Two obvious differences between Weddell seals and softshell turtles are that the latter are capable of aquatic gas exchange and have a much lower resting metabolic rate. It is not known how long softshells can remain under water without having to resort to anaerobic metabolism, but it has been estimated that loggerhead musk turtles (S. minor) can remain under water for two hours before exhausting oxygen stores (Gatten 1984). Therefore, it seems likely that softshells could take longer dives than they did in this study and still remain entirely aerobic.

What Limits Dives?

In every species of bimodal breather yet tested, aquatic VCO_2 exceeds aquatic VO_2. This phenomenon is well established in bimodal breathers (Wood and Lenfant 1976) and is due primarily to the high solubility of CO_2 in water, compared to O_2 (Graham 1990). In highly aquatic turtles, aquatic VCO_2 can exceed aerial VCO_2 (Chapter 1; Jackson et al. 1976). This is reflected in the low blood PCO_2 of the highly aquatic species discussed below. Conversely, the highest reported aquatic VO_2 is only 38% of total VO_2 (softshells, Chapter 1). It seems logical therefore, and is partially supported by the literature (see below), that highly aquatic turtles, like bimodally breathing fish, breathe air primarily to obtain oxygen. Turtles that are relatively poor at aquatic exchange, however, may be more like terrestrial vertebrates and may be more sensitive to hypercapnia than hypoxia (Gesell 1939).

It is well established in bimodally breathing fish that the stimulus for air breathing is aquatic hypoxia (Graham and Baird 1982, 1984; Smatresk and Cameron 1982a, 1982b), and that aquatic hypercapnia has little effect on aerial ventilation (Smatresk and Cameron 1982c; Graham 1983). Accessory breathing organs evolved in fish as organs of oxygen uptake, and most CO_2 is excreted across either the gills or the skin (Singh 1976; Smatresk and Cameron 1982a; Graham 1983).

In salamanders, there is evidence that the respiratory gas controlling ventilation depends on the habitat a given species occupies and its ability to exchange respiratory gases with the water. Aquatic species with a high capacity for aquatic gas exchange are less sensitive to high CO_2 levels than are more terrestrial species with reduced aquatic gas exchange capacities (Wakeman and Ultsch 1975).

The situation is less clear for other diving vertebrates. However, there is evidence that reptiles capable of high levels of aquatic gas exchange are similar to fish in that air breathing is controlled by oxygen and not carbon dioxide. The highly aquatic elephant trunk snake, Acrochordus javanicus, responds to breathing 8% CO_2 by a depression of ventilation and a nearly two-fold increase in cutaneous CO_2 excretion (Glass and Johansen 1976). Also, normal blood PCO_2 in softshells, stinkpots, painted turtles (Chrysemys picta), and common snapping turtles (Celydra serpentina) is inversely related to aquatic respiration in each species (Ultsch et al. 1984). Furthermore, during forced submergence in normoxic water, blood PCO_2 in softshells and stinkpots remained near control levels, while blood PCO_2 in the less aquatic painted and snapping turtles rose sharply (Ultsch et al. 1984). In this study, both I. spiniferus and S. odoratus respond to changes in aquatic PO_2 by altering ventilation frequency. Low

blood PCO_2's, attributed to high levels of aquatic CO_2 excretion and altered behavioral responses caused by manipulation of aquatic PO_2, suggest that it is O_2 and not CO_2 that limits dive duration in highly bimodal breathing turtles.

In reptiles that are poor at aquatic respiration, there is direct evidence indicating that CO_2 is the principal stimulus for air breathing. In C. serpentina, a comparison of blood PCO_2 and PO_2 during free diving indicates that increased arterial CO_2 is the principle stimulus for breathing in this species (Smits et al. 1987). Other evidence is largely circuitous in that it involves exposing turtles to aerial hypoxia and/or hypercapnia, conditions that are rarely, if ever, encountered in nature. Such studies more aptly describe what can be, not what is, involved in the control of ventilation. These studies have demonstrated that breathing either hypoxic or hypercapnic air can lead to increased aerial ventilation (Randall et al. 1944; Boyer 1963, 1966; Frankel et al. 1969; Lenfant et al. 1970; Jackson et al. 1974; Burggren 1975; Burggren et al. 1977; Jackson 1985; Funk and Milsom 1987; Wasser and Jackson 1988).

A final consideration involves the selection of turtles species for studies of respiratory physiology. Historically, broad generalizations have been made about turtle respiration based on observations involving a single family of turtles, the Emydidae. Painted turtles (Chrysemys) and various species of Pseudemys are easily obtainable from commercial sources and abundant throughout the United States. They display remarkable respiratory physiology that certainly is worthy of close scrutiny. However, members of this family are poor at aquatic respiration. It is clear that respiratory physiology and accompanying behavior have evolved differently in different groups of

turtles, and the differences appear to be related to aquatic respiration. The literature involving respiratory physiology in most bimodally breathing turtles is scant compared to the literature on emydid turtles. Furthermore, comparative studies involving several species of aquatic turtles also are rare. Such studies are needed in order to obtain a better understanding of the evolution of bimodal breathing in freshwater turtles.

SUMMARY

This is the first study to provide simultaneous measurements of aerial and aquatic oxygen uptake and carbon dioxide excretion in freely diving, bimodally breathing turtles. These measurements show that aquatic respiration is of considerable magnitude in all three species examined, particularly in terms of aquatic carbon dioxide excretion.

This is also the first study to relate cutaneous surface area to the partitioning of respiratory gas exchange. It is clear from these data that aquatic gas exchange increases with increasing cutaneous surface area.

This study has helped resolve the question of whether aquatic respiration is simply a byproduct of the morphology in turtles, or whether it is a distinct metabolic strategy of importance to the turtles that employ it, but providing a simple test involving the manipulation of aquatic PO_2 and subsequent monitoring of behavior. This test reveals marked behavioral changes in softshells and stinkpots associated with changes in aquatic PO_2, demonstrating PO_2 sensitivity, coupled with the regulation of lung ventilation in these species. In contrast, mud turtles do not alter lung ventilation when aquatic PO_2 changes, suggesting a lack of sensitivity to and perhaps a lack of importance of aquatic respiration in this species.

This study provides detailed diving and ventilatory behavioral data for stinkpots, softshells, and mud turtles. These data reveal that all three species typically undergo relatively short dives, and that differences in aquatic $\dot{V}O_2$ among the species are behaviorally displayed via differences in lung ventilation once surfaced, not through

differences in the length of dives. Softshell turtles, a group commonly suspected of naturally undertaking dives of tremendous duration, in fact typically undergo much shorter dives than was previously suspected. Softshells also are similar to tortoises and sea turtles in that they take a single breath per breathing period, and as such are the first freshwater turtles to display such a pattern, departing from the popular freshwater mode of several breaths in succession followed by apneic periods.

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