

## **Air breathing and gill ventilation frequencies in juvenile tarpon, *Megalops atlanticus*: responses to changes in dissolved oxygen, temperature, hydrogen sulfide, and pH**

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### **Synopsis**

This study quantified the air-breathing frequency (ABf in breaths h<sup>-1</sup>) and gill ventilation frequency (Vf in ventilations min<sup>-1</sup>) of tarpon *Megalops atlanticus* as a function of PO<sub>2</sub>, temperature, pH, and sulphide concentration. Ten tarpon held at normoxia at 22–33°C without access to atmospheric oxygen survived for eight days, and seven survived for 14 days (at which point the experiment was terminated) suggesting that the species is a facultative, rather than an obligate, air breather. At temperatures of 29°C and below ABf was highest and Vf was lowest at low oxygen partial pressures. Tarpon appear to switch from aquatic respiration to air breathing at PO<sub>2</sub> levels of roughly 40 torr. The gills were the primary organ for oxygen uptake in normoxia, and the air-breathing organ the primary mechanism for oxygen uptake in hypoxia. At 33°C, both ABf and Vf were elevated but highly variable, regardless of PO<sub>2</sub>. There were no mortalities in tarpon exposed to total H<sub>2</sub>S concentrations of 0–232 μM (0–150.9 μM H<sub>2</sub>S); however, high sulfide concentrations resulted in very high ABf and Vf near zero. Vf was reduced when pH was acidic. We conclude that air breathing provides an effective means of coping with the environmental conditions that characterize the eutrophic ponds and sloughs that juvenile tarpon typically inhabit.

### **Introduction**

Biotic and abiotic processes can reduce oxygen partial pressure (PO<sub>2</sub>) to low levels in aquatic environments with restricted circulation. However, approximately 374 fish species, less than 2% of all known fishes, have developed the ability to use atmospheric oxygen providing independence from fluctuations in dissolved oxygen availability (Graham 1997). These air-breathing fishes divide oxygen uptake between an accessory respiratory organ and the gills,

with respiratory allocation reflecting differences in their physiology, anatomy, habitat, and life history (Bicudo & Johansen 1979, Kramer & McClure 1980, McKenzie et al. 1991). Accessory respiratory organs include the mouth, buccal cavity, digestive tract, or gas bladder (Johansen 1966, Graham 1994). Accessory respiratory organs are most common in freshwater habitats. In marine habitats, the most common means of gaining atmospheric oxygen is through emergence, which may be forced upon some species by receding tides (Martin 1993). Intertidal species usually

obtain atmospheric oxygen by using a combination of cutaneous respiration and oxygen uptake at the gills during emergence (Gibson 1969).

Tarpon are large-bodied elopomorph fish that support economically important recreational fisheries in Florida and parts of the Caribbean. Spawning is thought to occur offshore (Crabtree et al. 1992, Crabtree 1995), with metamorphic larvae recruiting to estuarine areas after 2–3 months (Tucker & Hodson 1976, Smith 1980). Juvenile tarpon usually occur in small, stagnant pools and sloughs that have a wide range of temperature (12–36°C), salinity (5–40 ppt), pH (5.7–8.8), and oxygen concentration (anoxic to supersaturated) (Wade 1962, Rickards 1968, Chacón-Chaverri & McLarney 1992, J. David personal communication). Additionally, in extremely hypoxic water, accumulations of hydrogen sulfide are common (Luther et al. 1986, Abel et al. 1987). Their juvenile sojourn in stagnant water may explain why the tarpon, *Megalops atlanticus*, and the ox-eye tarpon, *M. cyprinoides*, are the only nektonic marine fishes that are capable of bimodal respiration (Graham 1997), and are the only marine fish that use respiratory gas bladders (Bone et al. 1995). The gas bladder has four rows of highly-vascularized tissue that enable tarpon to survive in water with low PO<sub>2</sub> (Babcock 1936, Liem 1989). Air breathing may also be associated with reduced pH and are exacerbated by warm temperatures.

Schlaifer & Breder (1940) and Schlaifer (1941) initially described the effects of different temperatures on air breathing in tarpon and concluded that tarpon were obligate air breathers, even though in some of the trials tarpon survived forced submergence for almost one week. In more recent works, fishes which could survive one (Graham et al. 1977) to two weeks (Magid 1966, Jordan 1976) of forced submergence have been considered facultative air breathers. Our study re-examines bimodal respiration in juvenile tarpon and provides information on the ventilation frequencies of the gills (aquatic respiration) and the gas bladder (air breathing) in relation to aquatic PO<sub>2</sub>, temperature, pH, and sulfide concentration.

## Materials and methods

### *Collection and maintenance of specimens*

Seventy-six juvenile tarpon (total length, TL, range 165–285 mm, mean TL = 218 mm; 50–332 g) were collected from three locations in South Florida during

the fall and winter of 1989, 1990, and 1991. Tarpon were maintained in a recirculating seawater system (salinity = 22 ppt, temperature range 19–33°C) and fed chopped herring, squid, or shrimp to satiation once weekly. A minimum of six weeks of acclimation to laboratory conditions preceded all experiments. An additional six-week acclimation to each experimental temperature preceded studies of air-breathing frequency (ABf) and gill ventilation frequency (Vf). Tarpon were moved from maintenance tanks to experimental chambers 24 h before observations were begun. For each trial, new fish were chosen haphazardly (but placed randomly in chambers for ABf and Vf experiments), and never used in two successive trials. Oxygen partial pressures were recorded with an array of 10 Clark polarographic electrodes (Clark et al. 1956) connected to a Hewlett-Packard data acquisition system (Torres & Somero 1988) at a sampling rate of once min<sup>-1</sup>. The electrodes were calibrated in air and nitrogen saturated water. Hundreds of hours of observation of tarpon held in large aquaria (2400 l), including still photography, were conducted to document general patterns of air-breathing behavior.

### *Obligatory air breathing*

Forced submergence tests were carried out on 10 fish to determine whether or not air breathing is obligatory. Experiments two weeks in duration were conducted in a 41.7-l annular respirometer that was connected to flowing water to ensure that aquatic oxygen levels remained near saturation. A small air space was provided for the fish during their first 24 h of acclimation to the chamber but was removed at the onset of the experimental period. Water temperatures ranged from 22°C to 26°C in seven tests and between 29°C and 33°C in three tests (ambient conditions in the laboratory were manipulated for other experiments). Fish were checked daily for signs of stress such as erratic swimming behavior or obvious physical injuries.

### *Air-breathing and gill ventilation frequency*

The air-breathing frequency (ABf in breaths h<sup>-1</sup>) of tarpon was observed at five discrete temperatures (19, 22, 26, 29, and 33°C) at PO<sub>2</sub>s ranging continuously from 1 to 159 torr (0.5–100% air saturation). Ten tarpon were placed in 40-l aquaria, one fish per aquarium, 24 h before each ABf trial. Each aquarium was flushed with flowing water that had been partially stripped of oxygen by saturation with nitrogen gas.

The supply of nitrogen was increased at the onset of each observation period so that  $\text{PO}_2$  varied throughout each trial, decreasing at rates of zero to  $30 \text{ torr h}^{-1}$ . The  $\text{PO}_2$  in the 10 aquaria was further manipulated using a manifold that randomly altered the level of aeration, such that the  $\text{PO}_2$  in each aquarium was independent of the others. Thus, the  $\text{PO}_2$  of each of the 10 aquaria was independent, and some were more variable than others within each trial.

Seven trials (10 fish each) totaling 18 h of observation were used at each temperature. Observation periods of 1, 1.5, 2 (2 repetitions at 2 h), 2.5, 3, and 6 h were utilized at each temperature. The variable time intervals were used to study the affect of the duration of the observation period on the observed ABf. An observer recorded the time that each air breath was taken to the nearest 0.1 min. The timing and frequency of air breaths were later compared with the continuous record of oxygen partial pressures recorded by the data acquisition system described above. At the conclusion of most trials, the gill ventilation frequency (Vf in ventilations  $\text{min}^{-1}$ ) of each tarpon was observed for 1 min. We also included data from several trials that were specifically conducted to increase the sample size of Vf observations but were not included in analysis of ABf. Tarpon used in the Vf-only trials were subjected to both the standard 24 h acclimation period and 1 h of observation before recording Vf.

ABf and Vf data were analyzed using a general linear model with a main effect (temperature) and one random effect ( $\text{PO}_2$ ), which was nested within temperature. When significant differences were detected between temperatures, Tukey's honestly significant difference test for unequal sample sizes was used as a post-hoc analysis to identify where those differences existed (Spjotvoll & Stoline 1973). Because observation period and variability among individuals within any given experiment did not significantly affect any result, all data were pooled for further analysis (see Geiger 1993). An interaction term ( $T * \text{PO}_2$ ) was also included as a second fixed effect. For ABf, each data point consisted of a mean ABf of one individual during a trial. In experiments where  $\text{PO}_2$  in an individual fish's aquarium varied by more than roughly 20% of saturation (dissolved oxygen concentration = 1 ppm, roughly 30 torr), two or more mean ABf's were calculated, based on all observations within 1-ppm increments. Thus, for each experiment, an individual fish might have one to six observations of ABf but only one observation of Vf.

During analysis, we noted that complex changes in both ABf and Vf occurred, particularly with respect to

$\text{PO}_2$ . We attempted to model the changes using non-linear regression analysis, but found that the predictive ability was generally low. As an alternative, we used breakpoint analysis followed by linear regression analysis for that portion of the data where changes were greatest: at low  $\text{PO}_2$ . Breakpoint analysis examines two way relations for discontinuities and can discern the value of the independent variable at which the discontinuity occurs. Regression equations for both ABf and Vf were calculated for all observations below the breakpoint  $\text{PO}_2$  at each temperature. Above the breakpoint, the rates of both Vf and ABf were independent of  $\text{PO}_2$ .

#### *Reactions to sulfide and pH*

We conducted five trials examining the relation between Vf and exposure to sulfide. Sulfide concentrations ranged from 0–232.1  $\mu\text{M}$  total sulfides (0–150.9  $\mu\text{M}$   $\text{H}_2\text{S}$ ). Nine tarpon were exposed to step-wise increases in sulfide. One or two fish per trial were used (one fish per aquaria, three to 12 one minute counts of Vf per fish). Five control fish not exposed to sulfide, one per trial, were also observed (repeated observations of each control fish were averaged to create a single data point). Total sulfides in the control aquaria never exceeded 5  $\mu\text{M}$ . A total of 68 observations of experimental fish were combined with the five control fish, resulting in 73 total counts of Vf for regression analysis. Results were based on total sulfides.

Sulfide concentrations (0–250  $\mu\text{M}$ ) were determined following the colorimetric method of Cline (1969). The change in Vf of tarpon exposed to hydrogen sulfide was tested following Bagarinao & Vetter (1989). Sodium sulfide stock (approximately 10 mM) in 15- to 20-ml aliquots was added to an aerated, 40-l sea-water aquarium containing one tarpon. Sodium sulfide rapidly dissociates and the hydrogen sulfide equilibrates as  $\text{HS}^-$  and  $\text{H}_2\text{S}$ , with a pK of 7.0 (Millero 1986). In each sulfide exposure experiment, pH was measured (range 6.8–7.8) and the ratio between toxic and non-toxic sulfide was determined. The addition of sodium sulfide caused pH to increase. After the addition of each aliquot, the pH was adjusted towards pH 7.5 by the addition of a single aliquot of either HCl or NaOH. In each trial, an aquarium that held one tarpon but no added sulfide, acid, or base was used as a control. The water in the control aquarium was stirred after each experimental manipulation of the tank holding the experimental fish to eliminate the potential bias of only stirring the water in the experimental aquaria. Approximately 10 min after adding the sodium sulfide

aliquots, each tarpon was observed for 1 min and Vf was determined. A water sample was taken to measure sulfide concentration, and the next aliquot of sulfide stock was added. This procedure was repeated until total sulfide concentration in the aquaria reached 100–250  $\mu\text{M}$ . The ABf of three fish exposed to sulfide in excess of 100  $\mu\text{M}$  were observed for 30 min. Because the reaction to sulfide was immediate in each trial, we felt that long-term exposure to make statistically valid observations was not warranted.

Oxygen partial pressure was measured for 1 min immediately after counts of Vf were made during hydrogen sulfide trials. The addition of low concentrations of sulfide ( $< 40 \mu\text{M}$ ) did not result in reductions in  $\text{PO}_2$ , but higher-than-normal voltage output from the oxygen electrode was recorded at  $\text{HS}^-$  concentrations above 40  $\mu\text{M}$ . We could not accurately measure  $\text{PO}_2$  at high sulfide levels, but we assumed that the aeration provided by an airstone was sufficient to maintain normoxia ( $\text{PO}_2 > 100$  torr).

The effects of the addition of HCl and NaOH upon Vf were also studied in six trials. The pH (4.1–8.9) was experimentally manipulated using the same methods as in the sulfide addition experiments with some exceptions. Acid or base was sequentially added to experimental aquaria, after which both experimental and control aquaria were stirred, and 10–15 min later Vf was counted. One control and one to four experimental fish per trial were used. Ninety-two counts of Vf in nine tarpon exposed to manipulated pH were conducted (seven to 12 observations per fish). In two of five control aquaria, pH varied slightly ( $\Delta\text{pH}$  was 0.02–0.19 pH units). Thus, in three cases, all counts of Vf were averaged, while in two cases, un-averaged data were used, resulting in eight control observations from five fish, and a total of 100 observations for the pH regression analysis.

## Results

Air-breathing tarpon approach the surface at an angle of about 30 degrees. An air bubble is expelled from beneath each operculum as the fish approaches the surface, suggesting that the gas bladder is emptied during ascent. When the mouth breaks the surface, the buccal and opercular cavities are greatly expanded, accompanied by an abduction of the operculi and gular plate. Tarpon then most often turn to one side and descend away from the surface at an angle of about 30 degrees.

As the fish descends from the surface the buccal and opercular cavities are compressed, presumably forcing most of the inspired air into the gas bladder, but often a small bubble is expired from beneath the most dorsal end of each operculum. An important conclusion resulting from these observations is that tarpon exhale air from their gas bladder before inhalation of the following breath. If denied access to the surface after exhalation, tarpon will be unable to maintain neutral buoyancy.

### *Forced submergence*

Seven of 10 tarpon survived forced submergence until experiments were terminated after 14 days; all tarpon survived at least 8 days. The three fish that died survived 8, 9, and 10 days at 22–26°C. At the termination of the 10 trials, all tarpon were negatively buoyant, which suggests that their gas bladders were at least partially deflated. We dissected the three fish that died prior to the end of the two-week trial and examined their gas bladders; no air was detectable. All 10 tarpon had abrasions on the anterior-most portion of the lower jaw, presumably from attempting to gulp air, and numerous abrasions (including torn fins) on the ventral surface of the body.

### *Air-breathing and gill ventilation frequency*

Temperature ( $F = 1116.5$ ,  $p < 0.001$ ),  $\text{PO}_2$  ( $F = 1573.1$ ,  $p < 0.001$ ), and an interaction term ( $\text{PO}_2 * T$ ) ( $F = 96.6$ ,  $p < 0.001$ ) all affected ABf (Figure 1). There was a rise in ABf at low  $\text{PO}_2$  at temperatures of 26°C and below; ABf was highest at  $\text{PO}_2$ s below about 40 torr. In experiments at 29–33°C, ABf was highly variable. At 33°C, when  $\text{PO}_2$  was below 40 torr, ABf was high.

ABf also varied with temperature within  $\text{PO}_2$  intervals. In normoxic water ( $> 80$  torr), ABf was highest at 33°C, lower at 29°C, and lowest with little variability at temperatures of 19–26°C (Table 1). When  $\text{PO}_2$  was between 40 and 80 torr, ABf increased gradually with increasing temperature. When  $\text{PO}_2$  was below 40 torr, variation of ABf was observed between temperatures, but no consistent trend was apparent.

Breakpoint and regression analyses showed that at 19 and 22°C ABf increased as  $\text{PO}_2$  decreased from about 80 to 0 torr, with  $\text{PO}_2$  explaining 21 and 24%, respectively, of the variability in ABf (Table 1). ABf was independent of  $\text{PO}_2$  at higher levels of dissolved

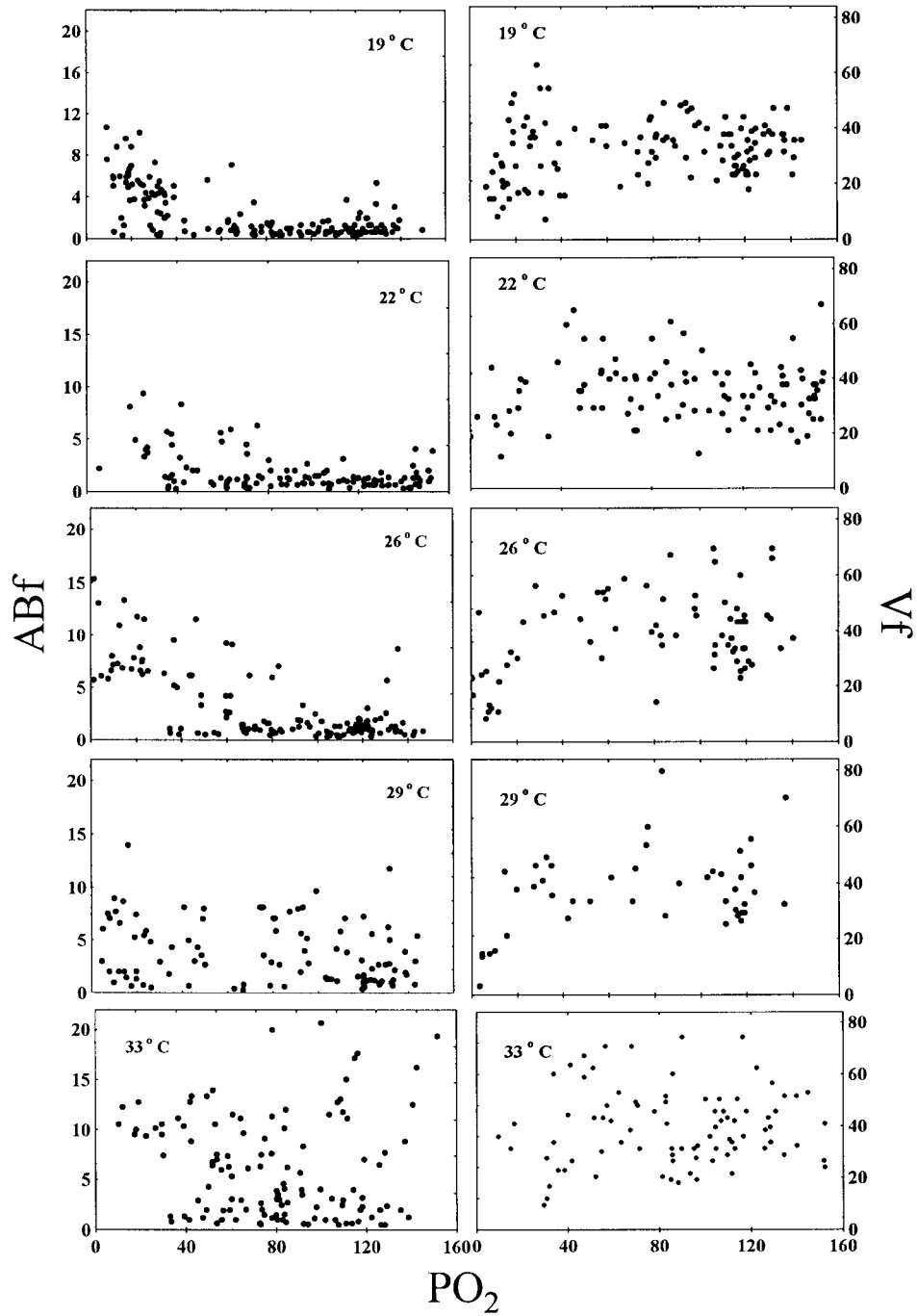


Figure 1. Scatterplots of air-breathing frequency (ABf in breaths h<sup>-1</sup>; left hand figures) and gill ventilation frequency (Vf in ventilations min<sup>-1</sup>; right hand figures) in relation to oxygen partial pressure (PO<sub>2</sub>) in torr. The temperature °C is indicated in each figure.

Table 1. Linear regressions for ABf (in breaths h<sup>-1</sup>) (top line of each pair) and Vf (in ventilations min<sup>-1</sup>) (2nd line of each pair) in tarpon based on breakpoint analysis. The regressions were calculated for all points between PO<sub>2</sub> = 0 and the breakpoint, above which the rates were independent of PO<sub>2</sub> (n = total sample size; n' = sample size for regression; p = significance level of the regression model; mean = mean for ABf and Vf at PO<sub>2</sub> = greater than breakpoint; ± sd, standard deviation).

T°C	n	Breakpoint	n'	r <sup>2</sup>	p	Equation	Mean	± sd
19	157	45	55	0.21	< 0.001	ABf = 7.73-0.13 * PO <sub>2</sub>	1.4	1.36
	124	37	36	0.19	< 0.007	Vf = 12 + 0.78 * PO <sub>2</sub>	31.5	8.17
22	121	94	94	0.24	< 0.001	ABf = 4.12-0.028 * PO <sub>2</sub>	1.4	0.98
	99	46	19	0.44	< 0.001	Vf = 18.2 + 0.61 * PO <sub>2</sub>	34.1	9.97
26	140	—	140	0.47	< 0.001	ABf = 7.52-0.55 * PO <sub>2</sub>	1.4 <sup>a</sup>	1.34
	73	40	19	0.55	< 0.001	Vf = 12.1 + 0.80 * PO <sub>2</sub>	35.4	10.36
29	101	—	101	0.07	0.009	ABf = 5.06 - 0.017 * PO <sub>2</sub>	3.4 <sup>a</sup>	3.11
	44	35	16	0.72	< 0.001	Vf = 7.6 + 1.24 * PO <sub>2</sub>	37.3	13.2
33	125	—	—	—	—	-ns-	6.7 <sup>b</sup>	6.34
	85	70	30	0.18	0.02	Vf = 27.4 + 0.37 * PO <sub>2</sub>	42.2	11.00

<sup>a</sup>Mean calculated for PO<sub>2</sub> > 110.

<sup>b</sup>Mean calculated for entire PO<sub>2</sub> range.

oxygen. In 26 and 29°C trials, ABf increased gradually as PO<sub>2</sub> declined over the entire range of PO<sub>2</sub>. However, at 29°C the r<sup>2</sup> value was so low, 0.07, that ABf was essentially independent of PO<sub>2</sub>. In 33°C water, ABf was independent of PO<sub>2</sub> over the entire range.

Oxygen partial pressure (F = 16.9, p < 0.001) and temperature (F = 16.7, p < 0.001) both had significant effects on Vf (Table 1), but the interaction term (PO<sub>2</sub> \* T) was not significant (F = 1.07, p = 0.435). Breakpoint analysis showed that at PO<sub>2</sub>s below 32–40 torr, Vf was directly related to PO<sub>2</sub> (Figure 1). Regression analysis showed that PO<sub>2</sub> explained 18–72% of the variability in Vf in hypoxic water. At PO<sub>2</sub>s above 40 torr, Vf showed little change with PO<sub>2</sub>. In normoxic water, the mean Vf increased from 31.5 min<sup>-1</sup> at 19°C to 42.2 min<sup>-1</sup> at 33°C. A similar increase was noticed in the intercepts (PO<sub>2</sub> = 0) of the regression equations although the pattern was less clear; 33°C was higher than 26°C (p = 0.041), but other comparisons were not significant (p = 0.239–0.998). Post-hoc analysis indicated that Vf was higher at 33°C than at 19–26°C (p < 0.001).

#### Reactions to sulfide and pH

The frequency of gill ventilation decreased as total sulfide concentration increased, and approached zero at very high sulfide levels (r<sup>2</sup> = 0.68, p < 0.001, Figure 2). Typical gill ventilation was replaced by coughs, or flow reversals across the gills, at high sulfide

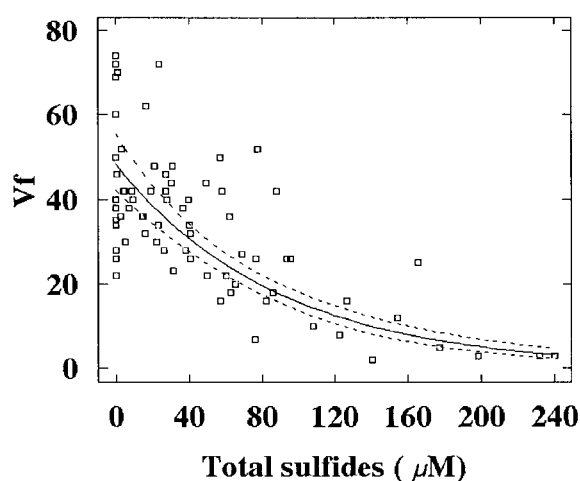


Figure 2. The relationship between gill ventilation frequencies (Vf) in ventilations min<sup>-1</sup> and total sulfide concentration (μM H<sub>2</sub>S) at 25°C. The regression equation was:  $Vf = e^{3.877 - 0.011X}$  where X = μM H<sub>2</sub>S. The solid line represents the regression model, and the dashed lines represent 95% confidence intervals (n = 73, r<sup>2</sup> = 0.677, p < 0.001).

concentrations. During our study, no tarpon died as a result of exposure to sulfide. In our limited observations of ABf at very high sulfide concentrations (> 230 μM), ABf was as high as 60 h<sup>-1</sup>, a higher frequency than observed for tarpon in nearly anoxic water containing no sulfide.

There was a weak positive relationship between pH and Vf (r<sup>2</sup> = 0.217, p < 0.001, Figure 3) over the range

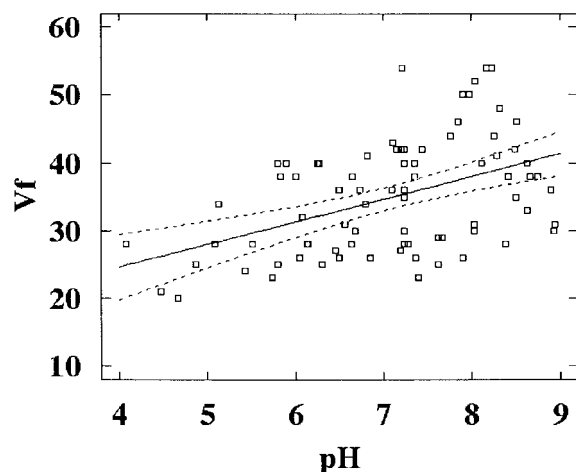


Figure 3. The relationship between gill ventilation frequencies ( $V_f$ ) in ventilations  $\text{min}^{-1}$  and pH at 25°C. The regression equation was:  $V_f = e^{3.877 - 0.011\text{pH}}$ . The solid line represents the regression model, and the dashed lines represent 95% confidence intervals ( $n = 100$ ,  $r^2 = 0.217$ ,  $p < 0.001$ ).

4.25–8.75. Gill ventilation frequency ranged between 20 and 30  $\text{min}^{-1}$  at pHs between 4 and 5. At pHs between 7 and 8,  $V_f$  was higher and more variable, ranging from 22 to 54  $\text{min}^{-1}$ . No upper or lower lethal pHs were observed.

## Discussion

Juvenile tarpon were continuous facultative air breathers over the range of temperatures and oxygen partial pressures we studied, in accordance with Gee & Graham's (1976) definition, rather than obligate air breathers as suggested by Schlaifer (1941). Ten tarpon survived in normoxic water for periods exceeding one week without access to atmospheric oxygen, and seven survived for the duration of a two week experiment. Oxygen for routine metabolism was obtained solely by aquatic respiration.

Air breathing by tarpon in normoxic water is probably related to the need for buoyancy, as in the South American catfish *Corydoras aeneus* (Kramer & McClure 1980). Tarpon expel gas from their gas bladder as they approach the surface. Thus, when a tarpon empties the gas from its gas bladder and then cannot reach the surface, it cannot reinflate the gas bladder. When denied access to the surface, tarpon became negatively buoyant in one to two days. Our observations

indicate that loss of buoyancy when fish were denied access to air was due to deflation of the gas bladder.

In air-breathing fishes oxygen uptake is partitioned between two systems, presumably utilizing the most energy efficient means available. In most species studied, ventilation of the gills increases as  $\text{PO}_2$  falls, but peaks between 40 and 80 torr. At oxygen tensions below this level, gill ventilation frequency and the percentage of oxygen obtained from water drops, often dramatically (Graham et al. 1978, Stevens & Holeton, 1978, Johansen et al. 1970). In gar, *Lepisosteus osseus*, a similar behavior appears to occur through reduction of ventilation volume without changes in  $V_f$  (Smatresk 1986). In all of these cases, the fishes simply switch to air breathing, which would be favored energetically. Non-air-breathing fish increase  $V_f$  (or volume) dramatically at low  $\text{PO}_2$ , because they have no alternative. This relationship was shown for a pair of air-breathing and non-air-breathing characoid fishes (Graham et al. 1978).

The interaction between temperature and  $\text{PO}_2$  on ABf in tarpon was most significant at low  $\text{PO}_2$ s and at high temperatures. Normally, physiological processes in ectotherms approximately double for every 10°C rise in temperature (Withers 1992) resulting in a  $Q_{10}$  of about two. For example, Horn & Riggs (1973) found that bowfin ABf doubled every 10°C. The overall mean ABf in our study increased from 1.4  $\text{h}^{-1}$  at 19°C to 6.7  $\text{h}^{-1}$  at 33°C: a  $Q_{10}$  of 3.1. However, if the total temperature range is examined interval by interval, a threshold effect is observed. No change was observed in ABf from 19°C to 26°C (ABf = 1.4  $\text{h}^{-1}$  over the range, Table 1), but the change in ABf between 26°C and 33°C was dramatic (1.4 to 6.7); a calculated  $Q_{10}$  of 9.4.

For comparative purposes, if  $V_f$  is examined as a function of temperature in normoxic water, little change is observed over the entire range of the study.  $V_f$  at 19°C (30.6  $\text{min}^{-1}$ ) and that at 33°C (42.9  $\text{min}^{-1}$ ) showed a  $Q_{10}$  of 1.27 for the range. Thus,  $V_f$  remains nearly constant with temperature, while ABf shows a step function above 26°C. The data imply that air breathing supplements gill ventilation at temperatures above 26°C. An alternate explanation is that stroke volume increases with temperature resulting in an increase in ventilation volume despite the absence of a change in ventilation frequency, as is observed in trout during exposure to hypoxia (cf. Kinkead & Perry 1990). Measurements of ventilation volume were precluded by our experimental design but obvious signs of distress, such as the gasping behavior noticeable in most non-air-breathing fishes facing hypoxia, were not detectable.

It is possible that both mechanisms contribute to the increased demand for O<sub>2</sub> at higher temperatures. However, based on our data and data on other air-breathing fishes, we hypothesize that in hypoxic waters, air breathing is the primary source of oxygen, while in normoxic water, the gills supply most of the oxygen at low temperatures, but in warmer waters air breathing becomes increasingly important.

Air-breathing frequency of the knifefish, *Gymnotus carapo*, increased from 5–10 h<sup>-1</sup> at 23°C to 40–90 h<sup>-1</sup> at 29–33°C, which yields a Q<sub>10</sub> of between eight and nine (Liem et al. 1984). The pattern of recruiting the air-breathing organ in warmer waters to help deal with elevated metabolism and lower ambient O<sub>2</sub> may be common among air-breathing fishes. Bowfin have a threshold where they switch from aquatic respirers to air breathers which is at 40 torr at 10°C, but at 90 torr at 30°C (Johansen et al. 1970). In tarpon, the threshold was not as distinct, but remained near 40 torr at all temperatures. The present study, along with those just described, suggest a strong interaction between temperature and PO<sub>2</sub> in governing the air-breathing response.

Tarpon decreased their gill ventilation rates in response to increased sulfide and decreased pH. Presumably, this was an avoidance behavior. Sulfide inhibits respiration by disrupting the function of both hemoglobin and cytochrome c oxidase. The toxic effects of sulfide may be a more immediate danger than hypoxia in habitats where both occur. Sulfide kills some organisms at concentrations near 1 μM HS<sup>-1</sup> (Hochachka & Somero 1984) whereas tarpon survived exposure to greater than 250 μM. *Rivulus marmoratus* will leap from the water and begin to respire cutaneously when H<sub>2</sub>S reaches 123 μM, but will not react to moderate hypoxia (1–2 ppm) (Abel et al. 1987). In a study of estuarine fishes by Bagarinao & Vetter (1989), only 2 of 11 fish species could survive exposure to 1.5 mM sulfide, one of which was *Gillichthys mirabilis*, a species capable of air breathing (Todd & Ebeling 1966).

The ability of tarpon to withstand a combination of harsh environmental conditions probably allows them to take advantage of the mangrove swamp during their critical juvenile period. Wade (1962) and Harrington (1958) reported the habitat of juvenile tarpon to be stagnant pools in mangrove-lined estuaries as well as *Spartina* marshes. These habitats contain abundant prey such as poeciliids, cyprinodontids, and juvenile mullet (Lewis et al. 1983), species known to be tolerant of both hypoxia and sulfide exposure (Bagarinao & Vetter 1989), or efficient at using the highly oxygenated surface layer to obtain oxygen (Kramer 1983).

Although at least 45 species of fishes may be captured in Florida's salt-marsh habitat (Durako et al. 1983), Lewis et al. (1983) collected only 14 fish species, including tarpon, from the upper mangrove swamp. Thus, prey species that are tolerant of low PO<sub>2</sub> and high sulfide concentrations can use the high marsh habitat as a type of refuge from most predators. Tarpon can live in hypoxic situations because they can supplement aquatic respiration with air breathing at PO<sub>2</sub>s below 50 torr and at PO<sub>2</sub>s below 10 torr, use the gas bladder as the principal respiratory organ. Air breathing also becomes increasingly important for tarpon when either temperature or hydrogen sulfide concentration increases. Predators such as tarpon that can deal with the metabolic hazards of hypoxia, elevated temperature, and elevated sulfides are afforded a rich supply of prey that allows them to grow rapidly and possibly minimize their own susceptibility to predation.

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