

Effects of hypoxia on oxygen uptake and surfacing behavior in the giant aquatic salamander *Siren lacertina*

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The greater siren (*Siren lacertina*) can acquire oxygen from both air and water (bimodal breathing). Currently, only physiological measurements have been made on bimodal breathing, with little work being done on the unique surfacing behaviors associated with this process. Flow-through respirometry was used to measure O₂ uptake in increasingly hypoxic water. In addition, video recordings were made to capture and quantify the surfacing behavior of greater sirens in response to progressive hypoxia. Greater sirens showed an increase in aerial O₂ uptake, a decrease in aquatic O₂ uptake, and no change in total O₂ uptake as the aquatic O₂ tension decreased. Also, greater sirens shifted only the number of surfacing trips they made as the aquatic O₂ tension decreased; all other surfacing behaviors remained unchanged. Greater sirens appear to maintain their metabolic rate despite the aquatic O₂ tension solely by modifying the number of trips they take to the surface.

Keywords: bimodal breathing; greater siren; *Siren lacertina*; respiration; surfacing behavior

Introduction

Oxygen is essential for metabolism in metazoan animals. Aquatic habitats, however, are generally considered to be oxygen-poor, primarily because of the low solubility of O₂ in water (approximately 28 times lower than in air) resulting in slow diffusion of O₂ into water (DeJours 1975). In addition, O₂ levels can be further reduced by seasonal increases in temperature and biological oxygen demand from decaying organic material especially in habitats of stagnant waters (Lynch et al. 1947, as cited by Ultsch 1976). Despite these factors, most aquatic species are still capable of effectively using skin and/or gills to acquire O₂ from the water. However, chronic or transient hypoxia has put pressure on some aquatic species to develop air breathing mechanisms (Graham 1994). This has resulted in a small group of both vertebrates and invertebrates, which are able to acquire O₂ from both the air and water, known as bimodal breathers. Bimodal breathers often use multiple respiratory structures including lungs, gills, and skin to exchange respiratory gasses (Belkin 1968; Jackson et al. 1976; Stone et al. 1992; Burggren and Infanatino 1994; Graham 1994). There is

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considerable variation among such species, with some using primarily only one route of gas exchange and some effectively using all three. Taxa that are capable of acquiring O₂ from both air and water include crustaceans (Henry 1994), fish (Graham 1994), amphibians (Boutilier et al. 1992), and reptiles (Bagatto et al. 1997). Of these groups, the amphibians are of interest because they are capable of using all three of the respiratory structures, either in concert or singly (Whitford and Hutchison 1967; Hutchinson et al. 1968; Guimond and Hutchinson 1972). The greater siren, *Siren lacertina*, is one such amphibian which uses all three respiratory structures to obtain O₂ (Guimond and Hutchinson 1973). Greater sirens are of particular interest because they frequently inhabit stagnant, semi-permanent habitats such as swamps or ditches which can become hypoxic during the summer months (Ultsch 1973, 1976; Mount 1975; Petranka 1998). During periods of hypoxia, bimodal breathing species such as greater sirens appear to rely more heavily on aerial respiration (Ultsch 1976).

Research into bimodal respiration in greater sirens has sometimes produced differing results, especially with regard to the measurement of metabolic rate. Reported values of O₂ consumption range from relatively low (7.15 μL g⁻¹ h⁻¹, aquatic) (Guimond and Hutchinson 1973) to high (21.28 μL g⁻¹ h⁻¹, aquatic) (Duke and Ultsch 1990). These differences highlight the difficulties which occur when trying to describe the metabolic rate of bimodal breathing species.

Although past studies have examined the effects of different aquatic O₂ tensions (PO₂) on O₂ uptake for greater sirens (Duke and Ultsch 1990), such studies did not address a central question: how do changes in aquatic PO₂ affect the surfacing behavior of greater sirens? Greater sirens are commonly found in burrows in benthic microhabitats (Petranka 1998; Schalk et al. 2010). In hypoxic waters, aquatic O₂ uptake is reduced relative to normoxic waters (Duke and Ultsch 1990). In addition, Ultsch (1976) showed that all weight ranges of greater sirens are obligate air breathers in the summer months in hyacinth communities. In hypoxic water greater sirens must therefore modify their normal benthic behavior (seen in normoxic waters) to obtain more O₂ from the air, but the mechanisms behind these behavioral changes are unknown.

Our study addresses both physiological and behavioral changes in greater siren respiration in response to aquatic hypoxia. Specifically, we address two central questions: (1) what are the effects of aquatic hypoxia on aquatic, aerial, and total oxygen uptake for greater sirens, and (2) what are the effects of aquatic hypoxia on the surfacing behavior of greater sirens? The behavioral modifications are put into the perspective of greater sirens as potential prey species. If greater sirens were preyed upon by other species, specifically at or above the water's surface, behavioral changes that accompany increased aerial respiration would be predicted to minimize time spent in that area of vulnerability (Kramer et al. 1983; Feder and Moran 1985).

Methods

Adult greater sirens, *S. lacertina*, were purchased from Nature Coast Exotics Inc., Brooksville, Florida. Weights ranged from 256 to 307 g, with a mean ± SE of 286 ± 8 g ($n = 5$, weighed prior to every experiment). Animals were kept in individual 31 × 60 × 35 cm³ aquaria in 20–25 cm of de-ionized water at 23 ± 1°C. Greater sirens

were fed night crawler worms (all they could eat in one meal) once per week. Animals were tested after 1 week of acclimation to laboratory conditions. Greater sirens were kept for no more than 10 weeks and were tested no more than once per week.

Oxygen uptake was measured utilizing a cylindrical flow-through respirometry chamber constructed from 9 cm diameter clear PVC pipe which was capped and plumbed at each end with a total length of 43.5 cm. In addition, a 5 cm diameter clear PVC chamber (length 7 cm) was built into the top of the 9 cm diameter flow-through respirometry chamber to create a static source of air. Water filled the entire 9 cm flow-through respirometry chamber, except for the 5 cm chamber used for the static air source. Paraffin oil (mineral oil) was not used to prevent O₂ diffusion from the air to the water at low aquatic O₂ tensions (see methods of Guimond and Hutchinson 1973) because preliminary results with no animal present in the respirometry chamber showed no significant changes in aquatic PO₂ (likely due to the small air-water interface, movement of the water, and short duration of the experiments). Water flowed through the system at 3 L h⁻¹ and the desired PO₂ was generated by either bubbling nitrogen (removes O₂) or air (adds O₂) through an equilibration column before that water entered the respirometry chamber. Greater sirens were acclimated in the respirometry chamber for at least 1 h; additional time was allowed for more active animals. All O₂ measurements were made using a polarographic, Clark-type O₂ platinum electrode and meter (Cameron Instruments OM-2). Aquatic O₂ uptake was calculated by measuring the PO₂ of the water entering and exiting the respirometry chamber housing the animal. Solubility values from DeJours (1975) were used in our calculations and oxygen uptake was reported as microliter O₂ per gram wet weight per hour (μL g⁻¹ h⁻¹). Aerial O₂ uptake was calculated by measuring the PO₂ in the air bubble above the animal's chamber before and after each experimental period, allowing us to calculate the amount of O₂ consumed from the air. Solubility values from DeJours (1975) were also used in the calculation of aerial O₂ uptake. Animals were measured at four aquatic O₂ tensions: 160 Torr (normoxia), and three levels of hypoxia: 120 Torr, 80 Torr, and 40 Torr. Temperature was held constant at 23°C ± 1°C. At each aquatic PO₂, the animals were measured for 1 h, divided into three 20 min repetitions.

Surfacing behavior was observed and measured using a video camcorder connected to a TV and a VHS recorder. Animals were placed in an aquarium similar to the one in which they were housed (31 × 60 × 35 cm³) with a water depth of 20 cm and allowed to acclimate overnight (16 + h). The tank was slightly modified in order to minimize disturbance as aquatic O₂ tensions were raised or lowered. Specifically, the aquarium was divided into two sections by a plastic divider (diameter = 1 cm, height = 21 cm): one without the animal (17 cm) and one with the animal (43 cm). The water was circulated by an aquarium filter which pulled water from the side of the behavioral tank with the animal to the side without the animal. After passing through the filter and spilling down into the side without the animal, the aquatic PO₂ was generated by an aquarium bubble wand where either air (adds O₂) or nitrogen (removes O₂) was bubbled through the water. This water then spilled over the plastic divider (separating the two sides of the tank) and into the side with the animal. Water on the side with the animal was approximately 1 cm lower than the plastic divider. This procedure ensured that the water containing the animal remained as still as possible, simulating the animal's natural habitat, but was at the desired PO₂. Animals were monitored for 1.5 h at four different aquatic O₂ tensions: 160 Torr (normoxia), 120 Torr, 80 Torr, and 40 Torr (hypoxia). Temperature was

held constant at $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$. At each PO_2 , the number of surfacing events (trips from the substrate to the surface and back to the substrate) was recorded along with the duration of each surfacing event, the duration of each surfacing event spent above water (breathing), and the number of breaths taken per surfacing event which were measured by movement of the lower jaw while the animal was above the air–water interface.

We used regression analysis (SAS Institute, Inc., 1985) to determine the relationship between aquatic PO_2 and aquatic O_2 uptake, aerial O_2 uptake, and total O_2 uptake. We also used regression analysis (SAS Institute, Inc., 1985) to determine the relationship between aquatic PO_2 and number of surfacing events per hour, duration of each surfacing event, duration of each surfacing event spent above water, and number of breaths taken per surfacing event. Significance was determined at the $p < 0.05$ level.

Results

All data proved to be normally distributed, allowing use of parametric statistics. Under normoxic conditions (aquatic PO_2 of 160 Torr), aquatic O_2 uptake by greater sirens was $7.8 \mu\text{L g}^{-1} \text{h}^{-1}$, comprising 41.3% of their total O_2 uptake. Aquatic O_2 uptake increased linearly with increasing aquatic PO_2 :

$$\text{Aquatic } \text{O}_2 \text{ uptake } (\mu\text{L g}^{-1} \text{h}^{-1}) = 0.0100(\pm 0.5360) + 0.0495(+0.0049) \\ * \text{ aquatic } \text{PO}_2(\text{Torr})$$

($F=102.34$, $df=18$, $p < 0.0001$, $r^2=0.8504$) (Figure 1). Aquatic O_2 uptake declined 77% from normoxia when aquatic PO_2 was lowered to 40 Torr (hypoxia).

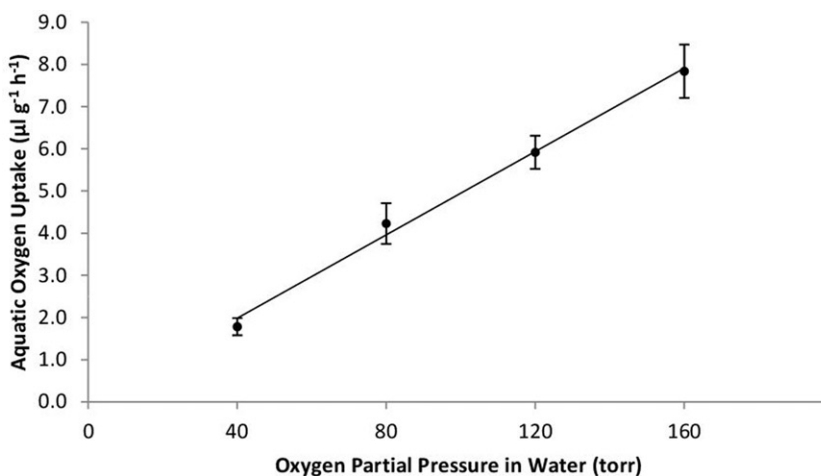


Figure 1. Rate of aquatic oxygen uptake \pm SE bars by *S. lacertina* at four different oxygen tensions. See text for equation ($n=5$, each measured at four different oxygen tensions).

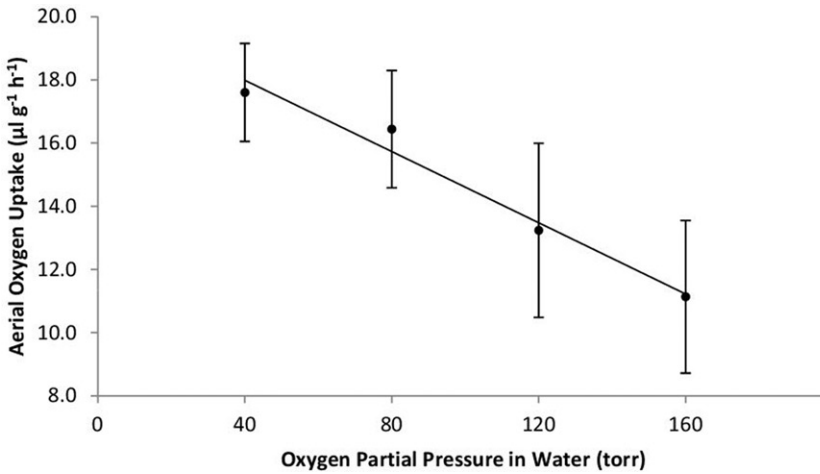


Figure 2. Rate of aerial oxygen uptake \pm SE bars by *S. lacertina* at four different oxygen tensions. See text for equation ($n = 5$, each measured at four different oxygen tensions).

Under normoxic conditions (aquatic PO_2 of 160 Torr), aerial O_2 uptake by greater sirens was $11.1 \mu L g^{-1} h^{-1}$, comprising 58.7% of their total O_2 uptake (Figure 2). Aerial O_2 uptake declined linearly with increasing aquatic PO_2 :

$$\text{Aerial } O_2 \text{ uptake } (\mu L g^{-1} h^{-1}) = 20.2400(\pm 2.5526) - 0.0563(\pm 0.02330) * \text{aquatic } PO_2(\text{Torr})$$

($F = 5.84$, $df = 18$, $p = 0.0265$, $r^2 = 0.2449$) (Figure 2). Aerial O_2 uptake rose 58.6% from normoxia when aquatic PO_2 was lowered to 40 Torr (hypoxia).

Total O_2 uptake (aquatic plus aerial) ranged from 18.9 to $20.7 \mu L g^{-1} h^{-1}$ for PO_2 of 160 and 120 Torr, respectively. However, total O_2 uptake was not significantly affected by aquatic PO_2 ($F = 0.08$, $df = 18$, $p = 0.7830$, $r^2 = 0.0043$).

The behavior of greater sirens was also affected by changes in aquatic PO_2 . The number of surfacing events per hour declined linearly with increasing aquatic PO_2 :

$$\text{Surfacing events per hour} = 3.5833(\pm 0.3877) - 0.01875(\pm 0.0035) * \text{aquatic } PO_2 (\text{Torr})$$

($F = 28.07$, $df = 14$, $p < 0.0001$, $r^2 = 0.6672$) (Figure 3). Other than the number of surfacing events, no other behavioral measure was affected by changes in aquatic PO_2 . Average surfacing event duration ranged from 10.5 s at 160 Torr to 11.8 s at 80 Torr (10.6 s at 120 Torr and 11.6 s at 40 Torr) and was not significantly affected by aquatic PO_2 ($F = 0.40$, $df = 12$, $p = 0.5370$, $r^2 = 0.0326$). The average duration of each surfacing event spent above the water–air interface also remained constant and was not significantly affected by changes in aquatic PO_2 , ranging from 2.0 s at 160 Torr to 2.7 s at 120 Torr (2.5 s at 80 Torr and 2.1 s at 40 Torr) ($F = 0.19$, $df = 12$, $p = 0.6698$, $r^2 = 0.0157$). Finally, the average number of breaths taken during each surfacing event for all aquatic PO_2 was 2. The number of breaths was not significantly affected by aquatic PO_2 ($F = 0.01$, $df = 12$, $p = 0.9285$, $r^2 = 0.0007$).

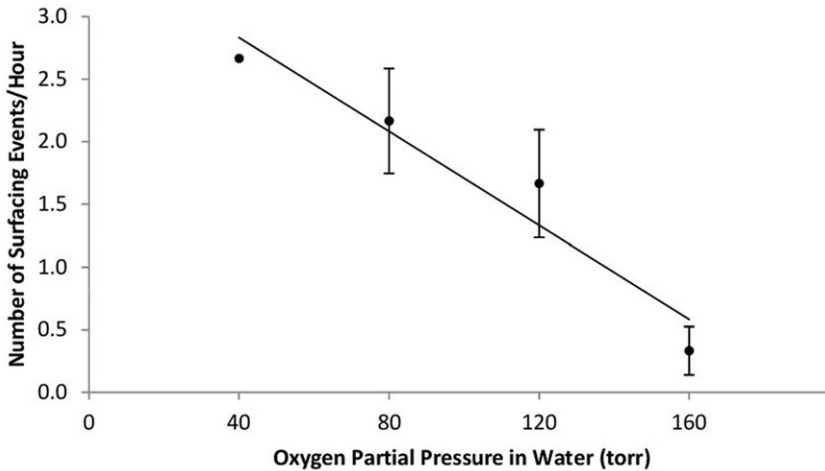


Figure 3. Mean \pm SE number of surfacing events per hour performed by *S. lacertina* at four different oxygen tensions. See text for equation. For PO_2 of 40, SE = 0 ($n = 4$, each measured at four different oxygen tensions).

Discussion

Greater siren aquatic oxygen uptake showed a significant positive relationship with aquatic PO_2 . As aquatic PO_2 was increased from hypoxia to normoxia, there was a significant linear increase in aquatic O_2 uptake (Figure 1). In addition, as the aquatic PO_2 was increased from hypoxia to normoxia, there was a significant linear decrease in aerial O_2 uptake (Figure 2). The absolute values of the slopes of the two regression lines (0.04950-aquatic; 0.05360-aerial) are very similar. These results suggest that as aquatic PO_2 declines, greater sirens are able to compensate for the O_2 they could no longer acquire from the water (aquatic) by going to the surface and acquiring that O_2 from the air. This is also evident when total O_2 consumption is examined, which reveals no relationship to aquatic PO_2 . Furthermore, our results suggest that regardless of the PO_2 of the water, greater sirens are able to effectively maintain their metabolic rates, simply by using different means (air and water) to obtain O_2 . In addition, greater sirens also appear to show a preference for aquatic respiration over aerial respiration. Greater sirens only shifted their O_2 consumption to the air as the water became hypoxic and aquatic O_2 was no longer available.

The aquatic O_2 uptake results of this study were similar to those of Guimond and Hutchinson (1973) for unrestrained greater sirens in a static respirometry chamber (termed aerial/aquatic respiration). At normoxia and $23^\circ C$, we measured aquatic O_2 uptake as $7.8 \mu L g^{-1} h^{-1}$, which was very similar to the $7.15 \mu L g^{-1} h^{-1}$ reported by Guimond and Hutchinson (1973) for adult greater sirens at $25^\circ C$. Aerial O_2 uptake reported in this study ($11.1 \mu L g^{-1} h^{-1}$) was somewhat greater than that reported by Guimond and Hutchinson (1973) at normoxia ($7.19 \mu L g^{-1} h^{-1}$). This difference is likely due to differences in experimental design. Our chamber was efficient at measuring aquatic O_2 uptake, but simultaneously made access to air easier (shorter distance to the surface), while the static tank used by Guimond and Hutchinson (1973) likely made access to air more difficult therefore resulting in a lower aerial O_2 uptake value.

Duke and Ultsch (1990) reported a total metabolic rate ($21.28 \mu\text{L g}^{-1} \text{h}^{-1}$) slightly higher than what we found ($18.9 \mu\text{L g}^{-1} \text{h}^{-1}$) for greater sirens in normoxic water. However, the sirens used by Duke and Ultsch (1990) were forced to submerge, which could possibly have led to elevated metabolic rates because forced submergence has been shown to affect dive performance in other animals by causing increases in metabolic rates (Boutilier and Shelton 1985; Bagatto and Henry 1999). The results provided by Duke and Ultsch (1990), Guimond and Hutchinson (1973), and the present study highlight the need for further investigation into the metabolic rate of greater sirens and how minute changes in the experimental design can affect this measurement.

The number of surfacing events per hour of greater sirens showed a negative linear relationship with aquatic PO_2 (Figure 3). However, other behavioral variables including surfacing event duration, duration of each surfacing event spent above the water–air interface, and number of breaths taken per surfacing event, were not significantly affected by aquatic PO_2 . Greater sirens appear to compensate for the O_2 that they are unable to acquire from the water at low aquatic O_2 tensions solely by travelling to the surface more frequently to acquire O_2 from the air. This is further supported by the negative linear relationship that aerial O_2 uptake shows with increasing aquatic PO_2 . The behavioral data in our study also suggests that greater sirens of approximately 286 g never solely rely on aquatic O_2 uptake because even in normoxic waters (PO_2 of 160 Torr), greater sirens still travelled to the surface to acquire O_2 .

Due to the low variation in most behavioral measurements and changes only in the number of surfacing events, we determined that the respiratory behavior of greater sirens is highly conserved. The fact that greater sirens only modify the number of trips to the surface and no other respiratory behaviors suggests that they could have evolved in an environment with a high threat of surface predation. The behavior of greater sirens greatly reduces the amount of time that they spend above the air–water interface. The only time spent at this level is what appears necessary for exchanging respiratory gases. It is possible that greater sirens could have evolved this highly conserved mechanism in response to predation from animals which prey upon species at the water's surface such as birds of prey and herons.

For future work we would suggest that the behavioral experiments conducted in this study be carried out for sirens at different temperatures and at different body weights to determine how these factors affect surfacing behavior. Weight is of particular importance as it could help to further explain this highly conserved mechanism of surfacing behavior. In addition, we believe that the surfacing behavior of two-toed amphiumas (*Amphiuma means*), common co-inhabitants of the same environments of greater sirens, should also be investigated. These two species appear as likely competitors due to the high level of overlap in their locality, habitat, diet, and morphology (Petranka 1998; Schalk 2010). It is likely that their respiratory strategies could help to explain how they are able to coexist despite being so similar in many ecological respects.

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References

- Bagatto B, Guyer C, Hauge B, Henry RP. 1997. Bimodal respiration in two species of Central American turtles. *Copeia*. 1997(4):834–839.
- Bagatto B, Henry RP. 1999. Exercise and forced submergence in the pond slider (*Trachemys scripta*) and softshell turtle (*Apalone ferox*): influence on bimodal gas exchange, diving behaviour and blood acid-base status. *J Exp Biol*. 202:267–278.
- Belkin DA. 1968. Aquatic respiration and under-water survival of two freshwater turtle species. *Respir Physiol*. 4:1–14.
- Boutilier RG, Shelton G. 1985. The effects of forced and voluntary diving on ventilation, blood gases and pH in the aquatic amphibian, *Xenopus laevis*. *J Exp Biol*. 122:209–222.
- Boutilier RG, Stiffler DF, Toews DP. 1992. Exchange of respiratory gases, ions, and water in amphibious and aquatic amphibians. In: Feder ME, Burggren WW, editors. *Environmental physiology of the amphibians*. Chicago: University of Chicago Press. p. 81–124.
- Burggren WW, Infantino RL. 1994. The respiratory transition from water to air breathing during amphibian metamorphosis. *Am Zool*. 34:238–246.
- DeJours P. 1975. *Principles of comparative respiratory physiology*. Amsterdam (The Netherlands): North-Holland.
- Duke JT, Ultsch GR. 1990. Metabolic oxygen regulation and conformity during submergence in the salamander *Siren lacertina*, *Amphiuma means*, and *Amphiuma tridactylum*, and a comparison with other giant salamanders. *Oecologia*. 84:16–23.
- Feder ME, Moran CM. 1985. Effects of water depth on costs of aerial respiration and its alternatives in tadpoles of *Rana pipiens*. *Can J Zool*. 63:643–648.
- Graham JB. 1994. An evolutionary perspective for bimodal respiration: a biological synthesis of fish air breathing. *Am Zool*. 34(2):229–237.
- Guimond RW, Hutchinson VH. 1972. Pulmonary, branchial and cutaneous gas exchange in the mud puppy, *Necturus maculosus maculosus* (Rafinesque). *Comp Biochem Physiol*. 42(2):367–392.
- Guimond RW, Hutchinson VH. 1973. Trimodal Gas Exchange in the Large Aquatic Salamander, *Siren lacertina* (Linnaeus). *Comp Biochem Physiol*. 46(1):249–268.
- Henry RP. 1994. Morphological, behavioral, and physiological characterization of bimodal breathing crustaceans. *Am Zool*. 34(2):205–215.
- Hutchinson VH, Whitford WS, Kosh RM. 1968. Relation of body size and surface area to gas exchange in anurans. *Physiol Zool*. 41(1):65–85.
- Jackson DC, Allen J, Strupp PK. 1976. The contribution of non-pulmonary surfaces to CO₂ loss in 6 species of turtles at 20°C. *Comp Biochem Physiol*. 55(3):243–246.
- Kramer DL, Manley D, Bourgeois R. 1983. The effect of respiratory mode and oxygen concentration on the risk of aerial predation in fishes. *Can J Zool*. 61(3):653–665.
- Lynch JJ, King JE, Chamberlain TK, Smith AL. 1947. Effects of aquatic weed infestations on the fish and wildlife of the Gulf States. *U.S. Dept Int Spec Sci. Rept*. 39:1–71.
- Mount RH. 1975. *The reptiles and amphibians of Alabama*. Auburn (AL): Auburn University Agricultural Experimental Station. p. 347.

- Petranka J. 1998. Salamanders of the United States and Canada. Washington (DC): Smithsonian Institution Press. p. 576.
- SAS Institute, Inc. 1985. SAS user's guide: statistics. Cary (NC): SAS Institute, Inc. p. 956.
- Schalk CM, Luhring TM, Crawford BA. 2010. Summer microhabitat use of the greater siren (*Siren lacertina*) and two-toed amphiuma (*Amphiuma means*) in an isolated wetland. *Amphibia-Reptilia*. 31:251–256.
- Stone PA, Dobie JL, Henry RP. 1992. Cutaneous surface area and bimodal respiration in soft shelled (*Trionyx spiniferus*), stinkpot (*Sternotherus odoratus*), and mud turtles (*Kinosternon subrubrum*). *Physiol. Zool.* 65(2):331–345.
- Ultsch GR. 1973. The effects of water hyacinth (*Eichhornia crassipes*) on the microenvironment of aquatic communities. *Arch Hydrobiol.* 72:460–473.
- Ultsch GR. 1976. Eco-physiological studies of some metabolic and respiratory adaptations of sirenid salamanders. In: Hughes GM, editor. *Respiration of amphibious vertebrates*. New York: Academic Press. p. 287–312.
- Whitford WG, Hutchinson VH. 1967. Body size and metabolic rate in salamanders. *Physiol Zool.* 40(2):127–133.