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 \(O_2\) 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 \(O_2\) uptake, a decrease in aquatic \(O_2\) uptake, and no change in total \(O_2\) uptake as the aquatic \(O_2\) tension decreased. Also, greater sirens shifted only the number of surfacing trips they made as the aquatic \(O_2\) tension decreased; all other surfacing behaviors remained unchanged. Greater sirens appear to maintain their metabolic rate despite the aquatic \(O_2\) 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 \(O_2\) in water (approximately 28 times lower than in air) resulting in slow diffusion of \(O_2\) into water (DeJours 1975). In addition, \(O_2\) 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 \(O_2\) 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 \(O_2\) 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 Infantino 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°C ± 1°C. At each PO₂, 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 PO₂ and aquatic O₂ uptake, aerial O₂ uptake, and total O₂ uptake. We also used regression analysis (SAS Institute, Inc., 1985) to determine the relationship between aquatic PO₂ 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 PO₂ of 160 Torr), aquatic O₂ uptake by greater sirens was 7.8 μL g⁻¹ h⁻¹, comprising 41.3% of their total O₂ uptake. Aquatic O₂ uptake increased linearly with increasing aquatic PO₂:

Aquatic O₂ uptake (μL g⁻¹ h⁻¹) = 0.0100(±0.5360) + 0.0495(+0.0049)

* aquatic PO₂(Torr)

(F = 102.34, df = 18, p < 0.0001, r² = 0.8504) (Figure 1). Aquatic O₂ uptake declined 77% from normoxia when aquatic PO₂ was lowered to 40 Torr (hypoxia).
Under normoxic conditions (aquatic $\text{PO}_2$ of 160 Torr), aerial $\text{O}_2$ uptake by greater sirens was 11.1 $\mu\text{L g}^{-1}\text{h}^{-1}$, comprising 58.7% of their total $\text{O}_2$ uptake (Figure 2). Aerial $\text{O}_2$ uptake declined linearly with increasing aquatic $\text{PO}_2$:

$$\text{Aerial } \text{O}_2 \text{ uptake } (\mu\text{L g}^{-1}\text{h}^{-1}) = 20.2400(\pm2.5526) - 0.0563(\pm0.02330)$$
$$\times \text{ aquatic } \text{PO}_2 (\text{Torr})$$

($F = 5.84$, df = 18, $p = 0.0265$, $r^2 = 0.2449$) (Figure 2). Aerial $\text{O}_2$ uptake rose 58.6% from normoxia when aquatic $\text{PO}_2$ was lowered to 40 Torr (hypoxia).

Total $\text{O}_2$ uptake (aquatic plus aerial) ranged from 18.9 to 20.7 $\mu\text{L g}^{-1}\text{h}^{-1}$ for $\text{PO}_2$ of 160 and 120 Torr, respectively. However, total $\text{O}_2$ uptake was not significantly affected by aquatic $\text{PO}_2$ ($F = 0.08$, df = 18, $p = 0.7830$, $r^2 = 0.0043$).

The behavior of greater sirens was also affected by changes in aquatic $\text{PO}_2$. The number of surfacing events per hour declined linearly with increasing aquatic $\text{PO}_2$:

$$\text{Surfacing events per hour} = 3.5833(\pm0.3877) - 0.01875(\pm0.0035)$$
$$\times \text{ aquatic } \text{PO}_2 (\text{Torr})$$

($F = 28.07$, 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 $\text{PO}_2$. 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 $\text{PO}_2$ ($F = 0.40$, 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 $\text{PO}_2$, 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 $\text{PO}_2$ was 2. The number of breaths was not significantly affected by aquatic $\text{PO}_2$ ($F = 0.01$, df = 12, $p = 0.9285$, $r^2 = 0.0007$).
Discussion

Greater siren aquatic oxygen uptake showed a significant positive relationship with aquatic PO₂. As aquatic PO₂ was increased from hypoxia to normoxia, there was a significant linear increase in aquatic O₂ uptake (Figure 1). In addition, as the aquatic PO₂ was increased from hypoxia to normoxia, there was a significant linear decrease in aerial O₂ 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 PO₂ declines, greater sirens are able to compensate for the O₂ they could no longer acquire from the water (aquatic) by going to the surface and acquiring that O₂ from the air. This is also evident when total O₂ consumption is examined, which reveals no relationship to aquatic PO₂. Furthermore, our results suggest that regardless of the PO₂ of the water, greater sirens are able to effectively maintain their metabolic rates, simply by using different means (air and water) to obtain O₂. In addition, greater sirens also appear to show a preference for aquatic respiration over aerial respiration. Greater sirens only shifted their O₂ consumption to the air as the water became hypoxic and aquatic O₂ was no longer available.

The aquatic O₂ 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°C, we measured aquatic O₂ uptake as 7.8 μL g⁻¹ h⁻¹, which was very similar to the 7.15 μL g⁻¹ h⁻¹ reported by Guimond and Hutchinson (1973) for adult greater sirens at 25°C. Aerial O₂ uptake reported in this study (11.1 μL g⁻¹ h⁻¹) was somewhat greater than that reported by Guimond and Hutchinson (1973) at normoxia (7.19 μL g⁻¹ h⁻¹). This difference is likely due to differences in experimental design. Our chamber was efficient at measuring aquatic O₂ 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 O₂ uptake value.

Figure 3. Mean ± SE number of surfacing events per hour performed by S. lacertina at four different oxygen tensions. See text for equation. For PO₂ of 40, SE = 0 (n = 4, each measured at four different oxygen tensions).
Duke and Ultsch (1990) reported a total metabolic rate (21.28 μL g⁻¹ h⁻¹) slightly higher than what we found (18.9 μL g⁻¹ 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 PO₂ (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 PO₂. Greater sirens appear to compensate for the O₂ that they are unable to acquire from the water at low aquatic O₂ tensions solely by travelling to the surface more frequently to acquire O₂ from the air. This is further supported by the negative linear relationship that aerial O₂ uptake shows with increasing aquatic PO₂. The behavioral data in our study also suggests that greater sirens of approximately 286 g never solely rely on aquatic O₂ uptake because even in normoxic waters (PO₂ of 160 Torr), greater sirens still travelled to the surface to acquire O₂.

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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