Scientific Research Publishing

Journal of Water Resource and Protection, 2023, 15, 23-32
https://www.scirp.org/journal/jwarp
ISSN Online: 1945-3108
ISSN Print: 1945-3094

Nitrogen Gas Saturation in Karst Springs Varies Throughout the Day

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How to cite this paper: Gross, M.A., Voorhees, J.M., Domagall, A.S. and Barnes, M.E. (2023) Nitrogen Gas Saturation in Karst Springs Varies Throughout the Day. *Journal of Water Resource and Protection*, **15**, 23-32.
https://doi.org/10.4236/jwarp.2023.152002

Received: January 7, 2023
Accepted: February 10, 2023
Published: February 13, 2023

Abstract

This experiment examined the fluctuations in nitrogen gas supersaturation throughout the day in three karst springs (upper, side, and lower) at McNenny State Fish Hatchery, rural Spearfish, Lawrence County, South Dakota, USA. Total gas pressures, oxygen percent saturation, and nitrogen percent saturation were recorded six times/day on eight days over a 26-day period in each of the three springs. Total gas pressure did not vary significantly throughout the day in any of the springs. However, percent oxygen and nitrogen saturation were significantly different throughout the day in all three springs. The highest mean (SE) nitrogen supersaturation value of 118.5 (1.1)% was observed in the lower spring at 07:00. The lowest mean nitrogen supersaturation values were 114.5 (1.1)% at 13:00 in the upper spring, and 114.2 (0.2)% and 113.1 (0.7)% at 15:00 in the side and lower spring, respectively. At 118% nitrogen supersaturation, gas bubble disease is likely to occur in fish, resulting in potentially high levels of mortality if untreated spring water was used for fish production. The results of this study indicate the importance of recording nitrogen gas levels at sunrise or early in the morning, when nitrogen is highest and oxygen is lowest, to obtain accurate and reproducible data.

Keywords

Karst Springs, Aquifer, Gas Supersaturation, Nitrogen, Aquaculture

1. Introduction

Karst landscapes form from the dissolution of soluble bedrock by surface and groundwater [1]. These landscapes are characterized by disappearing streams, springs, underground caves, and sinkholes [1] [2]. Karst forms in soluble sedi-

mentary rock layers such as carbonates, limestone ($CaCO_3$), gypsum ($CaSO_4 \cdot 2H_2O$) (**Figure 1**), and dolomite ($MgCO_3 \cdot CaCO_3$) and in evaporites, gypsum ($CaSO_4 \cdot 2H_2O$), and anhydrite ($CaSO_4$) [1] [2].

Limestone, gypsum, and anhydrite are common in the sedimentary formations of the Black Hills of South Dakota, USA, and these formations often display several karstic features. McNenny State Fish Hatchery, rural Spearfish, Lawrence County, South Dakota, USA is located on the Spearfish formation, which is primarily non-soluble red shale, siltstone, and fine-grained sandstone with soluble gypsum lenses [2] [3]. Underlying the hatchery are a range of sedimentary rock layers including mostly limestone of the Madison and Minnelusa formations [2].

The Madison and Minnelusa formations are both major aquifers in the northern Black Hills [1]. These aquifer units have created numerous sinkholes around the Black Hills and are the likely water source for the natural springs at McNenny State Fish Hatchery [4] [5]. The water in these springs is supersaturated with dissolved nitrogen, which is a common occurrence in ground water [6] [7]. This supersaturation likely occurs because of high pressure in the confined aquifers [7].

Nitrogen supersaturation in springs is of particular interest because of its well-documented negative effects on fish [8] [9] [10] [11] [12]. Aeration and degassing structures are used to reduce nitrogen gas levels and increase dissolved oxygen levels in spring water at McNenny Hatchery [13] [14]. Although nitrogen saturation is decreased with these structures, it still persists. Therefore, nitrogen levels in springs at the hatchery are still monitored at different times of the year and varying hours of the day. However, the hourly variation of nitrogen supersaturation, if it occurs, is unknown. Thus, the objective of this study was to document gas supersaturation levels from multiple karst springs located at McNenny State Fish Hatchery throughout the day.

2. Methods

2.1. Study Area

McNenny State Fish Hatchery, Lawrence County, South Dakota, USA is greatly influences by its local geology. At the surface there is siltstone, red shales, and sandstone, of the Spearfish Formation (**Figure 1**) and at a depth there is a range of sedimentary layer, some of which are easily dissolved such as limestones of the Madison and Minnelusa formations and some of which are fine-grained and do not allow water to pass through them (confining layers) [1] [2]. The confining layers mean that the limestone aquifers (Madison and Minnelusa) are under pressure and only recharged at higher elevations in the Black Hills. The numerous springs at and near the hatchery are the results of karstic collapse of the underlying limestone rock units allowing deep groundwater to rise to the surface [1] [2].

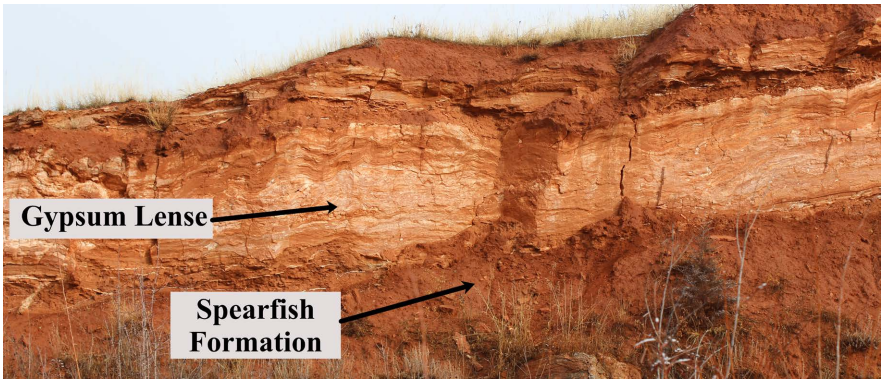

Figure 1. Example of gypsum lense in sedimenitary rock layer (Spearfish Formation) abundant at McNenny State Fish Hatchery.

2.2. Data Collection

Data collection occurred in three springs at McNenny Hatchery (approximate latitude 44˚33'32"N, longitude 104˚00'40"W, **Figure 2**). The springs were designated as upper, side, and lower, although this does not depict actual spring elevations (**Figure 3**). Approximate flows from the upper, side, and lower springs are 0.02, 0.01, and 0.03 m^3/sec, respectively [15]. Water chemistry for the springs is similar, with approximate total hardness as $CaCO_3$ of 360 mg/L, alkalinity as $CaCO_3$ at 210 mg/L, pH of 7.6, and total dissolved solids of 390 mg/L [16].

Water sampling at each of the three springs occurred six times a day at two-hour intervals, beginning at 07:00 and concluding at 17:00 (approximately sunrise and sunset).

Sampling occurred on eight days in 2021: 21, 26, 28 January and 4, 9, 18, 23, 25 February. At each sampling event, total gas pressure, dissolved oxygen, and water temperature were measured using a total gas pressure meter (Handy Polaris, OxyGuard, Farum, Denmark). Barometric pressure at every sampling event was also recorded, using measurements obtained from wunderground.com for Beulah, Wyoming, USA.

Total gas pressure was determined by taking total gas pressure percent saturation divided by 100 and multiplied by overall barometric pressure [18].

Where:

$$P_{TOTAL} = \frac{P_{SAT}}{100} \times P_{BAR} \tag{1}$$

Delta P was determined by taking total gas pressure minus barometric pressure [18].

Where:

$$\Delta P = P_{TOTAL} - P_{BAR} \tag{2}$$

Nitrogen saturation was determined using the following formula described by [18]:

$$N_2(\%) = \left[BP + \Delta P - \left(\left(O_2 / b_{O_2} \right) \times 0.5318 - P_{H_2O} \right) \Big/ \left(\left(BP - P_{H_2O} \right) \times 0.7902 \right) \right] \times 100 \tag{3}$$

Where:

N_2 = partial pressure of nitrogen gas in the water (percentage nitrogen saturation);

BP = local barometric pressure (mmHg);

O_2 = oxygen concentration (mg/L);

b_{O2} = Bunsen's coefficient for oxygen;

P_{H2O} = partial pressure of the water vapor (mmHg).

Figure 2. Location of McNenny State Fish Hatchery, rural Spearfish, Lawrence County, South Dakota, USA.

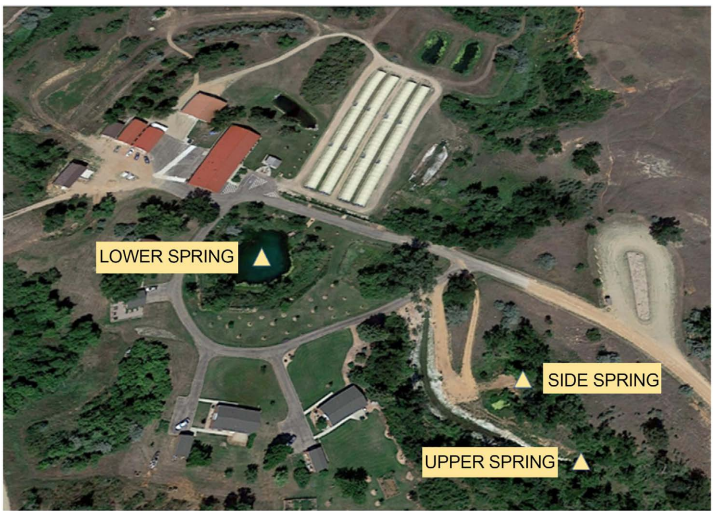

Figure 3. Aerial photo from Google Earth [17] showing location of the three springs on the hatchery grounds.

2.3. Data Analysis

Data were analyzed using SPSS (24.0) statistical program (IBM, Armonk, New York, USA). Significance was predetermined at $p < 0.05$. A two-way analysis of

variance was performed. If there was an interaction, then a one-way analysis of variance was performed with post hoc means separation test using Tukey HSD.

3. Results

Nitrogen gas saturation was not significantly different among the sampling dates (Table 1). However, total gas pressure in the side spring was significantly different among the sampling dates, with the lowest mean (±SE) percentages recorded on 21 January at 100.9 (0.1)% and the highest on 09 February at 101.7 (0.1)%. Mean (SE) total gas pressure ranged from 104.5 (0.2)% in the lower spring to 101.4 (0.1)% in the side spring. Percent oxygen saturation was not significantly different among the days in any of the springs.

Nitrogen supersaturation was significantly higher early in the morning at 07:00 and 09:00 compared to the sampling times later in the day (Table 2). The

Table 1. Mean (SE) percent saturation of total gas pressure, oxygen, and nitrogen at each sampling date (all sampling times each day combined) for three (upper, side, and lower) karst springs located at McNenny State Fish Hatchery, rural Spearfish, South Dakota, USA. Means followed by different letters across a row are significantly different (n = 6, $p < 0.05$).

Gas	Spring	Jan-21	Jan-26	Jan-28	Feb-04	Feb-09	Feb-18	Feb-23	Feb-25	Overall
Total Gas Pressure	Upper	101.9 (0.1)	102.3 (0.1)	102.3 (0.1)	102.4 (0.1)	102.3 (0.1)	101.9 (0.2)	102.2 (0.2)	102.2 (0.1)	102.2 (0.0)
	Side	100.9 (0.1) w	101.4 (0.2) zy	101.1 (0.1) wz	101.5 (0.1) zy	101.7 (0.1) y	101.6 (0.2) zy	101.5 (0.1) zy	101.6 (0.1) zy	101.4 (0.1)
	Lower	104.5 (0.2)	104.1 (0.4)	103.8 (0.4)	104.7 (0.4)	104.1 (0.5)	105.8 (0.6)	104.5 (0.6)	104.4 (0.4)	104.5 (0.2)
Oxygen	Upper	74.1 (0.4)	73.9 (0.8)	74.0 (1.1)	76.3 (1.3)	74.8 (1.0)	75.0 (1.3)	75.2 (1.8)	74.0 (0.9)	74.7 (0.4)
	Side	70.6 (0.5)	70.6 (0.7)	69.2 (0.9)	74.9 (4.3)	73.2 (1.2)	72.1 (1.1)	70.7 (1.0)	71.7 (1.3)	71.6 (0.6)
	Lower	79.9 (3.4)	78.3 (2.4)	81.8 (4.0)	83.6 (4.4)	84.8 (4.5)	87.3 (5.9)	79.3 (3.2)	85.3 (5.8)	82.5 (1.5)
Nitrogen	Upper	114.7 (0.1)	115.1 (0.2)	115.3 (0.1)	114.8 (0.3)	114.9 (0.3)	114.4 (0.3)	114.9 (0.3)	115.2 (0.2)	114.9 (0.1)
	Side	114.1 (0.1)	114.8 (0.2)	114.9 (0.2)	114.8 (0.3)	114.4 (0.2)	114.6 (0.2)	115.0 (0.2)	114.9 (0.3)	114.7 (0.1)
	Lower	116.4 (0.8)	115.9 (0.4)	115.1 (0.6)	115.7 (0.7)	114.2 (0.7)	116.0 (2.1)	116.7 (0.9)	114.9 (1.3)	115.6 (0.4)

Table 2. Mean (SE) percent saturation of total gas pressure, oxygen, and nitrogen at two-hour intervals during the day (all sampling days combined) for three (upper, side, and lower) karst springs located at McNenny State Fish Hatchery, rural Spearfish, South Dakota, USA. Means followed by different letters across a row are significantly different (n = 8, $p < 0.05$).

Gas	Spring	7:00	9:00	11:00	13:00	15:00	17:00	Overall
Total Gas Pressure	Upper	102.0 (0.1)	102.2 (0.1)	102.2 (0.1)	102.3 (0.1)	102.2 (0.1)	102.3 (0.1)	102.2 (0.0)
	Side	101.1 (0.1)	101.5 (0.2)	101.4 (0.1)	101.5 (0.1)	101.5 (0.1)	101.5 (0.2)	101.4 (0.1)
	Lower	104.2 (0.7)	104.0 (0.4)	104.1 (0.2)	105.2 (0.4)	104.4 (0.3)	105.0 (0.2)	104.5 (0.2)
Oxygen	Upper	71.1 (0.5) x	73.3 (0.8) yx	75.9 (0.6) wzy	77.1 (0.7) w	76.4 (0.7) wz	74.2 (0.6) zy	74.7 (0.4)
	Side	68.0 (0.4) z	69.3 (0.7) z	70.9 (0.7) wz	72.6 (0.6) wz	76.3 (2.9) w	72.6 (0.5) wz	71.6 (0.6)
	Lower	68.6 (1.3) y	72.4 (1.2) y	82.9 (1.9) z	90.1 (1.8) wz	93.5 (2.1) w	87.8 (1.9) wz	82.5 (1.5)
Nitrogen	Upper	115.5 (0.2) z	115.1 (0.2) wz	114.7 (0.2) w	114.5 (0.1) w	114.6 (0.2) w	115.1 (0.1) wz	114.9 (0.1)
	Side	115.0 (0.1) zy	115.2 (0.2) y	114.7 (0.2) wzy	114.4 (0.1) wz	114.2 (0.2) w	114.5 (0.1) wz	114.7 (0.1)
	Lower	118.5 (1.1) y	117.2 (0.3) zy	115.0 (0.4) wz	114.8 (0.7) wz	113.1 (0.7) w	114.9 (0.6) wz	115.6 (0.4)

highest mean (SE) value of 118.5 (1.1)% was observed in the lower spring at 07:00. The lowest mean nitrogen percentages were 114.5 (1.1)% at 13:00 in the upper spring, and 114.2 (0.2)% and 113.1 (0.7)% at 15:00 in the side and lower spring respectively. Total gas pressure was not significantly different throughout the day in any of the springs. However, percent oxygen saturation was significantly lower earlier in the day (07:00 and 09:00) for all three springs, compared to the highest readings at 13:00 and 15:00.

4. Discussion

This study is the first to examine the possible effects of time-of-day on nitrogen gas supersaturation in karst springs. The results of this study, whereby percent nitrogen was highest in the morning and lowest at mid-day, with percent oxygen saturation following an opposite pattern, and total gas pressure remaining relatively constant throughout the day, are similar to those described by Mahler and Bourgeais [19] in karst springs in Texas, USA. Kutty [20] and Francis-Floyd [21] reported similar results in aquaculture ponds, with the highest nitrogen and lowest oxygen levels at dawn. In contrast, Boyd *et al.* [22], monitoring gas pressures throughout the day in ponds fertilized and used to grow catfish, reported total gas pressure, percent nitrogen, and percent oxygen were all higher in the afternoon compared to morning.

In the springs at McNenny State Fish Hatchery the lowest oxygen levels were 69%, which is much higher than that reported in other springs or groundwater sampling locations [23] [24]. At the highest concentration of 116%, nitrogen supersaturation exceeds the general criteria of less than 110% as defined by the United States Environmental Protection Agency [7] [25]. At these levels of nitrogen supersaturation, gas bubble disease can occur, which can lead to fish mortality [7] [23] [24] [26] [27]. This makes the nitrogen gas-reducing structures [13] [14] used at McNenny Hatchery indispensable for fish production.

The supersaturation of nitrogen observed in the spring water in this study is not surprising. Supersaturation of nitrogen in ground and spring water is common [23], because when water is drawn downward into aquifers, atmospheric air is mixed with it under pressure and the gases become supersaturated [7] [24] [25] [28]. Specific to confined aquifer-fed karstic springs, the high nitrogen levels could be due to the short residence time of water in karst aquifers [27]. While it is possible that the high nitrogen gas levels observed in this study could be from the denitrification of nitrogen from fertilizers, septic tanks, agricultural, and municipality waste [29] [30] [31] [32], it is unlikely for the karst springs at McNenny State Fish Hatchery. The springs are near (<10 miles) their recharge area [1] [16], which is mostly publicly-owned coniferous forest used primarily for logging, along with light grazing, and less than 4% crop land [33]. Although there is likely some septic tank leakage, it is a probably a minor issue [34].

While significantly different among the sampling days, the difference in total gas pressure in the side spring from 100.9% to 101.7% is minimal and likely not

physically significant. Neither percent oxygen and nor percent nitrogen differed among the days sampled.

Gas pressure can change in karst springs during periods of high runoff and rapid recharge due to a higher water table, creating a need for longer-term studies [19] [35] [36]. This study examined only three springs at McNenny Hatchery, and these results may not be typical for the numerous other springs in the Black Hills or elsewhere. The springs at McNenny are mostly from the Madison aquifer [16] [34]. However, a karst spring less than one kilometer away releases water, half of which comes from the Madison aquifer and half from the Minnelusa [16] [34]. Thus, the results of this study may be site-specific. Also, with further monitoring it may be possible to formulate a model similar to Kamar and Sheng [37].

5. Conclusion

In conclusion, this study indicates the necessity of recording gas saturation levels early in the day to obtain accurate and repeatable measurements and minimize the possible effects of sunlight, photosynthesis, and respiration. The results of this study have sampling time implications for future studies examining long-term changes throughout the year or multiple years in karst spring gas saturation levels. In addition, these results can be used to designate sampling times for nitrogen supersaturation in fish hatcheries or other applications where accurate data collection is essential.

Acknowledgements

We thank Nathan Huysman, Eric Krebs, Thomas Mauck, and Michael Robidoux for their assistance with this study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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