

1 Effects of water quality on the abundance of *Lampetra aepyptera*

2 **Introduction**

3 Freshwater quality is a key factor in maintaining ecosystem functions and services
4 (Keeler et al., 2012). Ecosystem functions are processes carried out by an ecosystem that control
5 how energy and nutrients move throughout an environment. Ecosystem services are anything
6 within the ecosystem that makes something that is beneficial to humans like water and oxygen.
7 For example, wetlands filtrate incoming water, reduce nutrient and sediment loads, and provide
8 habitats to different organisms (Miller, 2020). These processes improve water quality and are
9 related to species biodiversity.

10 Water quality has a critical role influencing presence and abundance of organisms,
11 human health, recreational activities, and fishing. Water is an essential component of the
12 environment. It is critical to protect and maintain our water resources as it affects the overall
13 quality of the environment (Kumar et al. 2017). Within freshwater ecosystems, rivers are
14 essential natural resources for the development of human civilization and are constantly polluted
15 by industrial and domestic waste which causes a large change in the composition of the water
16 both through organisms and physiochemically (Kumar et al. 2017).

17 Water quality can be determined by measuring several physicochemical parameters such
18 as dissolved oxygen (DO), pH, water temperature, salinity, and electroconductivity (Bhateria and
19 Jain 2016). Dissolved oxygen is often described as one of the most important water quality
20 parameters, as decreasing DO, reduces aquatic organisms' availability to use oxygen (Dissolved
21 oxygen, 2019). Low oxygen, or hypoxia, is described as water conditions where oxygen is below
22 2mg/L; thus, negatively affecting aquatic organisms (Manitcharoen et al., 2020). Many studies

23 have focused on the effects of low DO or other water parameters to organisms such as caddisflies
24 (Benjamin et al., 2020), largemouth bass, and phytoplankton (Smith and Piedrahita, 1988).
25 Specifically, sensitive species such as *Trichoptera sp.* (caddisfly) are only present when water
26 parameters are optimal. On the other hand, organisms like the *Cyprinus carpio* (common carp)
27 are much less sensitive and can live in a wide range of parameters including a range of
28 temperature from 0 to 30 C° (Maiditsch and Ladich, 2014).

29 *Lampetra aepyptera* (Least brook lamprey) is a demersal fish species found in freshwater
30 streams and lakes and, unlike most species of lamprey, are non-parasitic. Lampreys are eel-
31 shaped fish with a skeleton made of cartilage and this particular species filter feeds. *Lampetra*
32 *aepyptera* breed by first constructing a nest made from rock and pebbles on the bottom of a body
33 of water. The eggs are then partially buried in the nest (*Lampetra aepyptera* summary, 2012).

34 Least brook lamprey are found in the central and eastern United States. In North Carolina
35 (NC) they are found in streams throughout the Neuse River Basin. Unlike other types of
36 lamprey, they only need freshwater as they are not anadromous. High quality of water and
37 substrate are required for lamprey especially for spawning areas (Lamprey). There is a need to
38 assess the effects of water quality on lamprey's fitness and it is critical to understand the
39 relationship between water quality and species abundance. In this research paper I intend to
40 evaluate how water quality parameters have an effect of lamprey abundance. I predict that
41 temperature will have the greatest impact on lamprey's abundance as it influences many
42 important water quality parameters.

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46 **Methods**

47 The Neuse River Basin stretches over 6,000 square miles from east to central NC and is
48 home to a plethora of aquatic species including around 100 species of fish. Some of these fish
49 species include the Least brook Lamprey and endemic species like the Carolina Madtom and
50 Neuse River Waterdog. Within this basin four streams were sampled for analyses of water
51 parameters and lamprey abundance. Little River had four different sampling sites and they are
52 located at 35.83754, -78.36003 (Riley Hill Road), 35.83084, -78.35475 (Green Pace Road),
53 35.82191, -78.35172 (Highway 97), and 35.80522, -78.37439 (UT). Buffalo Creek had two
54 sampling sites located at 35.88745, -78.43685 (Mitchell Mill Road) and 35.80656, -78.39964
55 (UT). The remaining streams all only had one sampling site and they are Rocky Branch at
56 35.976783, -78.629712, Mystery Lake at 35.78101, -78.35135, Snipes Creek at 35.777382, -
57 78.279880, and Hominey Creek at 35.86854, -78.40613. Water parameters were collected on
58 2/24/2021 for all sampled sites (N = 10). Soil Samples were collected on 2/24/2021 for all
59 samples sites other than Snipes Creek (N=9). Both samples were taken by using a plastic bottle
60 and scooping the required sample (soil or water) directly into the bottle. Water samples were
61 analyzed for temperature, pH, conductivity, and total dissolved solids (Turf-Tec PHEP5-n Hanna
62 pH electronic Pen), and nitrate-N, phosphate, Chlorine, Copper, Iron, and Chromium (LaMotte
63 water quality tests). Soil parameters measured were nitrate, phosphate, potassium, porosity, and
64 texture using LaMotte soil quality tests.

65 Lamprey sampling area was determined by scouting streams for two months and then
66 ranking them based on habitat features that promoted lamprey spawning including flow,
67 dominant substrate, subdominant substrate, and percent sedimentation. The sites that were

68 ranked the highest overall were chosen for sampling. The streams that were chosen for sampling
69 are part of the Neuse River basin, specifically the Little River sub basin, Middle Creek, Walnut
70 Creek, and Swift Creek. Lamprey were sampled for over a 2-month period twice a week.
71 Lamprey are sampled by walking down a section of the stream and looking for groups of
72 spawning lampreys. Lamprey discovered were collected and fin clips were taken to be analyzed
73 by the NC Resources Commission.

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75 **Results**

76 The Neuse River, Contentnea Creek, Walnut Creek, and Swift Creek (our comparative
77 creeks) have similar pH with a range of 5.6 to 7.5 (Table 1). Temperature range was 6.6 to
78 29.8°C. Dissolved oxygen levels had a range of 3.4 to 12.7 mg/L (Table 1). For sampled streams,
79 pH ranged from 6.73 at Mystery Lake to 7.67 at Hominey Creek and had an average of 7.05
80 (Figure 1). Temperature ranged from 9.4 C° to 13.9 C° and average is 11.79 C° (Figure 2).

81 Phosphate had the most variation among parameters tested (Table 2) as it varied from
82 undetected to overrange (Test range was 0-3 ppm). The average phosphate level (overrange
83 counted as 3 and undetected counts as 0) is 1.41 ppm. Nitrate had small variations among
84 sampling sites with most all being under 0.2 ppm (Table 2). All sites had a chlorine level of ≤
85 0.05 ppm while copper levels were ≤ 0.07 ppm for all sites other than Little River UT at 0.19
86 ppm, Mystery Lake UT at 0.11ppm, and Snipes Creek at 0.18ppm (Table 2). Iron had a range of
87 0.05 ppm (Hominey Creek) to 0.25 ppm (Mystery Lake) and had an average of 0.161 ppm
88 (Table 2). Chromium was undetected for all sites (Table 2).

89 Soil Samples were collected along the stream bank (Table 3) other than Snipes Creek
90 where no sample was taken. These creeks had a range of nitrate from 10 lb/a to 60 lb/a and an
91 average of 30 lb/a. Phosphates ranged from 25 lb/a to 200 lb/a with an average of 102.77 lb/a.
92 Porosity was relatively similar among sites with the range being 4.5% to 6.85% and an average
93 of 5.51%. Potassium was undetected at all sites sampled. Texture classification for each stream
94 soil was not included due to unclear laboratory results.

95 Lamprey were found at 4 of the 10 sites and those sites were Buffalo Creek (Mitchell
96 Mill Road), Rocky Branch, Little River (Highway 97), and Buffalo Creek (UT). (Figure 3). Only
97 one lamprey was found at each site other than Rocky Branch having three. Mating behavior was
98 observed at this site as well, showing that spawning will take place. While it is assumed
99 spawning could take place at the other sites with lamprey detected, it cannot be determined for
100 certain until it is observed as the lamprey could be passing through to go to a different stream.

101 **Discussion**

102 Temperature ranged from 9.4 C° to 13.9 C° and average is 11.79 C° among the sites that
103 were sampled (Figure 2). This means that temperature between all streams varied and could
104 influence what organisms live in the stream. Lamprey seems to be relatively unaffected by
105 temperature as streams as low as 10.5 C° and as high as 13.9 C° had lamprey observed which is
106 almost the range for all sites sampled. Temperature does affect other parameters such as pH and
107 dissolved oxygen. Thus, pH might have been influenced by temperature differently depending on
108 its value without temperature included. A site with low DO would be strongly affected by
109 increasing temperature resulting in progressive lower available oxygen. On the other hand
110 streams with high DO, would likely be less affected by increasing temperature. Dissolved
111 oxygen is one of the most important water quality factors as it can be the sole reason that fish

112 and other aquatic organisms can or cannot survive in water. Water that is classified as healthy
113 has a DO between 6.5-8 mg/L. Temperature influences DO concentration, as temperature
114 decreases there is increasing availability of dissolved oxygen. In contrast increasing temperature
115 decreases available oxygen in water (Dissolved Oxygen, 2019). This can have an effect on
116 lamprey abundance as DO levels can directly limit the amount of fish that can survive in a body
117 of water.

118 The pH varied between sites. When temperature increases, the pH of water tends to
119 decrease as the increased molecular vibrations cause more water to ionize, forming hydrogen
120 ions which decreases pH (Westlab, 2017). This is consistent with what is observed in Table 2 as
121 Little River (Highway 97) is the only outlier as it had the lowest temperature (9.4C°) among
122 sampled sites but had a pH of 6.94, which is average. The pH values between Table 1 and 2 were
123 similar as all values in table 2 are between the min and max for the three creeks tested other than
124 Hominey Creek at 7.67 (0.17 higher than the highest pH value in table 1).

125 Nitrate and phosphate concentration have a large impact on water quality. Nitrate and
126 phosphate travel via runoff to water ecosystems; high concentration of these nutrients increases
127 algal growth that could lead to eutrophication and fish kills (What is eutrophication, 2019). Mean
128 concentration of nitrate in water throughout the US ranges from 0.12 mg/L to 2.2 mg/L (EPA,
129 n.d). At concentrations below 1 ppm, eutrophication is not expected (Ozhan and Koray 2020).
130 For our streams measured, nitrate levels ranged from undetected to 0.17 ppm, meaning that
131 nitrate would not affect the water quality and would not promote algal growth. Phosphates range
132 from undetected to 3.02 ppm which is the biggest changes in value within a parameter
133 throughout all parameters. Five of the 10 sites had a phosphate level > 2.0 ppm with two sites
134 being overrange (>3.0 ppm; Table 2). Streams with concentrations of phosphate > 3.0 ppm will

135 likely have an effect on water quality and increase algal growth. High concentration of phosphate
136 in water is likely a result of road and farm runoff (EPA, 2019). Additionally, phosphate in soil
137 near the streams might affect phosphate in water. This can directly affect lamprey abundance as
138 eutrophication takes up the dissolved oxygen in the water which can kill off fishes including
139 lamprey if severe enough.

140 Copper is an essential nutrient at low concentrations; but, when its level gets too high it
141 can become toxic to aquatic organisms (Aquatic Life Criteria, 2019). Copper gets into water
142 from copper pipes corrosion, pesticides runoff, and antifouling paints that were used to coat ships
143 and buoys (Aquatic Life Criteria, 2019). Copper can naturally get into water as it may be
144 naturally deposited in the soil and can get into the water through erosion or weathering (Aquatic
145 Life Criteria, 2019). Copper levels as low as 1.4 ppm can cause a physiological stress response in
146 some fish that are fairly sensitive to metals (Solomon, 2019). Of the copper levels recorded none
147 come close to 1.4 ppm, meaning copper should not have a noticeable effect on any stream (Table
148 2).

149 Iron occurs naturally in the water at low levels and generally only rises to high levels due
150 to pollution. Iron is needed for photosynthetic and heterotrophic organisms as it is critical for
151 DNA replication, and hemoglobin production (Our Need for Iron, 2020). Iron also promotes
152 algal growth which can block sunlight for submerged macrophytes or lead to eutrophication. Iron
153 in high quantities can cause the pH of the water to become more acidic (Andromeda, 2019). Iron
154 was found under 0.20 ppm at all sampling sites meaning that iron should not be an issue for
155 harming aquatic life (Table 2).

156 Chlorine even in a small amount can cause chemical burns on the gills and can get into
157 the fish's body causing burns throughout their body (The Safest, 2021). These burns can cause

158 the fish to become stressed which compromises their immune response. Chlorine overexposure
159 at levels >0.3 ppm can cause respiratory issues in fish (Costa, 2019). This means all sites other
160 than Rocky Branch should have no problem with chlorine. It is important to point out that all
161 tests were only done once, meaning that while Rocky Branch was at 0.05 ppm and many other
162 sites were around 0.2 ppm – 0.3 ppm these levels could fluctuate drastically based on where in
163 the stream water was tested and if anything was recently dumped into the stream. Multiple tests
164 would need to be done to decide if chlorine is at high enough levels to cause issues in these
165 streams.

166 Due to water and soil connection, soil plays a role in influencing water quality by acting
167 as a sponge that can purify or filter water (Groh, 2021). Compounds (e.g., nutrients,
168 agrochemicals) will likely be transported into the water via runoff. Soil conservation practices
169 that aim to reduce water movement from the field to water sources have a direct influence in
170 water quality and corresponding water health. Nitrate had a range of 10 lb/a to 60 lb/a between
171 sites which are all ranked from low to average (Table 2). Phosphates ranged from 25 lb/a to 200
172 lb/a showing a similarly extreme range between sites as seen in water (Table 2). Interestingly the
173 two sites that had overrange phosphate soil levels (Above 3.0), Buffalo Creek (Mitchell Mill)
174 and Mystery Lake, had water phosphate levels of 25 and 75 lb/a detected. This supports the
175 theory that the soil and water parameters have little to no effect on each other. Two of the three
176 sites with highest soil nitrate values had no nitrate detected in the water. The data overall seems
177 to show that there is no connection between the soil and water parameters for nitrate or
178 phosphate.

179 DO was not measured at sampling sites; however, all 10 sampling sites are connected
180 either by geographical range or tributaries and streams to one of the four streams in Table 1. The

181 range of mean DO was 6.7 to 8.5 mg/L. Maximum DO have a range of 11.2 – 12.7 mg/L (Table
182 1). Minimum DO have a range of 3.4 to 6.3 mg/L. We can assume based on this data that the
183 four streams were very similar in DO and because of that the 10 sites would most likely have a
184 similar DO level to one another.

185 Lamprey were detected at four sites (Figure 3). However, these four sites have no water
186 quality parameters in common across the group. Rocky Branch is the only site where mating
187 occurred, so it can be inferred that Rocky Branch has one or multiple parameters that are
188 preferred by lamprey. Sampling of all parameters and lamprey was only performed once. This
189 means any conclusion drawn from these data may not hold true after sampling has been repeated
190 many times.

191 Two variables that were not measured that may have played a role in the effectiveness of
192 each stream for lamprey spawning are the sediment on the bottom of the stream and flow speed
193 of water. *Lampetra aepyptera* prefer slow-moving water when mating and lamprey make their
194 nests out of small stones, sand, and/or fine gravel. If the flow was too fast or the soil was too
195 hard to move, they may avoid spawning in that area or fail to spawn.

196 A limitation of this research is that the water and soil parameters were only sampled
197 once. Repeating tests multiple times provides the most accurate data and allows for outliers to be
198 discounted. Lamprey sampling in general is the biggest limitation as one sampling session does
199 not accurately display how many lampreys are in the area, and spawning times can last a few
200 months, meaning a stream with 0 lamprey could have many in the next few weeks. DO was not
201 measured at the sampled sites so we can only assume their values based on data from the past.
202 While this study can be useful to determine how water quality affects lamprey presence,
203 pollutants concentration needs to be analyzed to improve predictive power of lamprey spawning.

204 From the data gained from this study we can infer that Rocky Branch stream has adequate
205 conditions compared with the other streams that are conducive to lamprey presence. How water
206 quality affects lamprey abundance has not been determined based on limited data and lack of
207 connections between lamprey found and parameters among sites.

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	Contentnea Creek			Neuse River			Walnut Creek			Swift Creek		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Temperature (C°)	6.8	27.5	19.8	8.7	29.8	20.6	7.1	26.7	19.3	6.6	29.6	19.5

Dissolved Oxygen (mg/L)	4	11.4	7.2	6.3	12.1	8.5	6.2	11.2	8.5	3.4	12.7	6.7
pH	5.6	6.9	N/A	6	7.3	N/A	6.3	7.5	N/A	6.4	7.3	N/A

299 **Table 1:** Minimum, maximum, and mean temperature (C°), dissolved oxygen (DO, mg/L), and
300 pH measured at Contentnea Creek (35.5861, -77.8111), Neuse River (35.8479, -78.5302),
301 Walnut Creek (35.7493, -78.5345), and Swift Creek (35.6476, -78.6041)

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312 **Table 2:** Water parameters (pH, temperature, dissolved oxygen, nitrate, phosphate, chlorine,
313 copper, and iron) measured at Buffalo Creek (Mitchell Mill and UT), Rocky Branch, Little River
314 (Green Pace, Highway 97, Riley Hill, and UT), Hominey Creek, Mystery Lake, and Snipes
315 Creek No detection (-), Overrange (>)

Site	pH	Temperature (C°)	Nitrate (ppm)	Phosphate (ppm)	Chlorine (ppm)	Copper (ppm)	Iron (ppm)	Chromium (ppm)
Buffalo Creek (Mitchell Mill)	6.75	13.9	-	>	0.02	0.07	0.19	-
Buffalo Creek (UT)	6.76	13	0.03	-	0.01	0.07	0.16	-
Rocky Branch	7.36	11.8	0.13	2.07	0.05	0.02	0.12	-
Little River (Green Pace)	7.07	10.8	0.03	-	-	0.05	0.18	-
Little River (Highway 97)	6.92	9.4	0.17	2.41	0.02	0.03	0.21	-
Little River (Riley Hill)	7.09	11.6	0.03	-	0.03	0.03	0.15	-
Little River (UT)	6.8	12.7	0.03	0.04	0.02	0.19	0.09	-
Hominey Creek	7.67	10.5	0.07	0.56	0.02	0.02	0.05	-
Mystery Lake (UT)	6.73	12.7	0.10	>	-	0.11	0.25	-
Snipes Creek	7.36	11.5	0.03	3.02	-	0.18	0.21	-

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321 **Table 3:** Soil parameters (pH, temperature, dissolved oxygen, nitrate, phosphate, chlorine,
322 copper, and iron) measured at Buffalo Creek (Mitchell Mill and UT), Rocky Branch, Little River
323 (Green Pace, Highway 97, Riley Hill, and UT), Hominey Creek, Mystery Lake, and Snipes
324 Creek No detection (-)

Site	Nitrate (lb/a)	Phosphate (lb/a)	Potassium	Porosity
Buffalo Creek (Mitchell Mill)	60	25	-	6.68
Buffalo Creek (UT)	10	200	-	4.83
Rocky Branch	20	200	-	4.93
Little River (Green Pace)	20	75	-	6.85
Little River (Highway 97)	20	125	-	5.23
Little River (Riley Hill)	20	150	-	4.85
Little River (UT)	20	25	-	4.50
Hominey Creek	60	50	-	6.70
Mystery Lake (UT)	40	75	-	5.00

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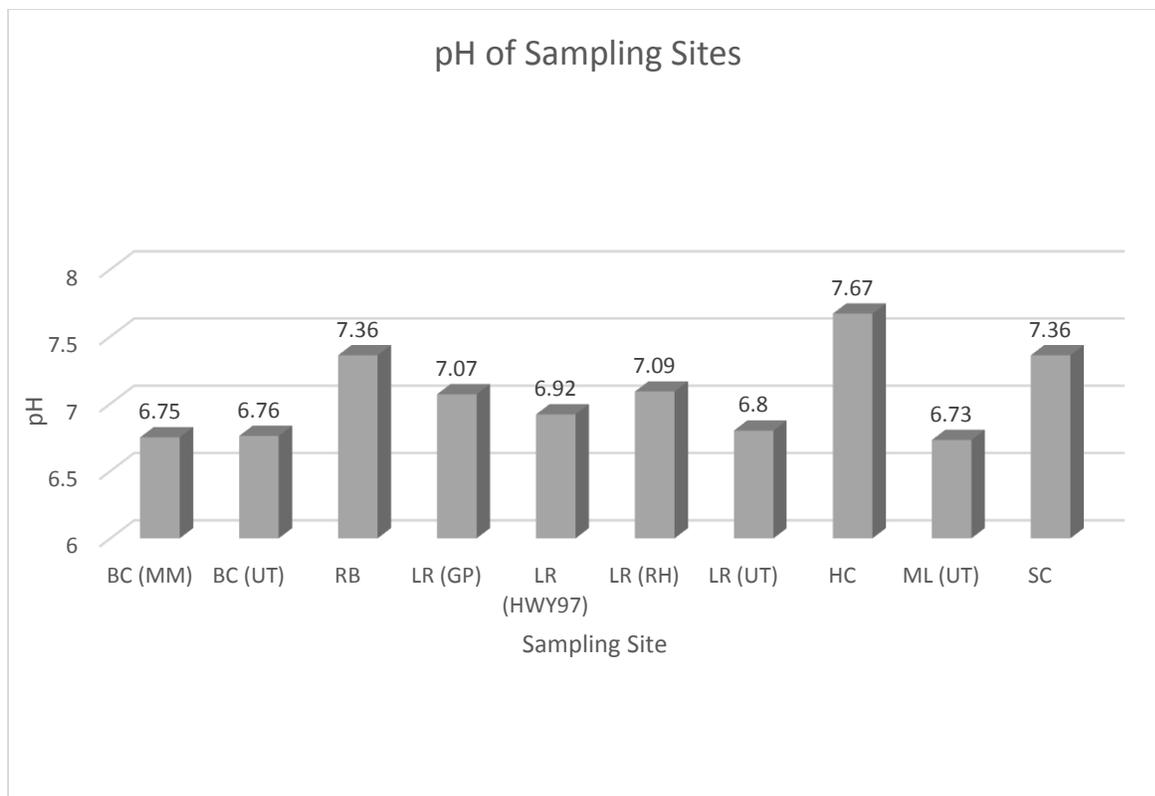
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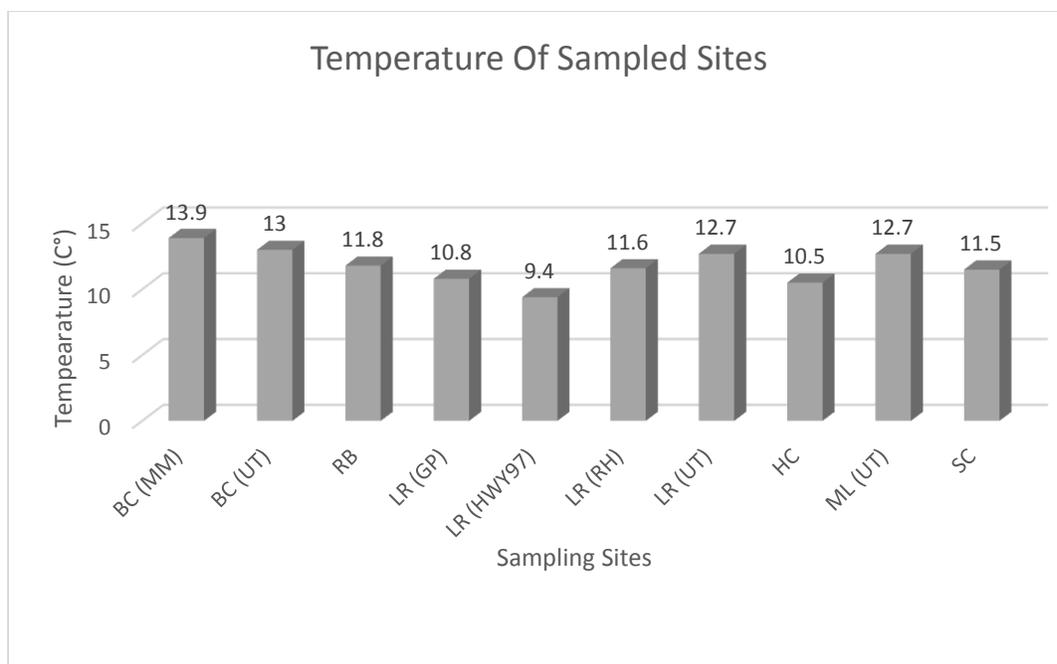
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339 **Figure 1:** pH from Sampling Sites. Site names were abbreviated and from left to right are
340 Buffalo Creek (Mitchell Mill Road), Buffalo Creek (UT), Rocky Branch, Little River (Green
341 Pace Road), Little River (Highway 97), Little River (Riley Hill Road), Little River (UT),
342 Hominey Creek, Mystery Lake (UT), and Snipes Creek.

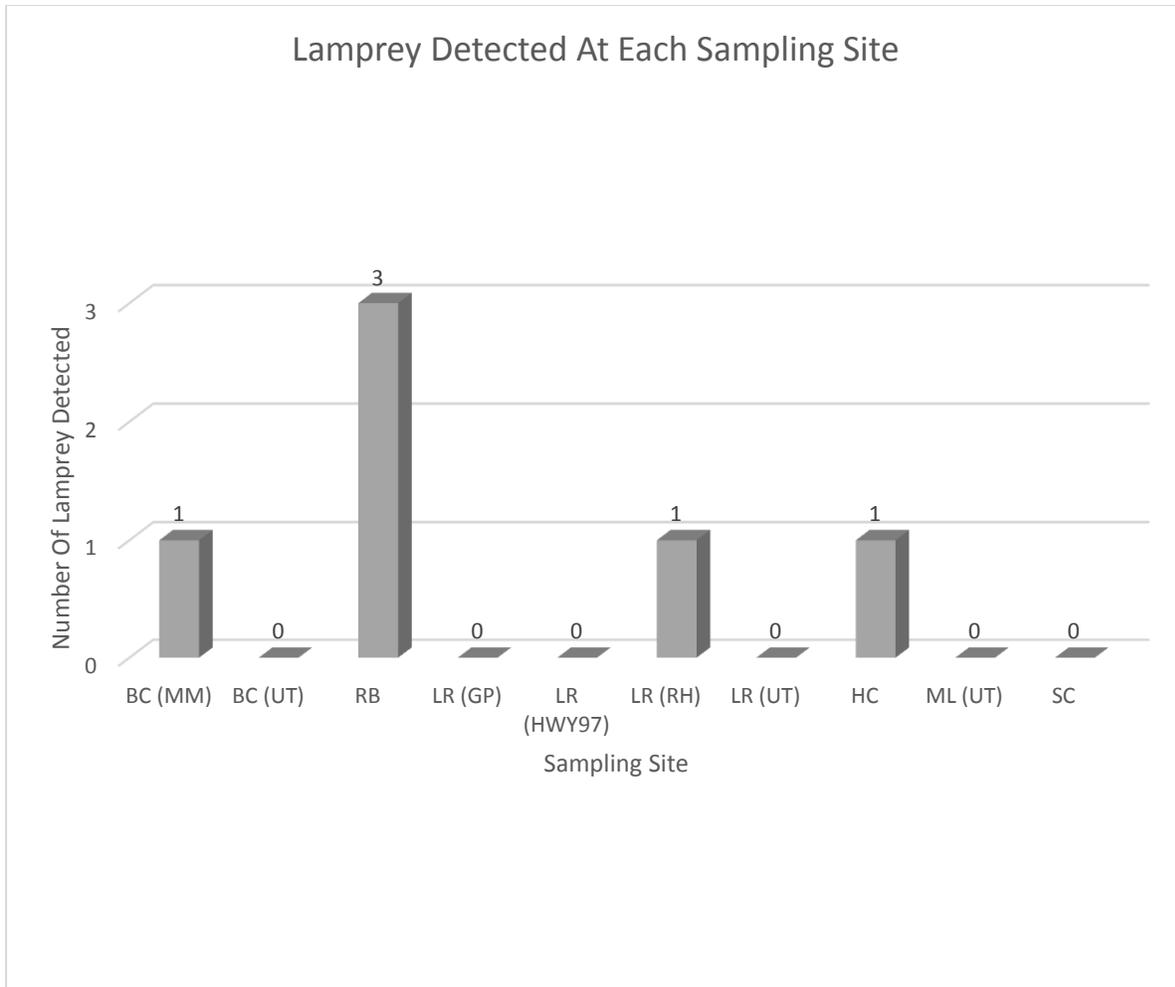
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345 **Figure 2:** Temperature from Sampling Sites. Site names were abbreviated and from left to right
346 are Buffalo Creek (Mitchell Mill Road), Buffalo Creek (UT), Rocky Branch, Little River (Green
347 Pace Road), Little River (Highway 97), Little River (Riley Hill Road), Little River (UT),
348 Hominey Creek, Mystery Lake (UT), and Snipes Creek.

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351 **Figure 3:** Lamprey observed at Each Sampling Site. Site names were abbreviated and from left
 352 to right are Buffalo Creek (Mitchell Mill Road), Buffalo Creek (UT), Rocky Branch, Little River
 353 (Green Pace Road), Little River (Highway 97), Little River (Riley Hill Road), Little River (UT),
 354 Hominey Creek, Mystery Lake (UT), and Snipes Creek.