De Silva_Semester Project

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Spawning habitat characteristics of the

Endangered Grotto Sculpin (*Cottus specus*) in hypogean streams

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Objectives

Grotto sculpin (*Cottus specus*) are a federally endangered troglomorphic fish species endemic to five cave systems of Perry County, Missouri (Adams et al., 2013). Their limited populations and restricted range make them very sensitive and susceptible to anthropogenic disturbance, and can be pushed to extinction following rapid population declines.Despite their importance, grotto sculpin life history and reproductive ecology has never been studied before. Understanding spawning habitat, minimizing disturbance and preserving existing habitat is vital to ensure the survival of this endangered species.

Habitat variability can be limited within hypogean streams. But anthropogenic disturbance can add sediments and contaminants from surface runoff which can alter the habitat and water quality along the stream channel (Bowles and Arsuffi, 1993).

For this project, we will evaluate observed spawning habitat characteristics and water quality to randomly selected sites along the stream channel to determine the preferred spawning habitat of the endangered grotto sculpin.

Based on findings from studies conducted on surface stream sculpin species (Simon and Brown, 1943; Johnston, 2001; Keeler and Cunjak, 2007), we hypothesize that grotto sculpin prefer to spawn in lower water temperatures, with higher dissolved oxygen concentrations, at below neutral water pH, and low conductivity levels. We also hypothesize that they prefer to spawn in shallow water depth with high water flow velocity. Additionally, we hypothesize that grotto sculpin prefer to spawn on bedrock substrate with minimal to no substrate depth.

Field Protocols

Three hypogean streams from Tom Moore, Mystery and Running Bull caves in Perry County, Missouri were studied to determine the spawning habitat of grotto sculpin. Nest sites were located by slowly walking upstream and visually inspecting rocks that had crevices and sediment cleared out underneath them (Figure 01). A nest site was defined by egg masses attached to the underside of a rock, with or without a fish guarding them (Figure 02). This method of site detection was determined based on similar spawning behavior of surface stream sculpin species (Smith, 1922; Simon and Brown, 1943; Morris, 1954; Savage, 1963; McCaleb, 1973; Goto, 1975, 1983; Jenkins and Burkhead, 1994)

After locating a nest site, water quality and habitat characteristic measurements were collected from each site. Water quality was measured using a Hydrolab MiniSonde 4a (Hydrolab Corporation. Austin, TX, USA). Parameters measured include: temperature (C^{o}), conductivity (µS/cm), dissolved oxygen (mg/L) and pH. For habitat characteristics; the substrate type around and under the nest was visually characterized into one of four types; bedrock, cobble, pebble, silt or mud. The substrate depth (cm) was measured using a standard ruler and pushing it into the substrate until it reached bedrock. Water depth (cm) was taken from the average of three measurements across the stream channel using a standard ruler. Water velocity (ms-1) was measured using a Flo-mate flowmeter (Model 2000, Marsh-McBirney Inc. Frederick, MD, USA). The wet and dry width (cm) of the stream channel was recorded using a standard tape measure. Additionally, site locations were recorded based on the cave's section number, which were then divided into three groups: upstream, middle and downstream.

Sampling methods were similarly repeated at random sites. Random sites were selected by walking upstream and randomly selecting rocks along the entire length of the stream channel. Sampling efforts continued until nests were no longer observed.

Response Variable(s)

The presence of nests will be used as the response variable. Based on the water quality and habitat data collected between nest and random sites, evaluating the differences between the two will help determine the preferred spawning habitat of grotto sculpin.

Predictor Variable(s)

Water quality and habitat characteristics were defined as the predictor variables, specifically:

- Water quality:
  - Temperature, conductivity, dissolved oxygen and pH (continuous variables).
- Habitat characteristics:
  - Substrate type around and under the nest (bedrock, cobble, pebble, silt or mud) (categorical variables)
  - Substrate depth, water depth, water velocity as well as wet and dry width of the stream channel (continuous variables)
  - Cave location (upstream, middle and downstream) (categorical variable)

Inferencial Procedure

Since nest site water quality and habitat characteristics will be compared to random sites, a generalized linear model (glm) within the binomial family will be used to evaluate the relationships between the response and predictor variables. In data collection, observed nest sites will be coded = 1 and Random sites = 0. Based on the binomial model output, we can then calculate the probability of observing a nest site given the observed water quality and habitat characteristics. Thereafter, determine the preferred spawning habitat of the grotto sculpin.

With the array of water quality and habitat predictor variables, multiple candidate binomial models will be fitted to cover every possible combination of response and predictor variables as an additive function. This will be done with a maximum of two predictors per model to minimize uncertainty.

We will then use Akaike's Information Criterion (AIC) model selection techniques to determine which candidate model(s) has the best approximation of the data-generating process from all models. The selection will be based on the lowest information criterion score and will be performed used the

"AICcmodavg" package in Base R. Thereafter, the model(s) outputs will be used to interpret the relationships between the response and predictor variables, calculate null hypothesis significance testing and to predict response based on changes in predictor variables.

All of this will be performed using Generalized Linear Models (glm) within the Binomial model family, in Base R (version 4.0.4).

Results

From our sampling methods, there were 25 nest sites observed across all three hypogean streams. Water quality and habitat data from these nest sites were recorded alongside 250 random sites dispersed across all three streams.

To fit Binomial models of all possible combinations of the water quality and habitat data, we used the following formula:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2$$

Where the response variable (y) was the presence or absence of nest sites given the predictor variables (x). Predictor variables were limited to two variables per model in order to reduce uncertainty.

Based on the AIC scores of all models (Table 01), model 36 had the lowest AIC score (54), which was 14 points lower than the next closest model. Therefore, of all possible model combinations limited to two predictor variables as additive functions, variables "Substrate around rock" and pH have the most influence on grotto sculpin spawning site selection.

To evaluate these relationships further, we calculated the probability of success of detecting a grotto sculpin nest for each of the predictor variables in model 36. This was calculated using th plogis function in Base R, based on the following formula:

$$p = \frac{exp(x)}{1 + exp(x)}$$

Based on these calculations at different levels of pH (minimum, median and maximum), we derived that grotto sculpin displayed 99.9% probability of nest site selection at pH = 6.26 (minimum) when substrate around nest was bedrock, a 32.5% probability at pH = 7.99 (median), and less than 1% probability of site selection at pH = 9.4 (maximum). Additionally, we also derived that when substrate around nest was not bedrock, the probability of nest site selection was 77.5% at pH = 6.26 and less than 1% at pH = 7.99 and 9.4.

For the null hypothesis that there is no significant effect of substrate around rock on nest selection, the p-value was < 0.05 thereby rejecting this null hypothesis. For the null hypothesis that there is no significant effect of pH on nest selection, the p-value was also < 0.05 thereby rejecting this null hypothesis as well.

We can therefore infer that the probability of successfully observing grotto sculpin nests is in areas around bedrock substrate in water that has lower pH (Figure 03). This corresponds to our original hypothesis for pH and substrate type.

Tables and Figures

Table 1: Top 5 lowest AIC scores

	Modnames	K	AIC	Delta_AIC	ModelLik	AICWt	LL	Cum.Wt
36	P36	6	52.03801	0.00000	1.0000000	0.9981503	-20.01900	0.9981503
14	P14	10	66.50901	14.47100	0.0007205	0.0007192	-23.25450	0.9988695
15	P15	10	66.50901	14.47100	0.0007205	0.0007192	-23.25450	0.9995887
34	P34	6	67.62953	15.59152	0.0004115	0.0004107	-27.81476	0.9999994
56	P56	7	80.89271	28.85470	0.0000005	0.0000005	-33.44635	1.0000000
30	P30	7	89.62800	37.58999	0.0000000	0.0000000	-37.81400	1.0000000

Figure 1: Nest sites were located by slowly walking upstream and visually inspecting rocks: (A) Hiking downstream to start sampling. (B) Measuring water quality and rock dimensions. (C) Photographing Grotto sculpin underwater.(D) Checking under rocks for Grotto sculpin nests.

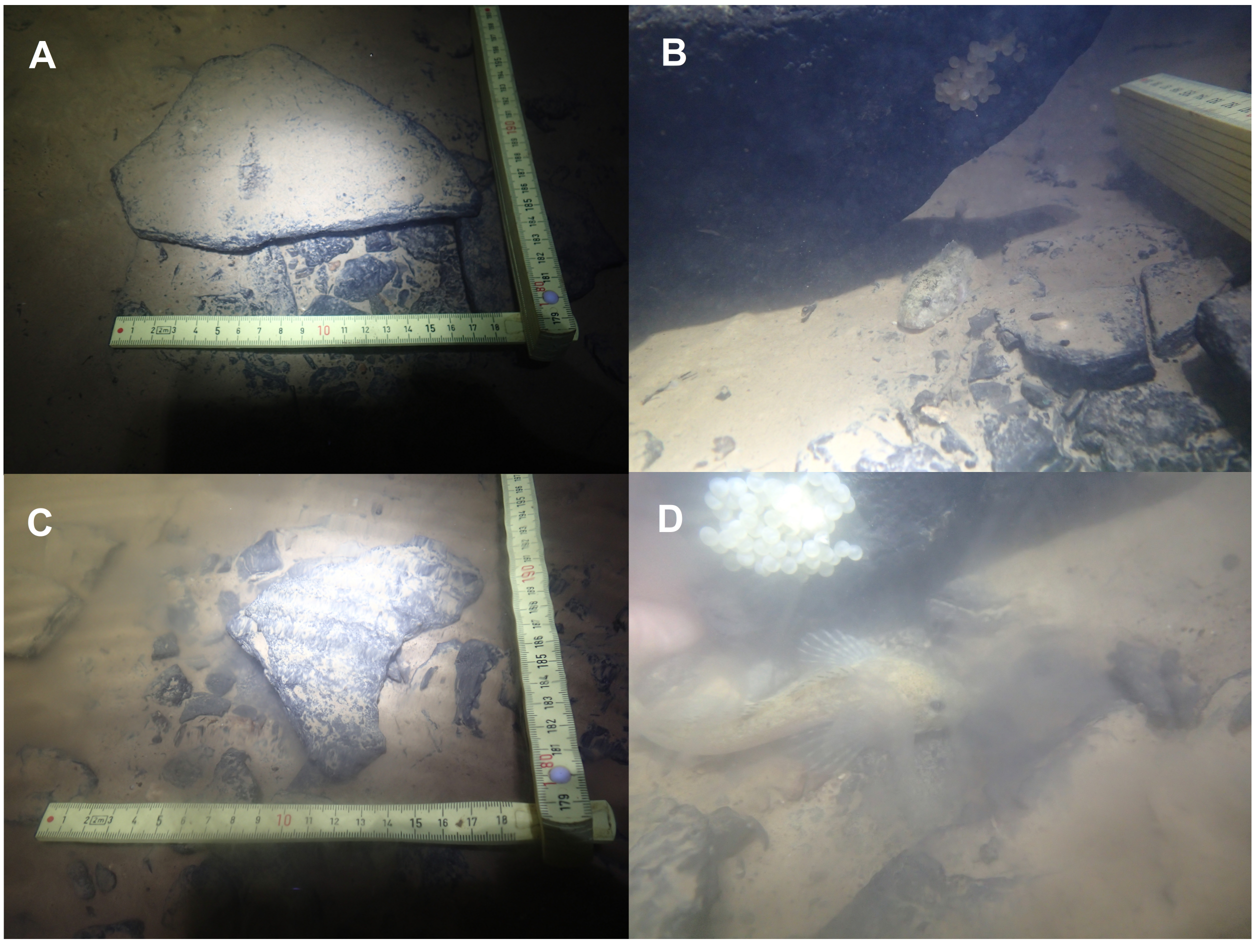

Figure 2: Grotto sculpin nests: (A, B) Nest sites viewed from above with cleared openings. (C, D) Egg masses attached to the underside of the rock with a fish guarding them

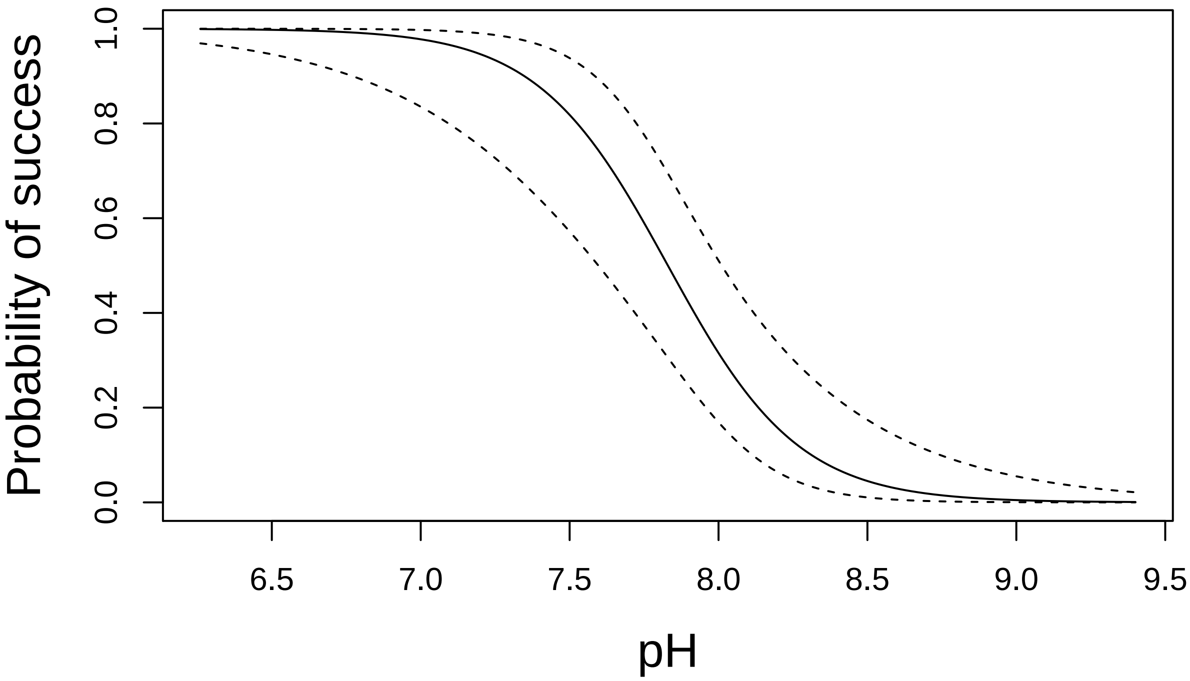

Figure 3: Probability of nest site selection in relationship to pH with substrate around as bedrock

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