The Effects of High Flow Rates on *Hexagenia limbata* (Ephermeroptera: Ephemeridae) and Their Burrows

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Introduction

Little is known about the burrows of the Giant Mayfly *Hexagenia limbata* ((Ephemeroptera: Ephemeridae) Serville, 1829) and the effects different variables can have on the burrow structure. Charbonneau and Hare (1998) considered the thin-spaced-chamber method (*e.g.*, Gallon *et al.* 2008) "fragmentary" because of the two-dimensional-like restrictions it puts on the construction of the burrow and thus the viewpoint (*e.g.*, Gallon *et al.* 2008; Pesch *et al.* 1995). In an attempt to create a more three-dimensional-like model, Charbonneau and Hare (1998) used x-rays to profile the burrow structures. The x-ray model did provide new insight into the burrow structure of *Hexagenia* spp. (*e.g.*, construction of the main burrow, abandoned burrows, and presumed feeding activity). In this proposed study, data obtained in multiple thin-spaced-chambers will compared to the data found by Charbonneau and Hare (1998) to determine whether the thin-spaced-chamber method actually alters the burrowing habits and structures.

If it is found that the use of the thin-spaced-chambers does not affect the burrowing behavior and structures of the nymphs, then this method will be used to determine the effects of high flow rates on the nymphs and their burrows. Many scientist predict, as a result of climate change, that there will be an increase in streamflow rate for many regions of North America (Climate Change: U.S. and Global Precipitation; Streamflow; Trends in Stream Temperature 2016; Hodgkins and Dudley 2006; Middelkoop *et al* 2001; Milly *et al* 2005; Novotny and Stefan 2007).

Literature Review

Species of the genus *Hexagenia* includes some of the best known mayflies. Their nymphal stages burrow in soft mud and later emerge as flying sub-adults ("subimagoes") *en masse*. Much research has been conducted on nymphal *Hexagenia* spp., including studies of their taxonomy, distribution, and ecology (Spieth 1941; Hunt 1953; McCafferty 1975; Berner 1977; Wallace and Merritt 1980; Schloesser and Nalepa 2002; and McCafferty *et al.* 2010; Devanna *et al.* 2013), methods for mass rearing (Fremling 1967 and Váncsa *et al.* 2015), use in sediment bioassays (Harwood *et al.* 2014), the function and importance of gills (Morgan and Grierson 1932), effects of varying degrees of temperature, oxygen content, salinity, food quantity, density, and photoperiod (Morgan and Wilder 1936; Lyman 1944; Nebeker 1971; Corkum and Hanes 1992; Giberson and Rosenberg 1992; Atkinson 1995; Winter *et al.* 1996; and Chadwick and Feminella 2001), and comparison of the effectiveness of various benthic samplers (Schloesser and Nalepa 2002). If the burrows are mentioned, most authors indicate only that *Hexagenia* spp. create U-shaped burrows. Only a few dealt with other aspects of the burrows (Hunt 1953; Wright and Mattice 1981; Charbonneau and Hare 1998; Gallon *et al.* 2008).

Although the rudimentary shape and structure of the burrows is U-shaped, Charbonneau and Hare's work (1998) making a 3D model using x-rays showed that the burrow is U-shaped only in the simplest of terms. While the main burrow does indicated a U-shape, the model shows that *Hexagenia* spp. actually create multiple burrows within the same system only in a matter of days. The burrows can serve many purposes, ranging from, but not limited to: feeding, metabolite removal, and avoidance of the toxic effects of sulfide in anoxic poor waters (Kristensen 1988; Riisgård and Larsen 2005; Meysman *et al.* 2006), biogeochemical cycling of nutrients (Canavan *et al.* 2006) and metals (Boudreau 1999), increase in oxygen concentrations (Gallon *et al.* 2008), and protection from predators (Spieth 1941; McCafferty 1975; Charbonneau and Hare 1998, Gallon *et al.* 2008).

Methods

Nymphs of *Hexagenia* spp., substrate, and water will be collected from Wildcat Creek. The upper 16 cm of substrate will be collected using an Ekman grab sampler (or a device of similar function that may be in storage). The sediment will then be sieved through 1-mm-mesh net to remove any macroinvertebrates and large debris, which will then be placed into the thin-spaced-chamber. Each thin-spaced-chamber will be only slightly wider than the width of the nymph in an attempt to make all portions of the burrow visible. The water will be held at 10°C to prevent emergence during the study. The thin-spaced-chambers will be immersed inside a large aquarium, which serves as a way to limit physiochemical variations in the water overlying the sediment (Gallon *et al.* 2008). The bottom portion of the side of the chamber will be covered, allowing no water flow, while the top will have a removable netting. At the minimum, the height of the chamber needs to reach 14 cm since the maximum recorded depth of a burrow of *Hexagenia limbata* is 12.7 cm (Hunt 1953), with a mean burrowing depth of 4–5cm (Hunt 1953; Charbonneau and Hare 1998; Gallon *et al.* 2008). The length of the chamber will be at a minimum 14 cm since the average burrow length for *Hexagenia limbata* is ~12 cm (Charbonneau and Hare 1998). An air stone will be used to provide oxygen. There is a positive correlation between nymph length and burrow length (Charbonneau and Hare 1998), therefore to make an accurate comparison between the two methods, nymphs of the same size as in the Charbonneau and Hare study (1998) will be used in this study, 25.0 mm ± 0.8, assuming a population of *Hexagenia limbata* can be found. Observations and photographs will be taken for the first three days to determine if the chamber method creates a "fragmentary" view (Charbonneau and Hare 1998). Some species will be allowed to develop their burrows longer in order to further document the structural change over time.

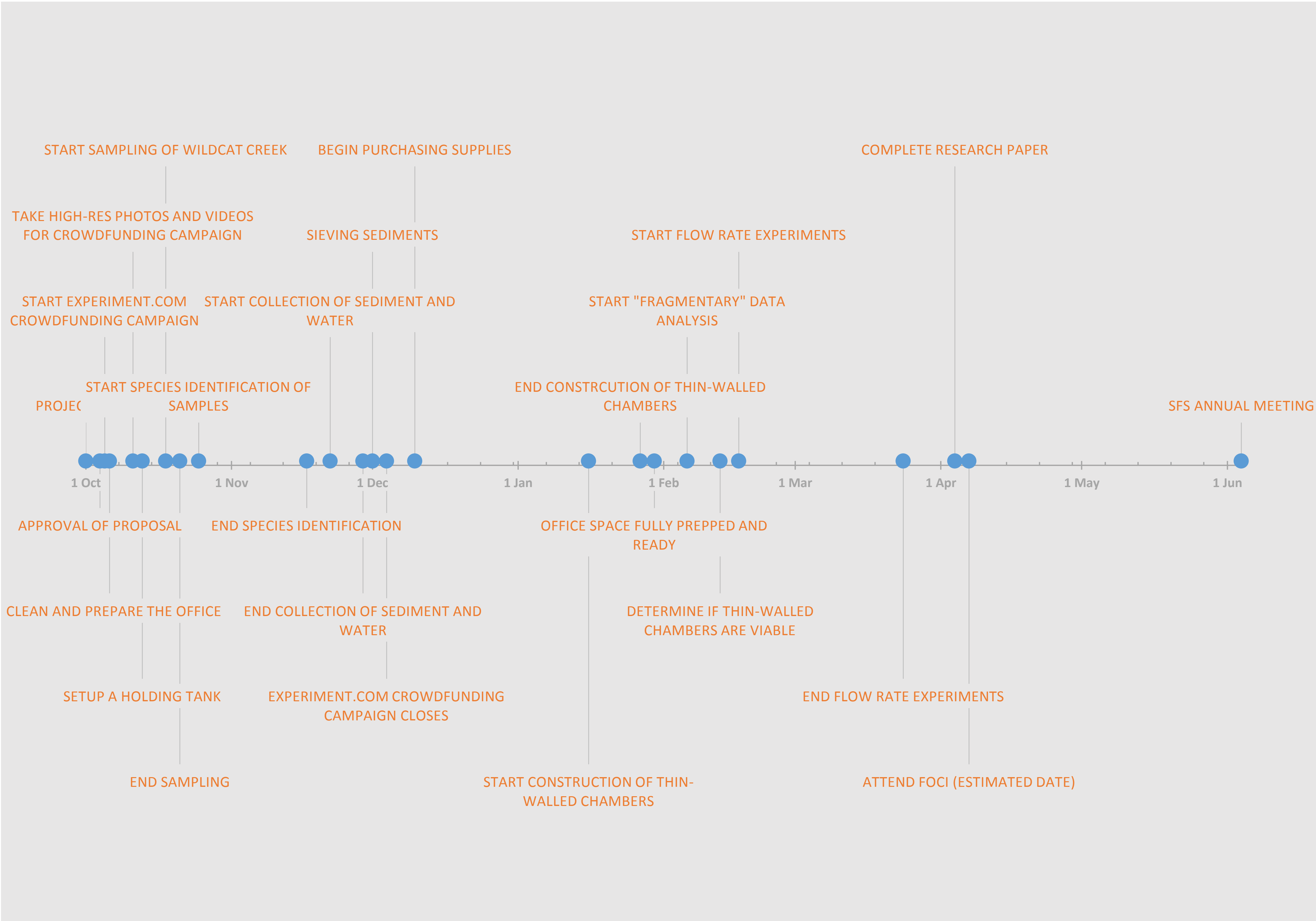
In order to test the effects of high flow rates on the nymphs and burrows, an aquarium circulation pump will placed in the aquarium, directed at the thin-spaced-chambers. The pump should have a variable speed to simulate different flow rate regimes. Flow rate meters will be placed inside to measure the change. Data, photographs, and video will be recorded to observe how the different flow rate regimes affect the nymph and the burrow and will then be compared to the burrows of the comparison test to discern the outcome. This portion of the study will also use nymphs with a length of 25.0 mm ± 0.8. Tests to determine effects of nymphal length will be attempted if time and resources permit.

Budget

Item	Cost	Number	Total Cost
Aquarium (10gal)	15	10	150
Plexiglass (1/4x24x24)	22	10	220
Mesh	15	1	15
Air Pump (Tetra 60-100gal)	20	2	40
Airline Tubing	5	1	5
Air Divider	7	1	7
Airstone (2 Pack)	4	5	20
Aquarium Fans (May not be needed)	20	10	200
Circulation Pump (?GPH)	17	10	170
Fish food	10	1	10
HOBO 64K Pendant (Temperature Data Logger)	60	3	180
Pendant Coupler	70	1	70
Boundary Layer Flow Meter	200	1	200
Wildco Might Grab Sampler	340	1	340
Travel to Site	75	1	75
SFS Student Membership (Early Rate)	290	1	290
Travel to SFS Annual Meeting	100	1	100
Hotel for SFS Annual Meeting	120	6	720
Total Cost of Research Project	**2812**		

* This is an estimated total cost and can be lowered if cheaper alternatives are found (*e.g.* grab sampler, flow meter, and hotel) or if items should not be included (*e.g.* SFS membership and travel and lodging to SFS meeting)

Timeline


START SAMPLING OF WILDCAT CREEK
BEGIN PURCHASING SUPPLIES
COMPLETE RESEARCH PAPER
TAKE HIGH-RES PHOTOS AND VIDEOS FOR CROWDFUNDING CAMPAIGN
SIEVING SEDIMENTS
START FLOW RATE EXPERIMENTS
START EXPERIMENT.COM CROWDFUNDING CAMPAIGN
START COLLECTION OF SEDIMENT AND WATER
START "FRAGMENTARY" DATA ANALYSIS
START SPECIES IDENTIFICATION OF SAMPLES
END CONSTRCUTION OF THIN-WALLED CHAMBERS
PROJEC
SFS ANNUAL MEETING
1 Oct
1 Nov
1 Dec
1 Jan
1 Feb
1 Mar
1 Apr
1 May
1 Jun
APPROVAL OF PROPOSAL
END SPECIES IDENTIFICATION
OFFICE SPACE FULLY PREPPED AND READY
CLEAN AND PREPARE THE OFFICE
END COLLECTION OF SEDIMENT AND WATER
DETERMINE IF THIN-WALLED CHAMBERS ARE VIABLE
SETUP A HOLDING TANK
EXPERIMENT.COM CROWDFUNDING CAMPAIGN CLOSES
END FLOW RATE EXPERIMENTS
END SAMPLING
START CONSTRUCTION OF THIN-WALLED CHAMBERS
ATTEND FOCI (ESTIMATED DATE)

PROJECT DETAILS	
DATE	MILESTONE
1-Oct-2016	Project Start
4-Oct-2016	Approval of Proposal
5-Oct-2016	Start Experiment.com Crowdfunding Campaign
6-Oct-2016	Clean and Prepare the Office
11-Oct-2016	Take High-Res Photos and Videos for Crowdfunding Campaign
13-Oct-2016	Setup a Holding Tank
18-Oct-2016	Start Sampling of WildCat Creek
21-Oct-2016	End Sampling
25-Oct-2016	Start Species Identification of Samples
17-Nov-2016	End Species Identification
22-Nov-2016	Start Collection of Sediment and Water
29-Nov-2016	End Collection of Sediment and Water
1-Dec-2016	Sieving Sediments
4-Dec-2016	Experiment.com Crowdfunding Campaign Closes
10-Dec-2016	Begin Purchasing Supplies
16-Jan-2017	Start Construction of Thin-Walled Chambers
27-Jan-2017	End Constrcution of Thin-Walled Chambers
30-Jan-2017	Office Space Fully Prepped and Ready
6-Feb-2017	Start "Fragmentary" Data Analysis
13-Feb-2017	Determine if Thin-Walled Chambers are Viable
17-Feb-2017	Start Flow Rate Experiments
24-Mar-2017	End Flow Rate Experiments
4-Apr-2017	Complete Research Paper
7-Apr-2017	Attend FoCI (Estimated Date)
4-Jun-2017	SFS Annual Meeting

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