



## Article

# Age Determination of Rocky Mountain Ridged Mussels (*Gonidea angulata*) in the Okanagan Basin, Canada

Paul Grant<sup>1,\*</sup>, Joy Wade<sup>1</sup>, Todd R. Lewis<sup>2</sup>, Rowshyra A. Castañeda<sup>1</sup>, Emma Branquinho<sup>1</sup>, Sean MacConnachie<sup>1</sup>, Stephen Wischniowski<sup>1</sup> and Barbara Campbell<sup>1</sup>

<sup>1</sup> Fisheries and Oceans Canada, Sidney, BC V8L 5T5, Canada; joywadefundyaqua@gmail.com (J.W.); rowshyra.castaneda@dfo-mpo.gc.ca (R.A.C.); emma.branquinho@dfo-mpo.gc.ca (E.B.); sean.macconnachie@dfo-mpo.gc.ca (S.M.); stephen.wischniowski@dfo-mpo.gc.ca (S.W.); barbara.campbell@dfo-mpo.gc.ca (B.C.)

<sup>2</sup> UWE Bristol—Frenchay Campus, Coldharbour Ln, Bristol BS16 1QY, UK; todd.lewis@uwe.ac.uk

\* Correspondence: paul.grant@dfo-mpo.gc.ca; Tel.: +1-250-217-5376

**Abstract:** Freshwater ecosystems and the biodiversity they support are facing unprecedented threats, exemplified by broad declines of freshwater mussels within a global biodiversity hotspot. The Rocky Mountain Ridged Mussel (*Gonidea angulata*) is an at-risk species in Canada, with limited information on population age structure. Maximum age of the species was found to be 50 years, by counting winter annuli and validated by isotopic oxygen analysis. Employing a Bayesian Generalized Linear Mixed Model (GLMM), results showed mussels from river habitat were predicted to have faster growth rates than mussels from lake habitats, highlighting the impact of local environmental conditions, including temperature variations, primary productivity, and water quality, on mussel growth dynamics. Of concern was the limited evidence of juvenile recruitment, with the majority of specimens potentially representing an ageing population. This pattern potentially signals an early warning of impending population decline. Our results underscore the necessity of monitoring age structure as a vital component of assessing population health of freshwater mussels and the importance of understanding local environmental conditions when determining age.

**Keywords:** conservation; mussels; age; mussel ecology; rocky mountain ridged mussel; western ridged mussel; *Gonidea angulata*



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## 1. Introduction

Freshwater ecosystems constitute a critical natural resource which provide essential ecosystem services and support significant, rich, and endemic biodiversity [1–3]. Despite their value, these ecosystems are among the most threatened on our planet, facing rapid global declines due to a combination of pressures such as pollution, habitat degradation, over-extraction of water, invasive species, and the impacts of climate change [1,4,5]. These pressures have led to increased threats to biodiversity, causing severe reductions in the range and population sizes of many freshwater species [4].

Freshwater mussels (Superfamily Unionoidea) represent one taxonomic group undergoing these global declines, particularly in North America, which is recognized as the global biodiversity hotspot for this group [3,6]. A common feature of these declines is a cessation of recruitment among mussel species, leading to ageing, non-viable populations, and, ultimately, the total loss of species as the remaining individuals perish [6]. For a mussel population to be considered healthy, evidence of recent recruitment should be apparent, consisting of a variety of age classes, from mature reproductive mussels to younger juvenile mussels [7]. Therefore, a significant knowledge gap hindering the understanding of what comprises a healthy mussel population is the population age structure, the relationship between shell length and age, as well as effect of local environmental factors on growth rate [7].

Although the specific causes of recruitment failure in mussels remain poorly understood [6], the reduction in their abundance and distribution has been consistently associated with human-induced threats to freshwater ecosystems [2,4]. Due to their sensitivity to a broad spectrum of environmental threats, freshwater mussels are considered to be key sentinel species, an indicator of the overall health and functionality of ecosystems [3]. They also provide vital ecosystem services, including water filtration, which enhances water quality, nutrient cycling, and contribute to the food web [8]. Consequently, monitoring freshwater mussels, with a particular focus on age distribution and recruitment within populations, serves as a critical indicator of population health, provides information on population viability and structure, growth rates, and acts as a barometer for the broader ecological health of freshwater ecosystems.

Rocky Mountain Ridged Mussel, *Gonidea angulata*, is an at-risk species of freshwater mussel endemic to western North America. Although the species was once commonly distributed within its range in the United States, it is now considered virtually absent from California and Nevada [9,10] and thought to be declining in Idaho, Oregon, and Washington [7]. In Canada, the species is mainly known to occur within the Okanagan basin in British Columbia, where it has been assessed as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) [10].

Despite known declines throughout its range, very little is known about the life history and age structure of this species. Ageing techniques for mussels involve counting growth rings, which are formed on an annual basis, corresponding to seasonal variations in environmental conditions. During periods of favourable conditions, typically spring and summer when water temperatures are higher and food resources are abundant, mussels grow more quickly depositing a layer of new shell material that is relatively thick and light in colour under transmitted light. Conversely, in less favourable conditions, growth slows down and thinner, darker layers are formed. The contrast between these layers form visible rings or annuli, which can enable precise ageing through cross sections of the shell. However, no studies have yet used stable oxygen isotopes to validate ageing techniques for our study species. The use of stable oxygen isotopes has been demonstrated to be an effective and accurate technique in ageing other mollusc species [11]. The inverse relationship between isotopic oxygen ( $^{18}\text{O}$ ) and temperature provides an ability to validate age by correlating higher isotopic oxygen with winter annuli. This approach not only validates ageing techniques, but also provides further insight into the life history of Rocky Mountain Ridged Mussel within the Okanagan basin.

## 2. Materials and Methods

### 2.1. Collection of Mussels

Rocky Mountain Ridged Mussel are characterized by unique morphological features that distinguish it from other mussel species found in its Canadian range, including a prominent ridge that extends from the umbo to the posterior margin of the shell. Rocky Mountain Ridged Mussel shells were collected from 2016 to 2023 in Okanagan Lake during surveys for this species at established index sites near Penticton [12]. Additional shells were collected from the Okanagan River between Oliver and Osoyoos in 2023. Only empty shells with both left and right valves attached were collected, given the current conservation concerns for the species. Efforts were made to select a broad range of shell sizes during collection events.

### 2.2. Age Determination and Validation

The left valve was measured using electronic callipers along the longest axis. A thin section of each shell was removed from the left valve at a 45° angle to the axis of longest growth. This region was selected to ensure the thin section cut would pass obliquely through the growth rings. Although Rocky Mountain Ridged Mussel shell valves are robust and can be easily handled, they are prone to delaminating when being sectioned and polished. In order to avoid delamination, epoxy was applied to the interior and exterior of

the valve along the 45° angle, forming a continuous thin layer along the blade path. Thin sections of 1 mm were made using an IsoMet 1000 precision sectioning saw (Buehler, Lake Bluff, IL, USA) and then were mounted on a glass slide using thermoplastic cement. Each thin section was then wet-polished using an Ecomet 3 grinder-polisher (Buehler, Lake Bluff, IL, USA) with 320 up to 600 grit silicon carbide paper. Once no saw blade marks were visible under a dissection microscope, a final hand polishing was conducted using aluminium oxide lapidary film (3 microns). After polishing, thin sections were 200–400 microns in thickness. Ages were then determined visually using a Leica dissecting microscope (Leica, Wetzlar, Germany) body on an illuminated base by counting winter annuli. Due to the potential for variation in clarity of bands across different counting axes from the umbo area to the mid-section and marginal edge, the whole thin section was examined.

Validation of annuli counts using stable oxygen isotopes was performed on a sub-set of mussels by the Canadian Centre for Isotopic Microanalysis (CCIM) at the University of Alberta for Secondary Ion Mass Spectroscopy (SIMS) technology. Once winter annuli were counted, thin sections were further reduced to 4 × 1 mm strips, targeting zones within each thin section that best illustrated an ageable pattern. High-resolution images were taken to assist in the interpretation of the annuli within the 4 × 1 mm strips. Measures of distance were compiled for postulated winter annuli and were compared to measures corresponding to <sup>18</sup>O values of ion beam targets.

### 2.3. Data Analysis

To investigate and describe the relationship between age and size of mussels, we used an ensemble model-based approach in R [13]. Boxplots of raw data were constructed to visualize differences between age class and length of mussels from lake and river. The linear relationship between age class and length was analyzed using Standardized Major Axis (SMA) testing, an established method for allometry [14,15]. Due to differences between river and lake sample size and possible problems in mean variance of data, we extended on SMA and applied a Bayesian Generalized Linear Mixed Model (GLMM) to confirm the hypothesis of size difference in mussels sampled from lakes and rivers. Bayesian GLMM has been used successfully on hierarchical ecological data for overcoming issues of mean variance, missing data, and small dataset sizes [16–20].

SMA used the R package *smatr* [21]. The SMA estimation assumed a linear relationship between  $y$  and  $x$  as  $Y = \alpha + \beta X$ , with the relationship becoming linear on the log-scale after transformation as follows:

$$\log y = \log \gamma + \beta \log x$$

We utilized Huber's M estimation in place of a least-squares method because it ensured a robust model fit whilst remaining inclusive for outliers [22]. Quantile and residual-fits plots were used to assess model fit [23], and Likelihood Ratio Tests (LRT) confirmed slope fit with 95% confidence intervals (CI) for coefficient estimates. The independent model used was the following:

```
ft1 = sma(Length ~ Age Class * Type, data = Allom, log = "xy", robust = TRUE)
```

This model reflects a typical GLM style formula, using Length as the response factor and Age Class by Type (lake or river) as predictor variables. It was log-transformed in both axis and sets Huber's M robust estimation.

For the Bayesian GLMM, Age Class and Length were long-formatted as integer count response factors, Type (lake and river) was used as a categorical predictor factor, and Specimen identification (ID) was applied as a categorical random effect. Data transformation used package *reshape2* [24]. Estimation was performed using Markov Chain Monte Carlo (MCMC) routines in software JAGS version 4.3.1 [25] through the R package *runjags* [26]. Models were expressed similarly to a frequentist log-linear model format and fitted as follows:

$$Y_i \sim \text{Poisson}(\mu_{ik})$$

$$\log(\mu_i) = a + \beta_1 \times \beta_2 \dots + a_i$$

$$a_i \sim N(0, 6^2_{Spec})$$

where  $k$  is the over-dispersion parameter,  $a$  is intercept,  $\beta_1$  and further  $\beta$  are fixed effect coefficients, and  $a_i$  is the randomized effect (Spec = Specimen ID). The independent model used was the following:

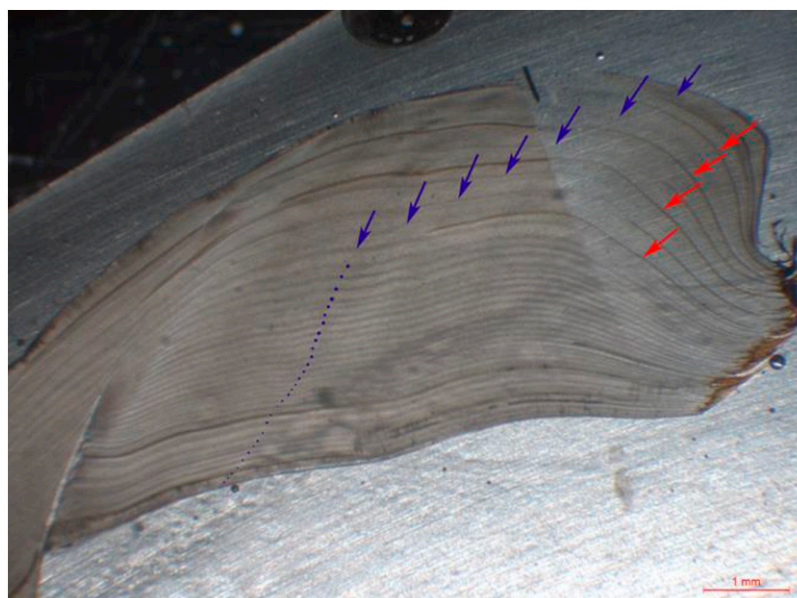
$$\text{Count} \sim \text{Factor} + \text{Type} + (1 \mid \text{Specimen})$$

Bayesian GLMM modelling comprised Poisson families running 40,000 iterations, 10,000 discarded for burn-in, and 4 chains. Minimally informed priors were set using templates within runjags modest automated uniform gamma distribution detected and set through JAGS (priors =  $\sim \text{dnorm}(0, 10^{-6})$ ). Convergence was assessed using MCMC trace plots of iterations retrieved from runjags and inspection of the Gelman-Rubin statistic potential scale reduction factor (psrf) [27]. Model assumptions of mean variance, log linearity, and potential autocorrelation were examined using convergence ensemble plots, and a correlation plot function within runjags. MCMC draws from posterior distributions were used for assessing model component relations. Factor interaction plots were constructed in ggplot2 [28] to contrast model coefficients and interpret findings.

### 3. Results

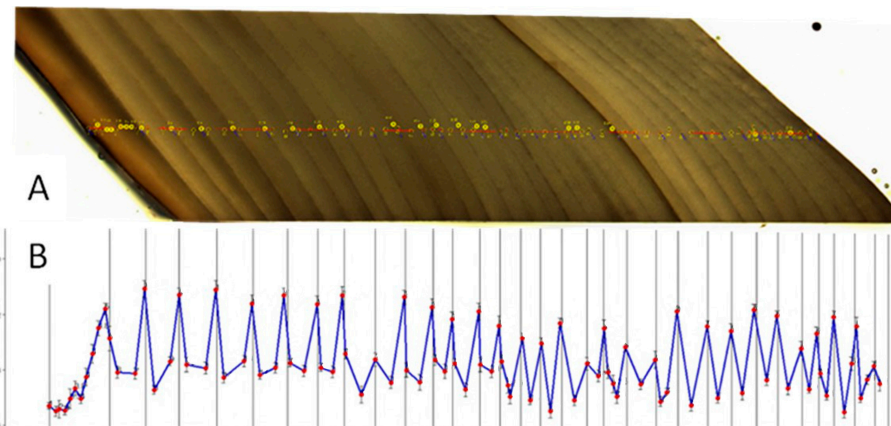
#### 3.1. Mussel Age Determination

Growth patterns on the cross-sections of Rocky Mountain Ridged Mussel shells were generally quite clear, but had some variation in clarity of bands across different counting axes from the umbo area to the mid-section and marginal edge. Often the mid-section was the clearest, with the umbo area displaying some ambiguity, particularly in the earliest years, which is possibly a result of torsion in growth during that phase. However, annuli growth was consistent and uninterrupted throughout the entire growth year. One issue that was encountered in the age estimation was cracks within growth zones of the umbo that looked deceptively like annuli (Figure 1). It is unknown if this is an artefact of using empty shells rather than those from live mussels. Therefore, the mid-section was consistently used for age determination in conjunction with the umbo.



**Figure 1.** RMRM growth pattern in umbo area showing discontinuous dark, fine bands (red arrows) between lighter, slightly thicker continuous bands (blue arrows) more in character with presumed annuli (blue dots).

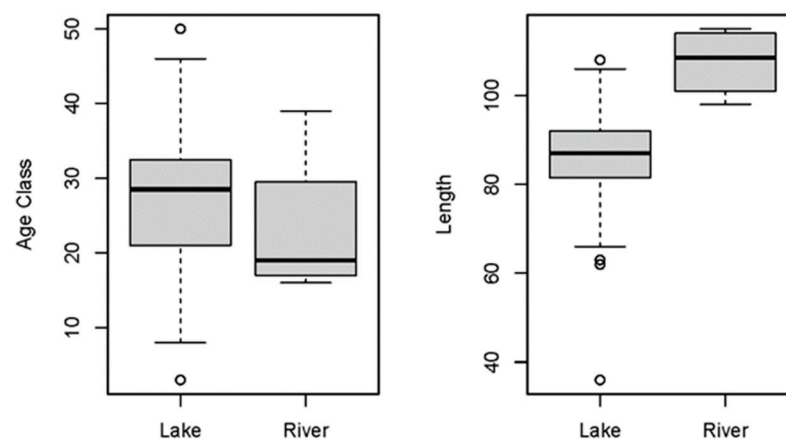
Nine samples from two specimens were submitted for secondary ion mass spectrometry (SIMS) assaying, containing samples from the umbo mid-section and marginal area. Measures of distance were compiled for postulated winter annuli and compared to measures corresponding to  $^{18}\text{O}$  values of ion beam targets. Again, mid-section results were the clearest and had the greatest alignment. Positive winter annuli counts were considered to be validated when there was alignment with postulated winter annuli and peak  $^{18}\text{O}$  values (Figure 2). While the validation sample size was small, the results indicated that the methods used to count winter annuli were successful due to the high frequency and consistency of the paired peak  $^{18}\text{O}$  values and winter annuli.



**Figure 2.** Mid-section thin section sample of (A) secondary ion mass spectrometry (SIMS) targets indicated by yellow circles. (B) postulated winter annuli represented by black vertical bars, while red dots indicate peak  $^{18}\text{O}$  value.

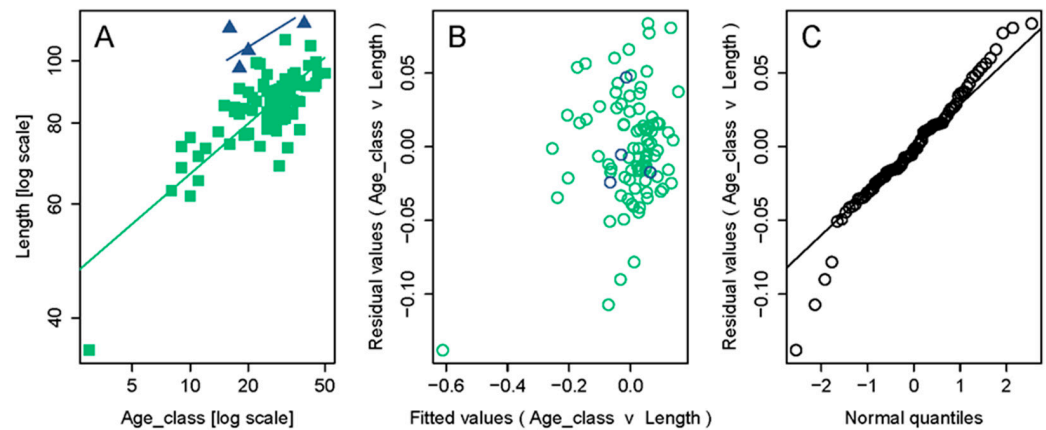
### 3.2. Mussel Age and Growth Patterns

A total of 98 mussel shells were collected and measured (lake:  $N = 94$ , river:  $N = 4$ ) from 2016 to 2023. A sample of 92 (88 lake specimens and 4 river specimens) were used for allometry (x6 lake specimens were removed due to incomplete measurements). From the sample, the maximum age class was 50 and the minimum was 3. Of the total 98 mussel shells, 42 specimens were 30 years old and older, including 16 that were 35 years and older. Conversely, 56 specimens were under 30 years old, including 11 that were between 8 and 15 years old, and 2 that were 6 and 3 years old. The maximum length of mussels was 114.1 mm, minimum was 35.64 mm, and mean was 86.07 mm. Initial boxplots of raw data revealed a difference in medians between lake and river, but overall spread of age class was similar (Figure 3). Length from the boxplots suggested that river specimens were larger in length than lake specimens (Figure 3).



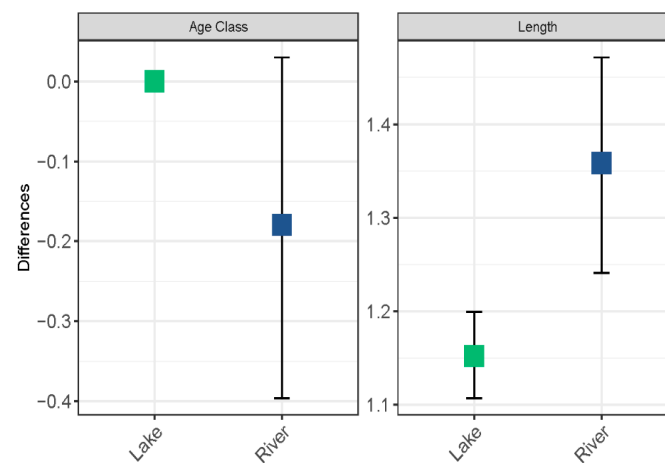
**Figure 3.** Boxplots of raw data showing Age Class and Length differences in mussels from Lake and River.

The SMA test showed a sufficient model fit for interpretation (Figure 4). An outlier of the smallest lake individual (length = 3) can be observed in the bottom left hand of the plot, yet this did not compromise the model fit. The slope fit of the model in the plot visually suggested that river mussel specimens are larger than lake specimens. However, LRT reported a non-significant test (LRT: 0.31,  $df = 1$ ,  $p = 0.57$ ), with coefficients showing significance for lake (R-squared: 0.61,  $p < 0.05$ ), but not for river (R-squared: 0.24,  $p = 0.511$ ).



**Figure 4.** Standardized Major Axis Test (SMA) showing River (blue triangles) being larger than Lake (green squares) specimens (A), and appreciable visual model fit from residual fits (green circles; lake, blue circles; river) (B) and quantile plot (black circles; residual values) (C).

To confirm the size difference statistically, a Bayesian GLMM was successfully deployed. The model fit results are reported in Supplementary Material 1.0 (Convergence Plots + Table). Convergence was successfully completed for all variables. Autocorrelation levels were minimal across all factors, helped by the hierarchical structuring nature of the GLMM. The coefficients and 95% CI are presented in a caterpillar plot (Figure 5). The plot confirmed the initial results in the boxplot of raw data. Modelled data revealed there were differences in mean age class, with river being smaller than lake. However, CI spread overlapped and signalled that age classes would likely be similar across the range of river and lake specimens (as per the boxplots). Length showed a robust and clear difference in mean (as per boxplots) and separated CI signalled a high probability that river mussels are predicted to have a faster growth rate than lake specimens (Figure 5).



**Figure 5.** Bayesian GLMM coefficients of effect of Lake or River on Age Class and Length. Plot confirms the size similarity of Age Class between treatment groups (represented by overlapping confidence intervals and data) and the difference in Length between treatment groups (represented by distinguished confidence intervals), with River having larger predicted specimens than Lake.

#### 4. Discussion

Freshwater mussels are a diverse and imperilled component of aquatic ecosystems, but little is known about their growth and longevity [29]. From a conservation perspective, a greater understanding of population structure, including shell length, age, and recruitment within freshwater mussel populations is critical to determine population health, potential risks of decline, and the effects of environmental stress [30,31].

While modern ageing and validation techniques [11,29,32] have increased our understanding of population structure with freshwater mussels, there is considerable variation in growth parameters across species. As a group, freshwater mussels are often portrayed as long-lived and slow-growing [33]. However, while some mussel species are known to reach advanced ages >50 years, many appear to have modest life spans of around 10 years [24]. Rocky Mountain Ridged Mussel is a relatively long-lived species, with maximum age ranges previously reported to be 20 to 30 years [10], 32 years old [32], and more recently 35 years old [7]. Results presented here include specimens up to 50 years old, which represent the oldest known specimens of Rocky Mountain Ridged Mussel.

To further add to the complexity of population dynamics, growth parameters within species of freshwater mussels are known to be greatly dependent on environmental conditions [31], with previous studies linking mussel growth rates to regional climate variability and environmental factors such as differences in temperature, primary productivity, and water quality [7,31,32,34]. Variation in productivity also occurs across habitat types, with river habitats tending to have, on average, higher productivity than that of lake habitat [35], and variations in shell shape and size associated with microhabitat conditions [31]. This aligns with the results in this study where Rocky Mountain Ridged Mussels within Okanagan River had faster growth rates, displaying greater shell lengths relative to their age, than specimens collected from Okanagan Lake. This variation highlights the importance of using data derived from local populations to inform specific spatial age structure dynamics and related conservation decisions.

One of the concerns with Rocky Mountain Ridge Mussel in the Okanagan Basin is the limited evidence of recruitment. While efforts were made to collect a broad range of shell sizes, only one shell was considered to be a juvenile (age = 3; 35.64 mm), with the vast majority of shells consisting of older individuals. While increase nutrient loading may have some initial benefits for productivity, extreme levels coupled with low water flows, high water temperatures, low dissolved oxygen, and high nitrogen (NH<sub>3</sub>) may result in survival problems for juvenile mussels [36], prevent recruitment, and result in eutrophication, which may kill adult mussels [3,6,36,37]. Temporal or spatial variability in recruitment of juveniles and remnant older populations therefore serve as an early warning of impending disaster for mussel populations [6,36].

Effective conservation efforts for mussels present a complex case problem that need to account for multiple factors, including their declining status, threats, and what constitutes healthy populations. Monitoring population dynamics for local mussel populations can provide some insight on population health and inform the development of targeted conservation measures to help ensure the sustainability of these endangered species. The above discussed factors of climate, environment, primary productivity, and water quality suggest future directions and data collection efforts to continue this work. Such further study may go some way to contrast *G. angulata*, its ecology, and eco-morphology with other freshwater mussel species and help complete our understanding of its natural history and conservation requirements.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d16070415/s1>, File S1: Convergence Plots + Table.

**Author Contributions:** Conceptualization, P.G., J.W. and S.M.; methodology, P.G., T.R.L., B.C. and S.W.; formal analysis, P.G. and T.R.L.; investigation, R.A.C. and E.B.; data curation, T.R.L.; writing—original draft preparation, P.G., J.W. and T.R.L.; writing—review and editing, P.G., J.W., T.R.L., R.A.C., E.B., S.M., S.W. and B.C.; project administration, P.G.; funding acquisition, P.G., S.M., R.A.C. and E.B. All authors have read and agreed to the published version of the manuscript.

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