Increased sexual dimorphism does not evolve in a fossil stickleback following ecological release from fish piscivores

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Abstract

Theory suggests that populations should expand their habitat- and resource-use niches when they are freed from interactions with competitors and predators–so-called ecological release. One way that this ecological release can manifest is through increased differences between males and females of the same species as they diverge into different niches. If divergent niche use drives corresponding divergent adaptation in in the traits used to exploit the divergent niches, then theory predicts an increase in sexual dimorphism for those traits, sometimes called character displacement between the sexes. Using a dataset of 16 traits collected from 18 temporal samples spread across 16,500 years of evolution of the fossil stickleback fish, *Gasterosteus doryssus*, we tested the prediction that release from predation inferred in this system would result in character displacement between the sexes. We used data from populations of extant stickleback (*Gasterosteus aculeatus*) to build an imputation model to classify individual sex in fossil specimens. Then, we quantified the extent of sexual dimorphism at each temporal sample, and tracked how it changed through time. NEED TO FINISH THIS

Keywords: character displacement, character release, evolutionary time series, *Gasterosteus doryssus*, missing data imputation

1 Introduction

Ecological release theory suggests that a population's niche should change when important species interactions like resource competition or predation are relaxed or removed (reviewed in Herrmann, Stroud, and Losos (2021)). Removal is posited to create ecological opportunity—i.e., aspects of the niche become newly accessible, and the focal population shifts and/or expands its resource use new resources (Parent and Crespi (2009); Herrmann, Stroud, and Losos (2021)). Ecological release may then be followed by adaptive morphological evolution as traits change to reflect the new niche (Parent and Crespi (2009); Herrmann, Stroud, and Losos (2021)).

For example, a population undergoing ecological release can experience disruptive selection on males and females stemming from intraspecific competition over newly accessible resources (Bolnick and Doebeli (2003); Bolnick and Lau (2008); Cooper, Gilman, and Boughman (2011)). This is predicted to result in intersexual divergence in habitat use and associated phenotypes (Schoener (1968); Shine (1989); Bolnick and Doebeli (2003); Butler and Losos (2007); Bolnick and Lau (2008); Cooper, Gilman, and Boughman (2011); but see Stuart et al. (2021); Blain (2022)). Such competition-driven "intraspecific character displacement" between the sexes is therefore one explanation for the evolution of sexual dimorphism (Pfennig and Pfennig (2012); De Lisle and Rowe (2015); De Lisle and Rowe (2017), De Lisle, Paiva, and Rowe (2018)).

Much of the theory and empirical data for character displacement between the sexes is based on release from resource competition specifically (e.g., Bolnick and Doebeli (2003); Cooper, Gilman, and Boughman (2011); Pfennig and Pfennig (2012); De Lisle and Rowe (2015); De Lisle, Paiva, and Rowe (2018)). However, release from predation might also drive the evolution of increased sexual dimorphism because an absence of predators should generate ecological opportunity (Reimchen and Nosil (2004); Parent and Crespi (2009); Herrmann, Stroud, and Losos (2021)).

Here, we tested the prediction that a release from predation results in the evolution of increased sexual dimorphism using a well-preserved, finely-resolved sequence of a fossil threespine stickleback fish (Gasterosteus doryssus). The sequence in this depositional environment is comprised of two lineages (Bell (2009); Cerasoni, Bell, and Stuart (2024)). Lineage I was a low-armor form with zero to one dorsal spines and highly reduced pelvises, on average. Lineage I lasted for at least 93,000 years before it was replaced by a second lineage, suddenly, likely on the order of years (Bell, Baumgartner, and Oslon (1985); Stuart et al. unpublished data). Lineage II appeared in the depositional environment fully armored, with complete pelvic girdles, two pelvic spines, and three dorsal spines (Bell, Travis, and Blouw (2006); Stuart, Travis, and Bell (2020)). It then immediately began evolving adaptive reduction in its armor traits (Hunt, Bell, and Travis (2008); Stuart, Travis, and Bell (2020)) until it

reached the same low-armor state previously held by lineage I.

The observation of 93,000 years of low-armor stasis in lineage I (Bell, Baumgartner, and Oslon (1985)) and the observation of rapid evolution of armor loss by lineage II, both suggest that this depositional environment lacked piscivorous fish like trout and other salmonids known to prey on modern threespine stickleback. Armor presence in extant threespine stickleback (Gasterosteus aculeatus) correlates strongly with the presence of vertebrate piscivores; populations with less predation pressure typically have less armor (Reimchen (1994), Reimchen and Nosil (2004); Bell et al. (1993); Roesti et al. (2023)). In our paleolake basin, only three fossil trout have been found in the same section of rock that has revealed >20,000 threespine stickleback fossils as well as occasional killifish (Fundulus nevadensis) (Bell (2009); Cerasoni, Bell, and Stuart (2024)). Thus, we reconstruct an evolutionar history in which it is likely that lineage II migrated from a nearby paleolake basin that had predators since it was armored when it arrived (Bell (2009); Cerasoni, Bell, and Stuart (2024)). Lineage II then experienced release from predators in the focal paleolake basin, generating the initial conditions of evolutionary models predicting the evolution of increased sexual dimorphism following ecological release. We test this prediction here.

A major challenge in the study of fossilized sexual dimorphism is assigning sex to individual specimens in the first place (Hone and Mallon (2017); Mallon (2017); Saitta et al. (2020)). This typically cannot be done directly, except for taxa whose sexes are distinguished by the presence or absence of sex-specific characters that preserve well. Instead, paleobiologists often resort to statistical detection of sex and sexual dimorphism, including tests for normality and bimodality in trait distributions (e.g., Mallon (2017)), mixture modeling (e.g., Mallon (2017)), divergence in growth curves (e.g., Saitta et al. (2020)), and emphasis on effect size statistics rather than significance testing (e.g., Saitta et al. (2020)). However, dimorphic signal can be masked by noise introduced by factors both biological and artifactual, including extended growth during ontogeny, age-based bias in survivorship, small sample sizes, time averaging, and taphonomic bias (Godfrey, Lyon, and Sutherland (1993); Koscinski and Pietraszewski (2004); Hone and Mallon (2017); reviewed in Mallon (2017); reviewed in Saitta et al. (2020)). Thus, the best approach to sex classification is likely one of total evidence (Saitta et al. (2020)), including comparison to closely-related, extant species of known sex (e.g., Hone and Mallon (2017); Saitta et al. (2020)).

For our study, we inferred sex in G. doryssus fossils by comparing multivariate morphological trait data from lineage II samples to the same multivariate trait set collected from multiple populations in the closely-related, extant Threespine Stickleback species complex (Gasterosteus aculeatus). Crucially, we determined G. aculeatus sex directly via dissection and/or PCR genotyping. This enabled us to use Multiple Imputation by Chained Equations (MICE; Buuren and Groothuis-Oudshoorn (2011)) to build a predictive multiple imputation algorithm (Little and Rubin (2002)) based on the multivariate morphology of G. aculeatus

individuals of known sex. We applied this algorithm to the fossil data to impute individual sex based on morphology, treating fossil sex as a missing variable. With sex assigned to fossil specimens, we fit a modified Ornstein-Uhlenbeck (OU) (Uhlenbeck and Ornstein (1930)) model using a Bayesian framework that accounts for uncertainty in sex classification to test for evolution of sexual dimorphism in each trait over ~16,000 years of lineage II.

2 Data

2.1 Fossil Specimen Data

We used Gasterosteus doryssus data that were previously reported by Stuart, Travis, and Bell (2020), Voje, Bell, and Stuart (2022), and Siddiqui et al. (2024). Briefly, the data were collected from fossil Series K from Quarry D (Cerasoni, Bell, and Stuart (2024)), dug from an open pit diatomite mine at 9.526° N, 119.094° W, near Hazen, Nevada, USA. Series K consisted of 18 samples taken at ~1000-year intervals, and mean sample times span ~16,363 years. Fish from series K were measured for 16 ecomorphological traits related to armor, swimming, and feeding (Table 1). Series K started at the previously documented horizon when lineage I was replaced by lineage II. The tempo and mode of lineage II armor reduction during this sequence suggests adaptive evolution by natural selection (Hunt, Bell, and Travis (2008)), and we focus on the multivariate evolution of sexual dimorphism by this second lineage.

The lineage II fossil data consist of 814 specimens of unknown sex sampled across the 18 K series samples. Figure 1 shows the sample size for each of the 18 samples. There are at least 22 specimens in each sample with a high of 67 specimens in sample 7.

Trait Name	Trait Code	Trait Description
Standard Length	stl	Distance from anterior tip of premaxilla to posterior end of last vertebra (i.e., the llhypural plate)
Dorsal Spine Count	mds	Number of dorsal spines
Dorsal Fin Ray Count	mdf	Number of bones in the dorsal fin posterior to the third dorsal spine (i.e., soft dorsal fin rays)
Anal Fin Ray Count	maf	Number of bones in the anal fin posterior to the anal spine (i.e., soft anal fin rays)
Abdominal Vertebra Count	mav	Number of vertebrae anterior to the first vertebra contacting an anal fin pterygiophore (Aguirre et al. 2014)

Trait Name	Trait Code	Trait Description
Caudal Vertebra Count	`mcv`	Vertebrae including and posterior to the first that contacts an anal fin pterygiophore (Aguirre et al. 2014)
Pterygiophore Count	`mpt`	Number of pterygiophores anterior to but excluding the pterygiophore under the third dorsal spine
Pelvic Spine Length	`lps.sc`	Length from the base of one pelvic spine at its pelvic girdle articulation to its distal tip
Ectocoracoid Length	`ect.sc`	Length from the anterior to posterior tips of the shoulder girdle base
Pelvic Girdle Length	`tpg.sc`	Length from the anterior to posterior tips along midline. If vestigial, the sum of longest axis along vestiges
Cleithrum Length	`cle.sc`	Length from dorsal tip to ventral tip on the anterior margin of the shoulder girdle
Premaxilla Length	`pmx.sc`	Length from the anterior tip of the premaxilla to the distal tip of its ascending process
Dorsal Spine Length	`Ds#.sc` `#=1/2/3`	Length from the dorsal spine base at the pterygiophore to its distal tip
Pterygiophore Length	`lpt.sc`	Distance from anterior to posterior edges of the pterygiophore immediately before ds3 (when present)

This table caption should be part of the table. Top row. at the very least, it should be the width of the table. -YS Table: Traits and trait descriptions. 'sc' denotes size correction of trait against standard length. Names of bones follow Bowne (1994) unless otherwise noted.

2.2 Extant Specimen data

To span the gamut of stickleback diversity for our predictive imputation model, we sampled modern stickleback from lakes containing generalist stickleback populations (Hendry et al. (2009); Bolnick (2011)) and from lakes containing benthic-limnetic species pairs (Baumgartner, Bell, and Weinberg (1988); Schluter and McPhail (1992)). The generalist specimens used here were collected by YES in 2013 and were previously described in Stuart et al. (2017) (Table S1). These samples were fixed in formalin, then stained for bone with Alizarin Red in 2013. Benthic and limnetic specimens were kindly loaned by D. Schluter and S. Blain at the University of British Columbia. The Schluter lab collected benthic and limnetic individuals from Enos Lake in 1988 and from Emily Lake, Little Quarry Lake, Paxton Lake, and Priest Lake in 2018 (Table S1). The Enos specimens had been fixed whole in formalin and stored in 40% isopropanol. The specimens from the other lakes were initially preserved

whole in 95% ethanol in the field before being gradually transferred to water then formalin in the lab and ultimately stored in 40% isopropanol. In 2019, we stained these specimens for bone using Alizarin Red.

We next replicated fossil data collection (Table 1) on these extant specimens. Standard length as well as pelvic-spine length on each side were measured with calipers. We used a dissection microscope to count dorsal spines, pelvic spines, dorsal-fin rays, and anal-fin rays. Right and left-side pelvic girdle lengths and ectocoracoid lengths were measured from ventral photographs taken using a Canon EOS Rebel T7 with a Tamron 16-300 mm MACRO lens mounted on a leveled Kaiser RS1 copy stand. Specimens were held in place for ventral photographs using a small tabletop vise with an attached scale bar. Lateral X-rays were used to measure dorsal spine length, number of pterygiophores anterior to the pterygiophore holding the third spine, length of the pterygiophore just anterior to the third spine, cleithrum length, and pre-maxilla ascending branch length. We also counted vertebrae from the X-rays: abdominal vertebrae were counted anterior to the first vertebra with a haemal spine contacting an anal fin pterygiophore. Caudal vertebrae were posterior, including the first vertebra with the haemal spine contacting the anal fin pterygiophore (following Aguirre, Walker, and Gideon (2014)). X-rays were taken with an AXR Hot Shot X-ray Machine (Associated X-ray Corporation) at the Field Museum of Natural History. Specimens were exposed at 35kV and 4mA. Small fish were exposed for 7s, medium fish for 8s, and large fish for 10s. We developed the film and scanned individual images of each fish using the B&W Negatives setting on an Epson Perfection 4990 Photo flatbed at 2400 dpi. Measurements from photographs and X-rays were taken with FIJI (Schindelin et al. (2012)) and its plugin ObjectJ (https://sils.fnwi.uva.nl/bcb/objectj/). We dissected individuals from the generalist populations (Table S1) to determine sex from the gonads. Individuals from the species-pair lakes (TableS1) were previously sexed by the Schluter lab, using either dissection or a genotyping protocol (Whom, personal communication).

2.3   Outlier analysis and size correction.

To check for outliers, we calculated within-group means and standard deviations for each trait separately for K series fossil specimens (pooled across samples) and for extant specimens (within generalist, benthic, or limnetic categories). We noted trait values greater than 3.0 standard deviations from the mean as potential outliers. We deemed 3.0 s.d. to be a reasonable threshold for detecting errors without excluding biologically relevant values. We checked whether these potential outliers were a result of data entry and collection error and corrected them if they were. We turned the remaining outlier trait values to NAs. We size-corrected continuous traits only, as they varied with size, unlike count traits that are fixed during early development. We regressed each trait on standard length using a mixed-

model regression, pooling all specimens, following Stuart et al. (2017). We appended the size corrected data to the uncorrected trait data frame and used all of our data (raw and size-corrected) to build the imputation model described next.

2.4 Missing data imputation, including fossil sex

Briefly, we used multiple imputation (Little and Rubin (2002)) to impute the sex of the fossils. Let $\boldsymbol{W}$ be an $(n_{extant} + n_{fossil}) \times 1$ vector of the covariate sex of the stickleback fish and $\boldsymbol{Y}$ be an $(n_{extant} + n_{fossil}) \times K$ matrix of the K phenotypes of interest. Because the sex of the fossilized stickleback fish is unobservable, we further define $\boldsymbol{W} = (\boldsymbol{W}_{extant}^T, \boldsymbol{W}_{fossil}^T)^T$ where $\boldsymbol{W}_{extant}$ and $\boldsymbol{W}_{fossil}$ are the $n_{extant} \times 1$ and $n_{fossil} \times 1$ vectors of the observed extant sex and missing fossil sex, respectively. We imputed missing sex for the fossil data by sampling from the posterior predictive distribution $P(\boldsymbol{W}_{fossil}|\boldsymbol{W}_{extant}, \boldsymbol{Y})$ using the multiple imputation by chained equations (MICE) algorithm (Buuren and Groothuis-Oudshoorn (2011)) with predictive mean matching, implemented in R (CITATION). Traditionally, the choice for the number of completed dats sets is a relatively small number such as $M = 5$ or $M = 10$. However, Zhou and Reiter (2010) recommend a larger number of imputed data sets if users intend on performing Bayesian analysis after imputation. Therefore, we imputed $M = 100$ complete datasets. Greg, PRESUMABLY this also imputes missing data in the other traits? We should probably say that explicitly. -YS Greg, AKHIL'S METHODS FOR VALIDATION OF THE IMPUTATION MODEL GOES HERE

We then pooled all of the draws from the posterior distribution across all the imputed data sets to estimate the posterior distributions of our parameters of interest (i.e., the trait values), rather than using Rubin's combining rules, following Zhou and Reiter (2010). We then dropped extant fish from our imputed data sets and used these posterior distributions in our downstream analyses of fossil sexual dimorphism.

2.5 Modeling fossil stickleback trait means through time for males and females

The goal of our analysis was to estimate fossil trait means through time, by sex, while incorporating uncertainty across imputations in the classification of sex. As we explain in this section, we are implementing a complex model structure to account for the fact that the rate of change in the mean of the trait over time may be a non-constant value. In addition, we are using multiple imputation procedure to account for the fact that the sex is unobserved in the fossils in addition to other pieces of information that are missing. We implement a Bayesian approach because these methods are more effective for analyzing hierarchical model structures as well as incorporating missing data.

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To estimate fossil sample means through time, for a given imputed dataset, we let W_{ij} be the imputed sex and $\boldsymbol{Y}_{ij}$ be the $K \times 1$ vector of phenotypes for stickleback fossil j at time t_i where $i = 1, \ldots, T$ and $j = 1, \ldots, n_t$. In addition, we denote $Y_{K,ij}$, the last variable in $\boldsymbol{Y}_{ij}$, to be the standard length of each fish individual, a measure of body size.

$$Y_{K,ij} \overset{iid}{\sim} \begin{cases} \mathcal{N}(\mu_{K,ft_i}, \ \sigma_K^2), & W_{ij} = \text{Female} \\ \mathcal{N}(\mu_{K,mt_i}, \ \sigma_K^2), & W_{ij} = \text{Male} \end{cases}. \tag{1}$$

Continuous traits will likely have some correlation with standard length (i.e., allometry) (Huxley (1932))(Voje, Bell, and Stuart (2022))(Voje et al. (2014)). We account for this by adding an additional parameter, γ_k. In addition, the continuous traits ds1, ds2, ds3, lps, tpg are unique in that these traits tend to be lost over time, results in a proportion of zeros in our empirical dataset. For these random variables, we add binary variable Z_{ij}, where $Z_{k,ij} = 1$ means that the trait is lost, and $Z_{k,ij} = 0$ means the trait is observed. For all other continuous traits, we assume $Z_{k,ij} = 0$

If $Y_{k,ij}$ is a continuous trait, then, potentially conditioning on $Z_{k,ij} = 0$,

$$Y_{k,ij} \overset{iid}{\sim} \begin{cases} \mathcal{N}(\mu_{k,ft_i} + \gamma_k Y_{K,ij}, \sigma_k^2), & W_{ij} = \text{Female} \\ \mathcal{N}(\mu_{k,mt_i} + \gamma_k Y_{K,ij}, \sigma_k^2), & W_{ij} = \text{Male} \end{cases}. \tag{2}$$

In other words, (2) only considers the subset of fossils where the given trait is present when performing a data analysis. For the traits with potential loss, we model the $Z_{k,ij}$ via a $Bernoulli(p_{k,ij})$ distribution where

$$\text{logit}(p_{k,ij}) = \begin{cases} \alpha_{k,ft_i} + \beta_k Y_{K,ij} & W_{ij} = \text{Female} \\ \alpha_{k,mt_i} + \beta_k Y_{K,ij}, & W_{ij} = \text{Male} \end{cases}, \tag{3}$$

and logit is a function to convert the probability to the log-odds, to allow for easier modelling of linear techniques while still ensuring any probability predictions are guaranteed to be between 0 and 1.

If $Y_{k,ij}$ is a discrete trait, the conventional modelling method is to fit a Poisson distribution. However, the empirical fossil data violate the assumption that the Poisson variance is equal to its mean, so we fitted discrete traits to a generalized Poisson model ((**GeneralizedPoisson?**)). Specifically, if $X \sim GP(\lambda, \alpha)$, then

$$P(X = x) = \begin{cases} \frac{(1-\alpha)\lambda[(1-\alpha)\lambda+\alpha x]^{x-1} \exp\{-((1-\alpha)\lambda+\alpha x)\}}{x!} & (1-\alpha)\lambda + \alpha x \geq 0 \\ 0 & (1-\alpha)\lambda + \alpha x < 0 \end{cases}, \tag{4}$$

where GP denotes a generalized Poisson distribution with $E(X) = \lambda$ and $Var(X) = \frac{\lambda}{(1-\alpha)^2}$.

In addition, we modeled two discrete traits, abdominal vertebrae number (mav) and caudal vertebrae number (mcv) to vary with standard length. This is because these are vertebral counts that made along the anterior-posterior access of the fish; these vertebrae contribute to the length of the fish directly and may evolve in a correlated fashion. If $Y_{k,ij}$ is one of the above traits, then

$$Y_{k,ij} \sim \begin{cases} GP(\lambda_{k,ft_i} = \exp\{\mu_{k,ft_i} + \gamma_k Y_{K,ij}\}, \alpha_k), & W_{ij} = \text{Female} \\ GP(\lambda_{k,mt_i} = \exp\{\mu_{k,mt_i} + \gamma_k Y_{K,ij}\}, \alpha_k), & W_{ij} = \text{Male} \end{cases}. \tag{5}$$

For the other discrete counting traits, we set $\gamma_k = 0$ because we expect that trait counts are set during early ontogeny and should not change with size in adult fish. Thus,

$$Y_{k,ij} \sim \begin{cases} GP(\lambda_{k,ft_i} = \exp\{\mu_{k,ft_i}\}, \alpha_k), & W_{ij} = \text{Female} \\ GP(\lambda_{k,mt_i} = \exp\{\mu_{k,mt_i}\}, \alpha_k), & W_{ij} = \text{Male} \end{cases}. \tag{6}$$

We point out that counting trait means are represented by $\exp\{\mu_{k,ft_i}\}$ and $\exp\{\mu_{k,mt_i}\}$. This distinction helps in our modelling procedure because μ_{k,ft_i} and μ_{k,mt_i} are represented on the real number line. This allows for easier modelling by linear techniques, and that any mean for the discrete traits is strictly positive. In addition, to ensure proper sampling from our posterior distribution of interest, we transform $\phi_k = \log\left(\frac{\alpha_k - \max_{i,j}(-\lambda_{k,ij}/y_{k,ij})}{1-\alpha_k}\right)$ where
$$\lambda_{k,ij} = \begin{cases} \exp(\mu_{k,ft_i} + \gamma_k Y_{K,ij}) & W_{ij} = \text{Female} \\ \exp(\mu_{k,mt_i} + \gamma_k Y_{K,ij}) & W_{ij} = \text{Male} \end{cases}.$$

To account for the possibility that there is a time-dependent (i.e., evolutionary trend) trend in μ_{k,ft_i} and μ_{k,mt_i}, we further set

$$\mu_{k,gt_i} = \beta_{0,kg} + \beta_{1,kg}t_i + u_{k,gt_i}, \tag{7}$$

for $g \in \{f,m\}$, where $\beta_{0,kg}$ and $\beta_{1,kg}$ are regression parameters of phenotype Y_k for each sex, and u_{k,gt_i} is the corresponding residual. For the continuous phenotypes with potential lost trait values, we model $\alpha k, gt_i$ in (3) by

$$\alpha_{k,gt_i} = \beta_{0,p,kg} + \beta_{1,p,kg}t_i + u_{k,p,gt_i}. \tag{8}$$

For convenience, we drop the subscript p for all model descriptions to come, and assume same modelling techniques apply. To account for potential correlations between the residuals for a given trait k and sex g, we fit an Ornstein-Uhlenbeck (OU) process (Uhlenbeck and Ornstein (1930)). More specifically, we defined $du_{k,gt} = u_{k,g(t+dt)} - u_{k,gt}$, the change in u_k, gt for a

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given trait k and sex g over a miniscule time period dt. The OU process is defined as

$$du_{k,gt} = -\kappa_k u_{k,gt} dt + \tau_k dW_t, \qquad (9)$$

where κ_k is a parameter associated with the correlation between $u_{k,gt}$ and $u_{k,g(t+dt)}$, τ_k is the standard deviation of the OU process, and W_t is a standard Brownian motion. As shown in Uhlenbeck and Ornstein (1930), the closed form solution for the stochastic differential equation in (9) is

$$u_{k,gt_i} \overset{iid}{\sim} \mathcal{N}\left(u_{k,gt_{i-1}} \exp\{-\kappa_k(t_i - t_{i-1})\}, \frac{\tau_k^2(1 - \exp\{-2\kappa_k(t_i - t_{i-1}))\}}{2\kappa_k}\right) \qquad (10)$$

for $i = 2, \cdots, T$. In a traditional OU process, the initial value u_{k,gt_1} is assumed to be a (potentially unknown) constant. This unknown constant can therefore be considered a latent variable, further justifying our Bayesian approach, which better incorporates prior information and uncertainty from missing data imputation in the estimate of latent variables.

We chose the following priors, designing them to be weakly informative such that the posterior distributions could be influenced by the data, if warranted. For $k = 1, \cdots, K$,

$$
\begin{aligned}
u_{k,gt_1} &\overset{iid}{\sim} \mathcal{N}(0, \tau_{0,k}) \\
\sigma_k &\overset{iid}{\sim} \mathcal{N}(0, 10)I_{\{\sigma>0\}} \\
\tau_k &\overset{iid}{\sim} \mathcal{N}(0, 10)I_{\{\tau>0\}} \\
\tau_{0,k} &\overset{iid}{\sim} \mathcal{N}(0, 20)I_{\{\tau>0\}} \\
\kappa_k &\overset{iid}{\sim} \mathcal{N}(0, 1)I_{\{\kappa_g>0\}} \\
\gamma_k &\overset{iid}{\sim} \mathcal{N}(0, 5) \\
\beta_{0,kg} &\overset{iid}{\sim} \mathcal{N}(0, 100) \\
\beta_{1,kg} &\overset{iid}{\sim} \mathcal{N}(0, 3)
\end{aligned}
$$

$$(11)$$

We also note that, for the discrete phenotypes in equations (5) and (6), there is no σ_k, and it is not sampled, and for the continuous phenotypes in equation (2), there is no ϕ_k and it is not sampled.

We follow the method outlined in Zhou and Reiter (2010) to obtain R draws from the posterior distributions of the model parameters given our observed data and imputed values. More specifically, for the jth imputed data set where $j = 1, \cdots, M$, we obtain $\frac{R}{M}$ draws from the posterior distribution using an MCMC algorithm with a sufficient burnin period, and

then we combine these M MCMC draws to say that we have a total of R draws from the overall posterior distribution of interest.

2.6 Quantifying sexual dimorphism, and testing for increased sexual dimorphism through time.

We quantify sexual dimorphism at each time $t_i : i = 1, \cdots, T$ by calculating the difference in mean trait size for males and females at each time for a continuous phenotype, $SD_{k,t_i} = \mu_{k,m,t_i} - \mu_{k,f,t_i}$ and for a discrete phenotype $SD_{k,t_i} = \lambda_{k,m,t_i} - \lambda_{k,f,t_i}$. For simplicity, we will define sexual dimorphism for both continuous and discrete traits as $SD_{k,t_i} = \mu_{k,m,t_i} - \mu_{k,f,t_i}$. Because we are performing a Bayesian analysis, we do not obtain a single point estimate of sexual dimorphism at a particular time, but rather R samples from the posterior distribution of sexual dimorphism given the observed fossil and extant data. Therefore, our analysis will be conducted by calculating $SD_{k,t_i,r} = \mu_{k,m,t_i,r} - \mu_{k,f,t_i,r}$ for $r = 1, \ldots, R$ and then performing our data analysis on these samples. Presence (or absense) of sexual dimorphism for trait k can be indicated by estimating the posterior probability of positive sexual dimorphism $P(SD_{k,t_i} > 0|\boldsymbol{y}) = \frac{1}{R} \sum_{r=1}^{R} I(SD_{k,t_i,r} > 0)$. Figures 2.6 and 2.6 plot these posterior probabilities across time for each of the armored traits and non-armored traits, respectively. The closer the $P(SD_{k,t_i} > 0|\boldsymbol{y})$ is to 1, the larger the evidence that the average trait size for males is larger than females, while if $P(SD_{k,t_i} > 0|\boldsymbol{y})$ is close to 0, the larger the evidence that the average trait size for females is larger than males, both indicating strong presence of sexual dimorphism. On the contrary, $P(SD_{k,t_i} > 0|\boldsymbol{y})$ around 0.5 indicates that the average trait size is roughly equivalent for both males and females, providing little evidence of dimorphism.

Then, to test our prediction that ecological release should result in an increase in sexual dimorphism through time, we calculated Kendall's τ coefficient for each trait (Kendal (1938)). Kendall's τ assesses the ordinal association between a set of bivariate observations, where $\tau > 0$ indicates concordance between the observations (i.e., as x increases, y also tends to increase; here, x is time and y is sexual dimorphism.) Kendall's τ varies between -1 and 1, and can be thought of as behaving like a correlation. Values farther from zero indicate a stronger relationship.

$\boldsymbol{\tau}_k = \{\tau_{k,r} : r = 1, \cdots, R\}$ for trait k where τ_r is the estimate of the Kendall's τ correlation for $\mu_{k,m,t_i} - \mu_{k,f,t_i}$ versus time t_i for the rth MCMC sample from the posterior distribution. Matt, I think there's an absolute value problem here. Dimorphism can increase of M-F gets more positive or more negative. I've indicated that in the text below, but I could use some help refininig the explanation -YS For each trait, the proportion of values in $\boldsymbol{\tau}_k$ that are greater than 0 ($\frac{1}{R} \sum_{r=1}^{R} I(\tau_{k,r} > 0)$) may indicate that males have gotten relatively larger than females, indicating more pronounced dimorphism. For each trait, the proportion of

values in $\boldsymbol{\tau}_k$ that are less than 0 ($\frac{1}{R}\sum_{r=1}^{R} I(\tau_{k,r} < 0)$) may indicate that females have gotten relatively larger than males, indicating more pronounced dimormphism.

3 Results

The extant data used here consist of a total of 367 specimens all with known sex (Table S1). Of these, there are 202 and 165 female and male specimens, respectively.

3.1 Missing Data Imputation

Greg, WE NEED RESULTS SECTION DESCRIBING THE RESULTS OF AKHIL'S MULTIPLE IMPUTATION, BOTH THE TESTS OF HOW WELL THE MODEL WORKED CLASSIFYING MODEL SAMPLES, AND SUMMARY STATISTICS OF FOSSIL IMPUTATION RESULTS -YS

3.2 Sexual Dimorphism

Table **??** shows the posterior probability of the differences in the means between male and female specimens at each time point. Probabilities near 0.5 indicate very little difference in the means whereas probabilities far from 0.5 indicate sexual dimorphism with values of 0 and 1 indicating larger mean values for females and males, respectively. These results are shown graphically in figure **??**.

Figure **??** shows the posterior mean difference for males versus females (values above 0 indicate means that were greater for males versus females). The full posterior distributions over time for all traits are shown in the appendix.

3.3 Changes over time

We can also assess the strength of the dimorphism by calculating the proportion of posterior samples that are greater than a prespecified threshold (0.5??, idk what this could be.) -MS

Table **??** shows the probability that the posterior distribution of the differences in the mean is greater than the posterior distribution of the differences at the first time point. Values near 0.5 indicate very little difference between the distributions whereas values closer to 0 and 1 indicate changes in the distributions with values of 1 meaning the difference in means has shifted towards males having a larger mean and values of 0 indicating the difference in means has shifted towards larger means in females. These results are shown graphically in figure **??**.

4 Discussion, Future work and conclusions

We predicted that release from predators would result in niche expansion and increased sexual dimorphism, based on several studies of modern stickleback. For example, in lakes where sculpin competitors are absent and stickleback (Roesti et al. 2023) See Spoljaric and Reimchen 2008, page 512 right column for references and discussion of differences between benthic males and limnetic females. Male stickleback are benthic and littoral (Wootton 1976).... Reimchen papers in general good for this section. Moreover, tooth wear data from the lineage II sequence suggest that individuals in this lineage began eating more planktonic prey over time, expanding toward an open-water niche from the benthic, bottom-feeding niche they started with (Purnell et al. 2007). That this expansion into open water by lineage II coincided with armor reduction is further indicative of a limnetic system with fewer salmonid piscivores (Schluter and McPhail (1992); Vamosi and Schluter (2004); Roesti et al. (2023)).

5 Acknowledgements

6 Data and Code Statement

All code for reproducing the analyses in this paper is publicly available at https://github.com/Akhil-Ghosh/SticklebackProject Is this true? Matt's code is at this GitHub page? -YS

7 References

8 Supplementary Material

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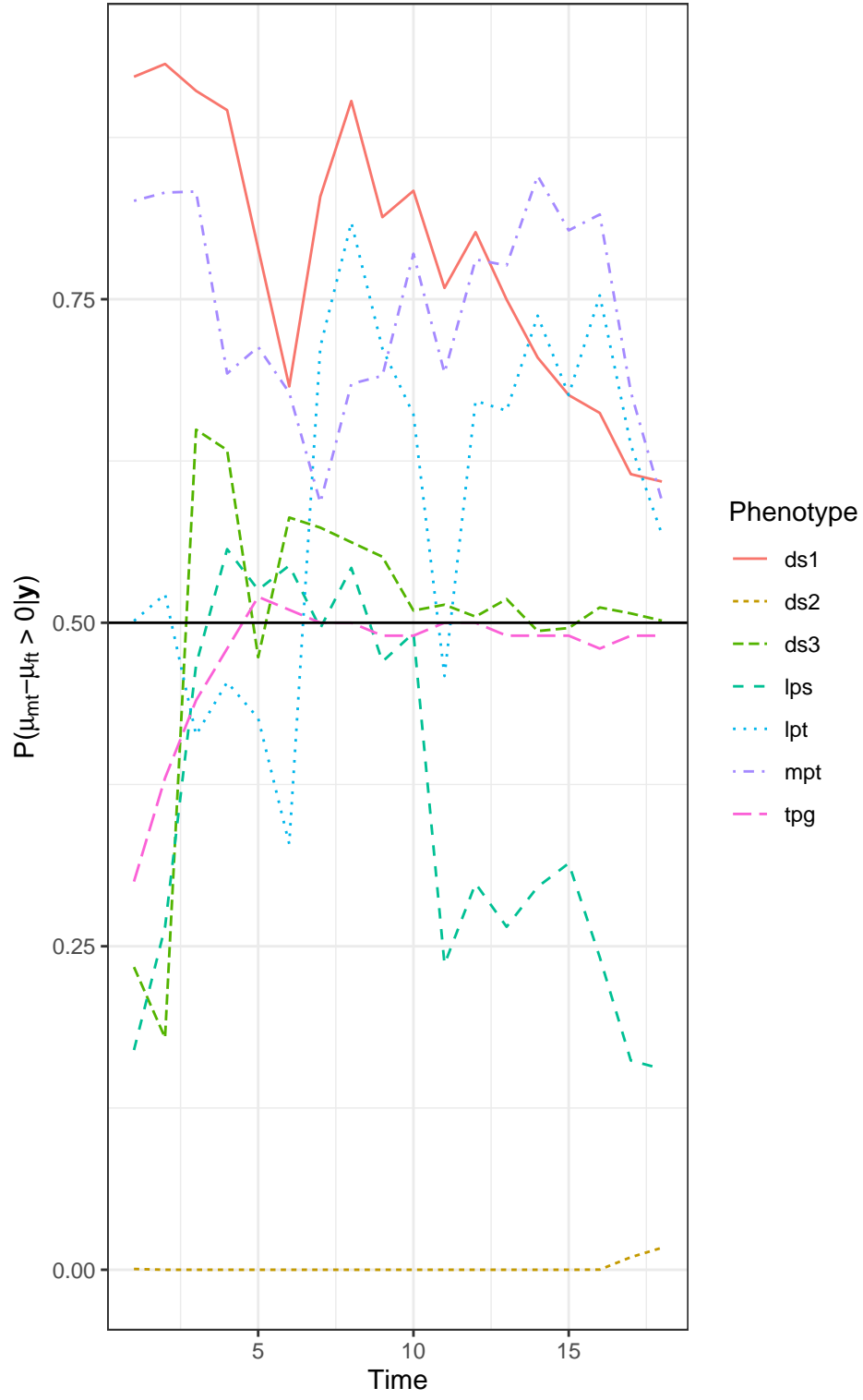
Figure 1: Posterior probability of positive sexual dimorphism for armor traits
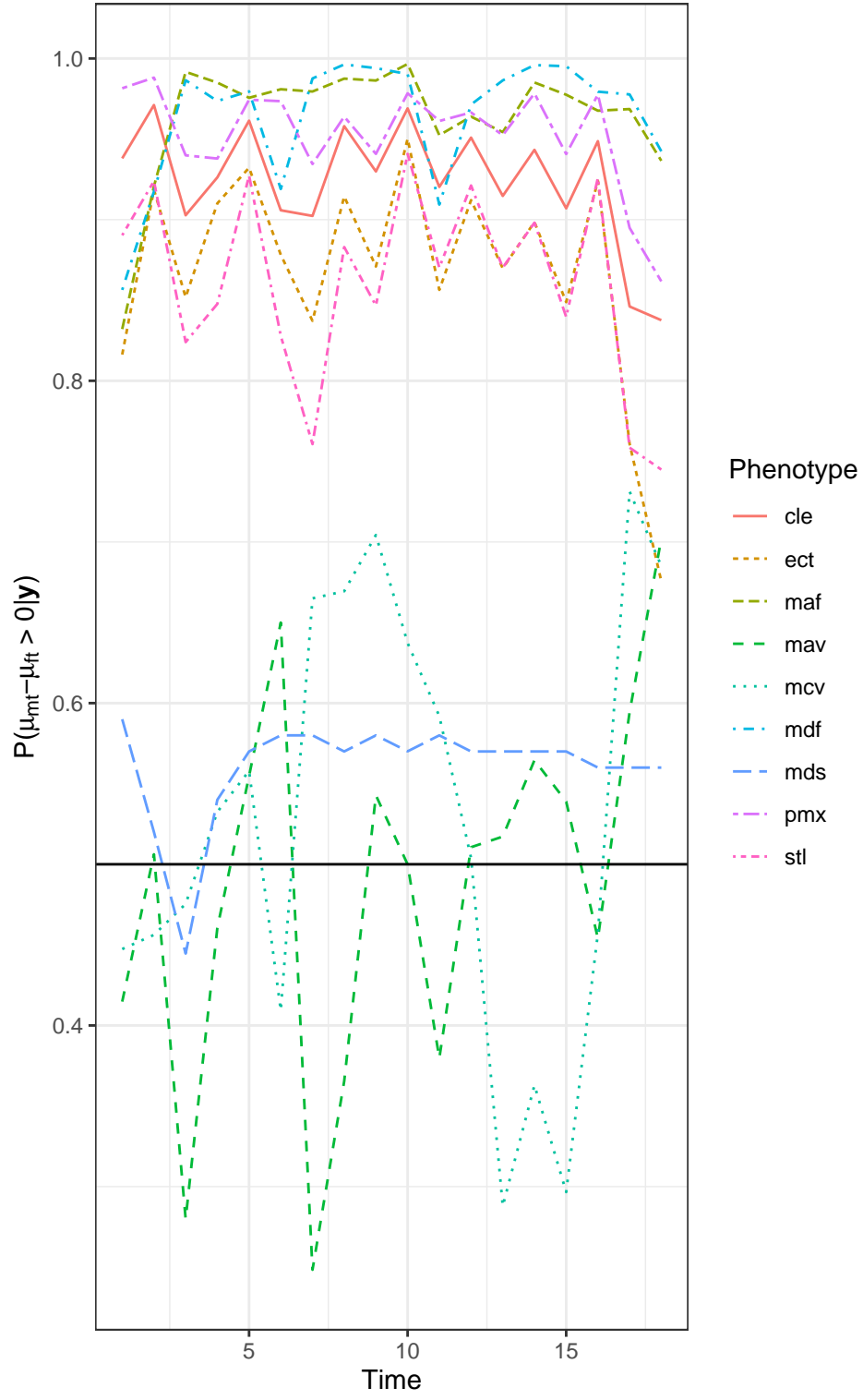
Figure 2: Posterior probability of positive sexual dimorphism for non-armor traits