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The Role of Predation in the Evolution of Sexual Dimorphism in *Gasterosteus aculeatus*

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Marine Biology

by

Christina Puzanghera

Committee in charge:

Professor Diana Rennison, Chair
Professor Andrew Allen, Co-Chair
Professor Ron Burton

2022

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University of California San Diego

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The Role of Predation in the Evolution of Sexual Dimorphism in *Gasterosteus aculeatus*

by

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Sexual dimorphism —morphological differences between the sexes, contributes to morphological variation within a species and has been shown to occur in a variety of taxa. One mechanism hypothesized to generate dimorphism is differential niche partitioning between the sexes, which leads to selection acting differently on each sex. Differential predation is one factor that has been suggested to accompany sex-specific niche divergence. However, most studies investigating predation and sexual dimorphism have been conducted in the field, which has prohibited a direct test of the contribution of differential predation. To directly test for the contribution of differential predation to the evolution of sexual dimorphism, I utilized data from a manipulative predation experiment. In the experiment, a highly variable population of hybrid benthic-limnetic stickleback were either exposed to cutthroat trout predation or left in a trout-free control treatment for one generation.

Morphological trait distributions were then compared between the two generations to compare the pattern of evolution between the sexes and estimate the strength of selection on sexual dimorphism. When looking at overall dimorphism we did not find a significant effect of predation. However, when looking at specific traits we found a trend towards a significantly greater amount of dimorphism in predation ponds for two traits, anal fin length and caudal peduncle depth. We also found evidence of differential patterns of selection between males and females when exposed to predators, with males experiencing more selection due to predation. These results together suggest that predation might play some role in the evolution of sexual dimorphism in stickleback.

INTRODUCTION

Sexual dimorphism, defined as morphological differences between the male and female of a species, is a topic of research that has been long investigated for its role in adaptive evolution and speciation (Darwin 1874; Frayer & Wolpoff, 1985; Leutenegger & Cheverud, 1985; Hedrick & Temeles, 1989; Bolnick, 2003). While it can be difficult to understand what causes sexual dimorphism, previous research has shown that sexual dimorphism may be caused by two main mechanisms: the more commonly studied mechanism, sexual selection (Price 1984; Leutenegger & Cheverud, 1985; Fairbairn 1997; Preziosi and Fairbairn 2000; Ferguson and Fairbairn 2000; Badyaev and Martin 2000, Anderson 1996) and differential selection accompanying niche differentiation (Slatkin, 1984; Mealey 2000; Hedrick and Temeles 1989).

Sexual selection refers to when competition for mates causes trait changes that increase an organism's reproductive output (Anderson, 1996). One example of this is found in the peacock. Male peacocks have large ornate tails in comparison to their rather plain female counterparts. This exaggerated tail is used in important mating behaviors. Male birds with the largest and most ornate tails tend to attract the most mates and produce the most offspring, thus increasing their reproductive fitness (Petrie & Halliday, 1994). Natural selection is the process where heritable traits which improve an organism's ability to survive and reproduce become more common in a population over a long period of time. Natural selection is also thought to generate sexual dimorphism. It is well known that the occupation of different niches by different species or populations can lead to trait divergence (Rundell & Price, 2009; McPhail, 1969). The same mechanism is hypothesized to drive trait divergence between the sexes within a species or population when the two sexes experience different types or degrees of selection. For example, differential niche use is found in the amphibious sea-krait *Laticauda Colubrina*; in this species

feeding differences between the sexes, where the female of this species eats much larger prey, have caused females to evolve to be much larger and have a larger feeding apparatus (Shine et al., 2002). Differential niche use, when the two sexes exhibit differential resource use and/or experience habitat or diet preferences may also lead to the sexes experiencing different environmental factors including climate, parasitism, predation exposure, or competition (Reimchen & Nosil, 2001; Hedrick & Temeles, 1989; Slatkin 1984). These different selective conditions could then lead to divergence between the sexes in additional traits unrelated to foraging (Reimchen & Nosil, 2001). While there are many good examples of sexual dimorphism driven by sexual selection (Petrie & Halliday, 1994; Price, 1984; Ferguson & Fairbairn, 2000; Anderson & Vitt, 1990; Acharya, 1995), research on differential selection and niche differentiation is limited to fewer studies (e.g. Reimchen & Nosil, 2001; Slatkin, 1984; Götmark et al., 1997). Thus, we know little about the type of selective agents that may generate sexual dimorphisms.

One species in which sexual dimorphism has been described is the three-spined stickleback (*Gasterosteus aculeatus*) (Kitano et al 2007; Aguirre et al., 2008). Stickleback are considered a model organism for several biological fields and have been studied extensively in evolutionary biology. Several traits have been found to vary between the sexes including body size and shape, jaw, fin, and head morphology (Kitano et al., 2007; McGee & Wainwright, 2013). For several traits in stickleback, sexual dimorphism has also been shown to be heritable, which means it can evolve over time (Leinonen et al 2011). The sexual dimorphism found in this species has been hypothesized to be caused, at least in part, by differential selection accompanying niche divergence. When taking advantage of different habitats, male and female

fish could be exposed to distinct environmental factors including different temperatures, predators, and parasites.

Differential predation in particular, is thought to contribute to the evolution of sexual dimorphism in stickleback as predation regime often covaries with habitat/resource usage. Invertebrate predators of stickleback dominate the littoral environment, whereas vertebrate predators of stickleback (diving birds and salmonids) are predominantly found in pelagic habitats (Vamosi, 2002). Differential exposure to predators between these habitats has already been suggested to underlie the divergence of several traits between benthic and limnetic stickleback ecotypes, which specialize in the exploitation of the littoral and pelagic environments respectively (Vamosi & Schluter, 2004). Thus, if males and females differentially utilize littoral and pelagic habitats, exposure to these different regimes could promote the evolution of dimorphism.

Previous research in stickleback exploring the role of differential predation as a mechanism underlying sexual dimorphism has been limited to surveys of wild populations, which have found that predation can drive the evolution of sexual dimorphism through shifts in ecology driving sex-specific selection (Reimchen & Nosil, 2004). Although useful, field surveys are uncontrolled, and several environmental factors may co-vary with predation regime, which precludes identification of the precise mechanisms driving sexual dimorphism. In contrast, a controlled experiment allows tests of the contributions of individual factors to the evolution of sexual dimorphism. To explore the role of predation in the evolution of sexual dimorphism in stickleback I used a manipulative predation experiment. In the experiment populations of F2 hybrid benthic-limnetic stickleback were assigned to either a cutthroat trout predation treatment or a no trout control. I then compared the degree of sexual dimorphism present in each treatment

before or after one generation of selection. I predict that if the sexes have a difference in their encounter rates due to differential resource use, sexual dimorphism should increase in the trout treatment ponds relative to paired controls.

METHODS

Trait measurements for the project were taken from fish that originated from a selection experiment conducted from 2012 -2013 see Rennison et al., 2019 for full details. Briefly, the experiment was run at the artificial ponds facility at the University of British Columbia campus. F1 hybrid benthic-limnetic sticklebacks were generated through *in vitro* fertilization in the lab. In May of 2012 reproductive F1s were introduced into ten ponds in the facility. In the ponds, the F1 fish were allowed to breed naturally, and these matings generated the F2 hybrids used in the selection experiment. The F2 fish again bred naturally the following spring, generating an F3 generation. The F2 fish were sampled before any effect from predation would occur in September 2012. The F3 fish were sampled the following September 2013. In September 2012 after the first sample was taken two cutthroat trout, a natural stickleback predator, were introduced into each of four ponds (the predation treatment ponds) and five ponds were kept as non-predation controls. Predation and control ponds were paired based on the F1 hybrid family that was introduced, with one family per pond pair. The fish sampled during these periods were juveniles three to four months old. In total, 500 fish from generation one and 500 fish from generation 2 were sampled. In the end, 947 samples were usable for morphological measurement.

The stickleback specimens were stained with alizarin red, which binds to calcium and highlights boney elements. Standardized pictures were taken of the left side of each stained fish and a set of 14 traits commonly found to be variable among stickleback populations were selected to be measured from the photos. The traits measured were: first dorsal spine length, second dorsal spine length, third dorsal spine length, dorsal fin length, anal fin length, pelvic spine length, head length, mouth length, eye diameter, body depth, standard length, total lateral

plate number count, snout length and pectoral insertion length. Measurements were collected using the program ImageJ. All measurements were converted from pixels to centimeters using the ruler in each image to set the scale.

All statistical analyses were performed in R (version 3). Since traits scale with body size, the raw data was size corrected using the following equation:

$$\gamma_i = \chi_i - \beta(L_i - \underline{L}),$$

where γ_i is the size-adjusted trait, χ_i is the original trait, β is the regression coefficient of the un-adjusted trait values on standard length, L_i is the standard length of the individual and \underline{L} is the average length of the sample (3.5 cm). This size-corrected data was then used for all subsequent analyses.

To identify traits that were sexually dimorphic an LDA analysis was performed from fish in the F2 generation, here trait values of males were compared to females in multidimensional space. To confirm a significant difference between the sexes an ANOVA was run on each trait that was suggested by the LDA to be sexually dimorphic. Eight traits were confirmed to be sexually dimorphic (head length, caudal peduncle depth, eye diameter, dorsal fin length, anal fin length, third dorsal spine length, snout length, and mouth length) before selection (i.e. in the F2 samples), thus only these traits were used in the subsequent analyses

A PCA analysis of the entire data set (F2 and F3 generations) was then performed using these eight size corrected traits. Individuals were grouped by sex to visualize overall dimorphism (Figure 1), the first axis was confirmed to separate the sexes. So this process was repeated with the data subset into control and predation groups (Figure 2). Eigenvalues were extracted from the independent treatment and control PCAs to examine overall shifts in dimorphism between

generations. We used this data to calculate the overall dimorphism (D) in each pond for each generation using the following equation (1):

$$D_{gn} = F_{gn} - M_{gn}$$

where F is the mean female eigenvalue and M is the mean male eigenvalue, g indicates the generation and n the pond number. Significance testing was done using an ANOVA with pond set as a random effect. To estimate the change in dimorphism (ΔD) we compared estimates of D across one generation within each pond using equation (2):

$$\Delta D_n = D_{2n} - D_{1n}$$

where n indicates pond number. To determine whether there was significant difference in the change in overall dimorphism (ΔD) between treatments (predation and control) we used an ANOVA with family set as a fixed effect. We next examined the change in dimorphism of individual traits. We again used equation 1, but now the F represents the mean female trait value and M , the mean male trait value (rather than the mean eigenvalue). To estimate change in dimorphism between generations equation 2 was again used with the trait-specific estimates of D used as input. To test for the significance of the change in dimorphism by trait we used a series of t-tests. To test for a treatment effect, we used ANOVAs with family as a random effect.

We then sought to characterize the pattern of selection on individual traits within each sex. To do this we estimate the trait mean for each pond number, sex, and generation and the pooled standard deviation for each trait and each pond across the two generations. We estimated standardized univariate selection differentials (intensities, s') between generations for each sex using the following equation (3):

$$s' = (\bar{x}_{f3} - \bar{x}_{f2}) / \hat{\sigma}_{pooled},$$

where \bar{x}_{f3} is the trait mean of a pond in the second (F3) generation is \bar{x}_{f2} the trait mean of a pond in the first (F2) generation and $\hat{\sigma}_{pooled}$ is the pooled standard deviation of both time points. This yielded independent estimates of s' for males (s'_m) and females (s'_f) for each pond and trait. To examine whether there was a significant effect of predation on the pattern of selection within a sex we compared predation and control ponds within a family, with significance tested by ANOVA with family as a random effect.

RESULTS

An LDA analysis of the F2 cohort (pre-selection) identified eight traits that were significantly differentiated ($p < 0.05$) between the sexes (Table 1). A PCA analysis based on these eight putatively sexually dimorphic traits separated males and females along the first axis with 33.1% of the variance explained by PC1 (Figure 1). Thus, the difference in mean PC1 eigenvalues between males and females reflected the overall magnitude of sexual dimorphism. For seven of the eight traits males had larger trait values than females (Figure 1). When the PCA was split into treatments both the control and treatment groups separated along the first axis with 31.9% and 34.5% of the variance explained by PC1 respectively (Figure 2).

Table 1: Magnitude of dimorphism - difference between males and females, for fourteen traits. Those traits that were statistically significant are indicated in bold.

Trait	Magnitude of Dimorphism	F statistic	P value	DF
Plate Number Count	0.028	0.076	0.782	445
First Dorsal Spine	-0.010	1.418	0.234	449
Second Dorsal Spine	-0.005	2.581	0.109	450
Third Dorsal Spine	-0.008	17.619	<.0001	437
Dorsal Fin Length	0.033	19.105	<.0001	443
Anal Fin Length	0.027	27.355	<.0001	450
Body Depth	0.001	0.137	0.712	451
Mouth Length	0.013	43.160	<.0001	451

Table 1 Continued

Snout Length	0.011	29.915	<.0001	451
Caudal Peduncle Depth	0.003	5.1168	0.024	451
Eye Diameter	0.004	7.545	0.006	451
Head Length	0.019	41.152	<.0001	451
Pectoral Fin Insertion Length	-0.0002	0.027	0.870	451
Right Pelvic Spine Length	-0.013	3.344	0.068	451

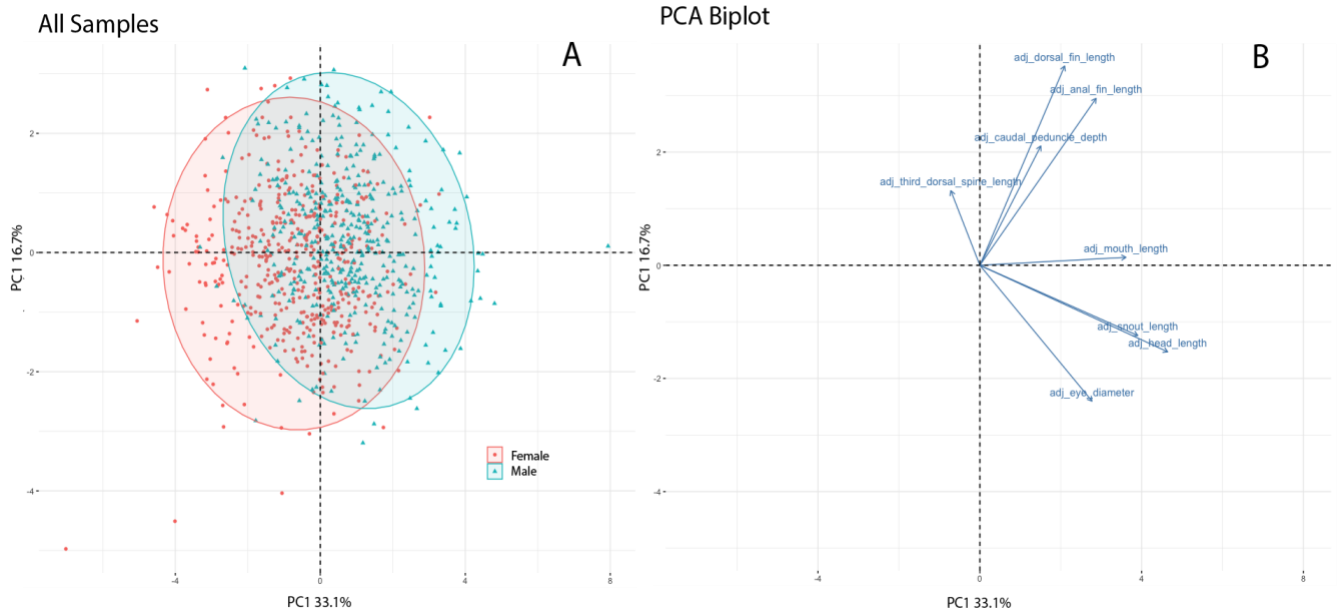


Figure 1: (A) PCA of seven putatively dimorphic traits for all F2 fish. Males and females are indicated in blue and red respectively. (B) PCA biplot depicting the trait loadings. Traits larger in females are on the left and traits larger in males are on the right.

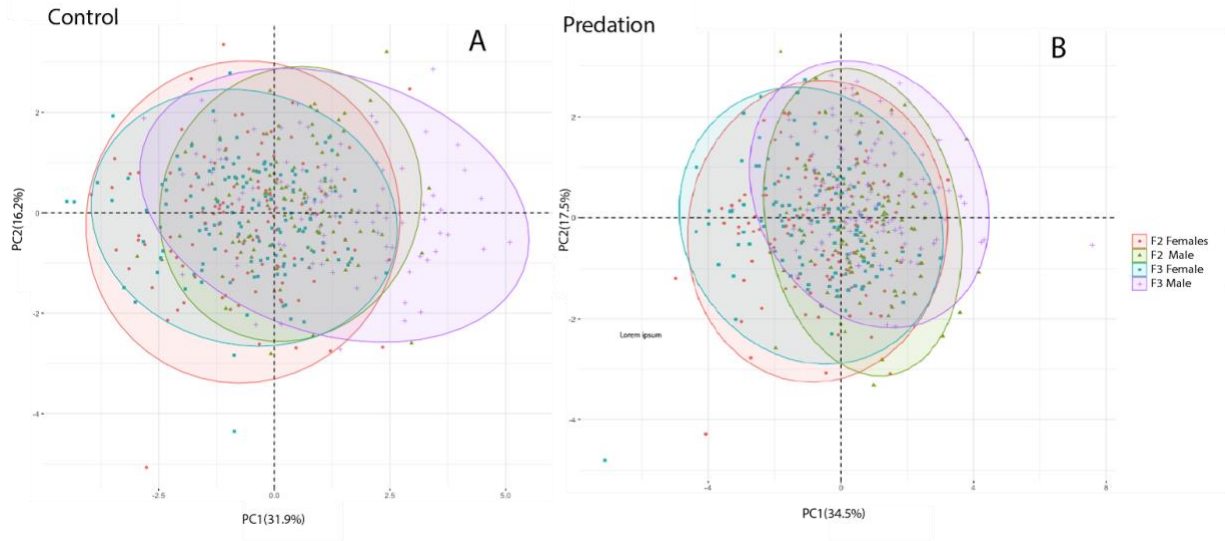


Figure 2: PCA plot of treatment males and females from the F2 and F3 generations (indicated by color) from (A) Control Ponds and (B) Predation Treatment Ponds.

The eigenvalue distance between males and females (overall dimorphism) was compared between the F2 and F3 generations to estimate the pattern of evolution. Between generations, there was evidence of a significant increase in overall dimorphism across all 10 pond replicates (mean difference in dimorphism between generations = -0.88 ± 0.27 SE, $t_9 = -3.24$, $p = 8e-04$; Figure 3). Interestingly, the pattern of evolution did not consistently differ between control and predation treatment ponds; the increase in dimorphism was greater in some control ponds relative to paired predation ponds, but was smaller in other pairs; thus, there was no significant treatment effect for the pattern of overall evolution (mean difference between treatments = -0.097 , $t_4 = -0.24$, $p = 0.8232$; Figure 4).

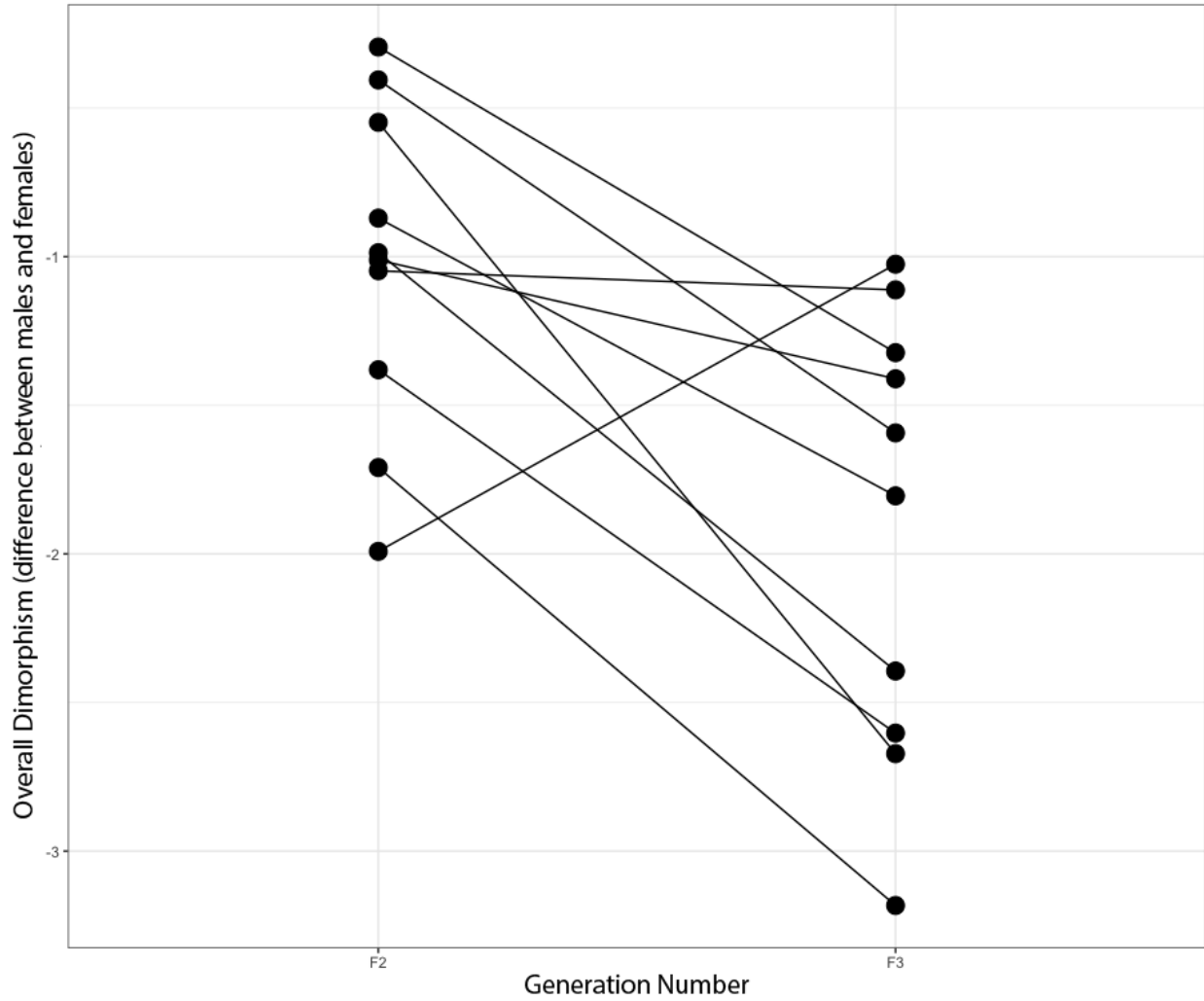


Figure 3: Average overall dimorphism in the F2 and F3 generations. Here dimorphism is estimated as the difference in eigenvalues from PCA 1 between males and females and a negative value reflects more dimorphism.

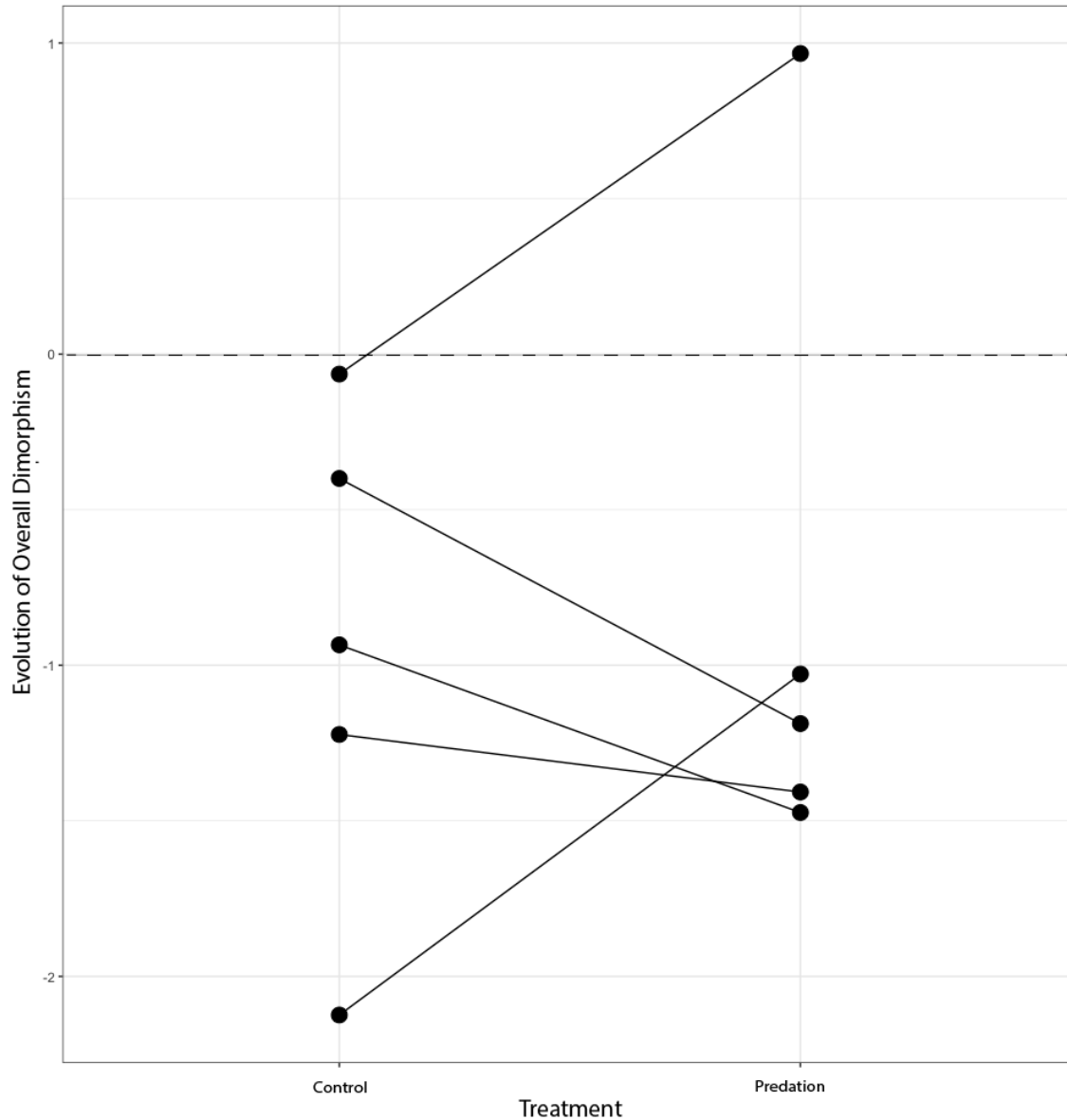


Figure 4: Change in overall dimorphism as estimated using PC1 eigenvalues for paired control and predation ponds. Paired ponds are indicated by a connected line. Negative values indicate increased dimorphism.

The pattern of evolution for dimorphism was next considered trait by trait. There was a significant difference in dimorphism between generation F2 and F3 for two traits: caudal peduncle depth and anal fin length (Figure 5, Table 2). However, when the change in dimorphism for control ponds was compared to that in paired predation ponds there was no significant treatment effect for any of the eight traits. Although, for the anal fin and caudal

peduncle there was a trend toward a significantly ($p < 0.1$) greater increase in dimorphism for predation ponds (Figure 6 and Table 2). Interestingly, when the evolutionary response was estimated separately for each sex, there was evidence of several significant treatment effects in males (Figure 7, Table 3). Specifically, there was a significant difference in evolutionary response between treatments for eye diameter, caudal peduncle depth, head length, and mouth length in males. In comparison, for females, only the evolutionary response for mouth length was significantly different between treatments (Figure 8, Table 3).

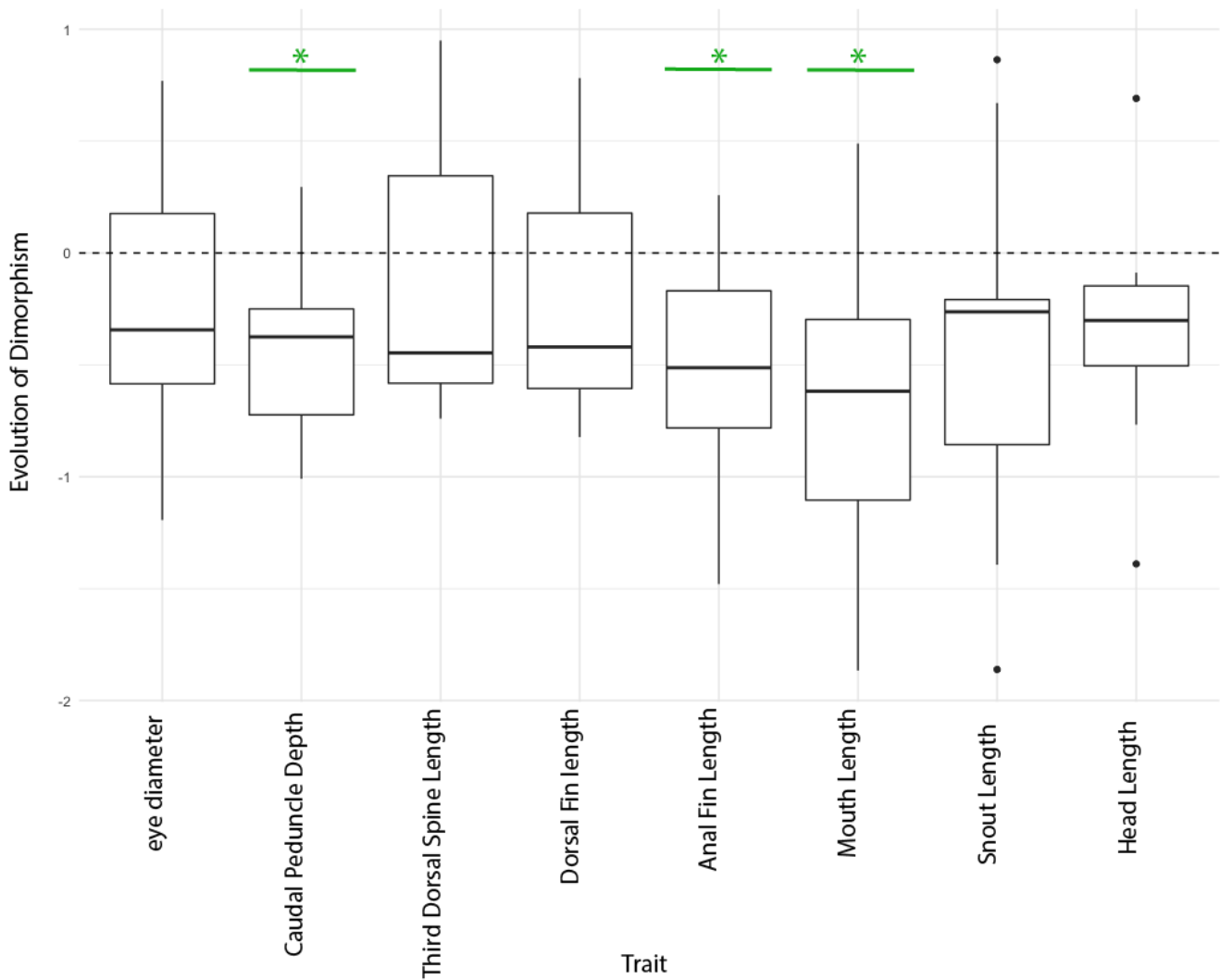


Figure 5: Evolution of dimorphism as calculated for each relevant trait. The green bar and star indicate significance ($p < 0.05$). Negative values indicate increased dimorphism.

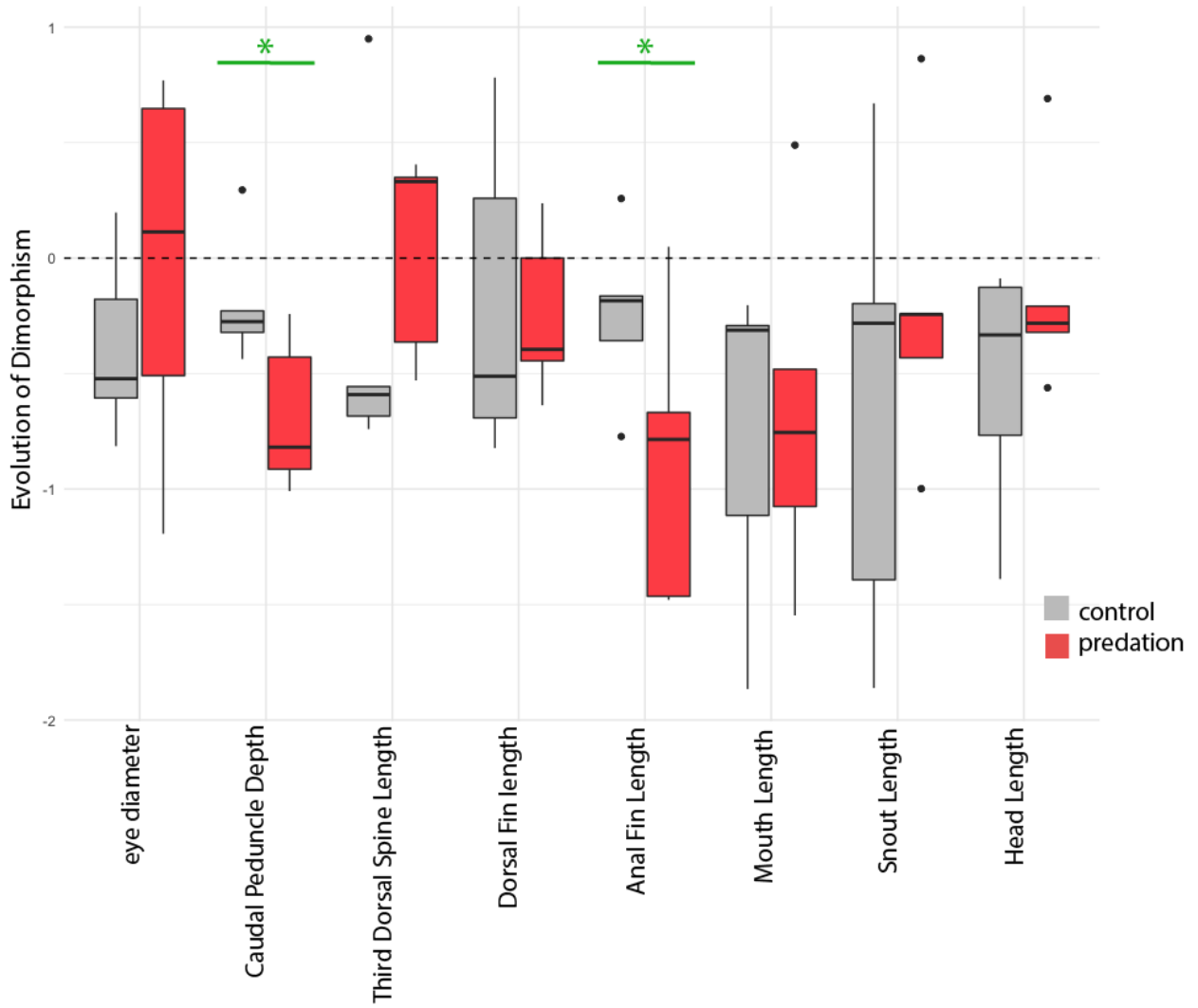


Figure 6 Evolution of dimorphism as calculated for control and predation treatment ponds. The green bar and star indicate a trend toward significance ($p < 0.1$). Negative values indicate increased dimorphism.

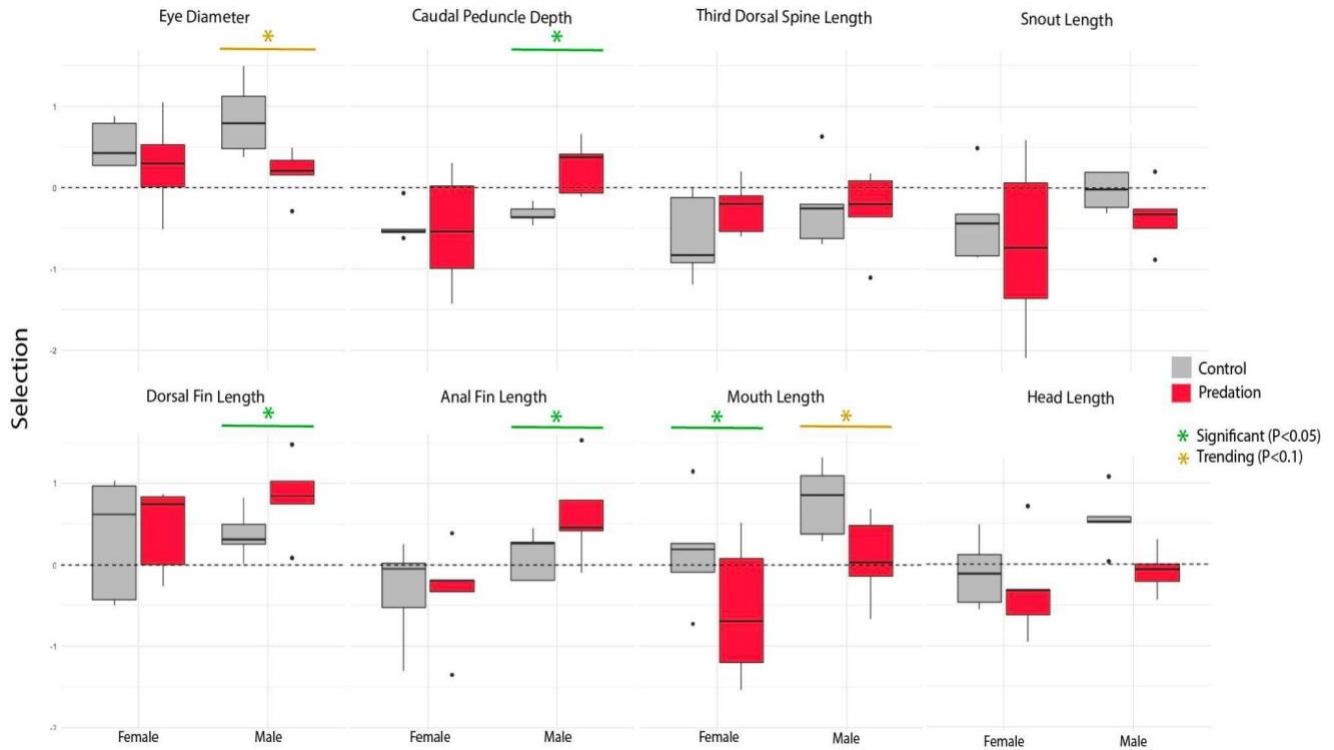


Figure 7: Evolutionary response (selection) separated by sex and treatment. Significant ($p < 0.05$) comparisons are indicated in green; trending ($p < 0.1$) comparisons are indicated in yellow.

Table 2: (A) Overall pattern of evolution of dimorphism (B) Treatment effect on evolution of dimorphism. Significant or trending toward significance values are indicated in bold. * Indicates p value less than 0.1 ** indicates less than 0.05 *** indicates less than 0.01

Effect	Trait	Estimate	P value	F statistic	DF
Overall evolution of dimorphism	Anal Fin Length	-0.557	0.016**	-2.977	9
	Caudal Peduncle Depth	-0.438	0.006***	-3.566	9
	Dorsal Fin Length	-0.222	0.208	-1.358	9
	Eye Diameter	-0.210	0.325	-1.0419	9
	Head Length	0.339	0.074*	2.022	9
	Mouth Length	-0.716	0.010	-3.232	9

Table 2 Continued

	Snout Length	-0.412	0.155	-1.553	9
	Third Dorsal Spine Length	-0.143	0.467	-0.760	9
Treatment effect on dimorphism	Anal Fin Length	-0.61	0.093*	4.838	4
	Caudal Peduncle Depth	-0.707	0.066*	6.297	4
	Dorsal Fin Length	-0.591	0.870	0.031	4
	Eye Diameter	-0.476	0.272	1.621	4
	Head Length	-0.512	0.1613	2.945	4
	Mouth Length	-0.371	0.695	0.178	4
	Snout Length	-0.569	0.4103	0.844	4
	Third Dorsal Spine Length	-0.707	0.391	0.924	4

Table 3: Treatment effect on selection for each trait and sex. Significant or trending toward significance values are indicated in bold. * Indicates p value less than 0.1 ** indicates less than 0.05 ***indicates less than 0.01

Sex	Trait	Estimate of selection	P value	F statistic	DF
Female	Anal Fin Length	-0.193	0.386	0.947	4
	Caudal Peduncle Depth	-0.068	0.847	0.042	4
	Dorsal Fin Length	-0.252	0.397	0.900	4

Table 3 continued

	Eye Diameter	-0.015	0.966	0.002	4
	Head Length	0.365	0.256	1.753	4
	Mouth Length	-0.722	0.009***	21.919	4
	Snout Length	-0.315	0.509	0.525	4
	Third Dorsal Spine Length	0.101	0.817	0.061	4
Male	Anal Fin Length	-0.624	0.023**	12.840	4
	Caudal Peduncle Depth	0.578	0.017**	15.774	4
	Dorsal Fin Length	-0.673	0.009***	22.439	4
	Eye Diameter	0.498	0.089*	4.983	4
	Head Length	-0.052	0.831	0.052	4
	Mouth Length	-0.708	0.052*	7.517	4
	Snout Length	-0.487	0.181	2.615	4
	Third Dorsal Spine Length	0.456	0.104	4.384	4

DISCUSSION

Sexual dimorphism is thought to be driven in part by niche differentiation (Sá-Pinto et al., 2017). This is because sex-specific niche differentiation exposes the sexes to different ecological conditions allowing natural selection to act on each sex differently and cause divergence. We sought to test the contribution of predation to the evolution of sexual dimorphism using a controlled manipulative experiment. The study revealed that the overall dimorphism (across all traits) between males and females increased across generations regardless of treatment. Interestingly, there was not strong support for greater overall dimorphism in the predation treatment relative to the control, as only three of the five predation replicates exhibited a greater increase in overall dimorphism across generations. When dimorphism was considered for individual traits, the dimorphism for two traits, anal fin length and caudal peduncle depth, was found to significantly increase across generations irrespective of treatment. This again suggests that there is some evolution of dimorphism that occurs regardless of predation. However, when predation treatment was considered, a trend towards a significant effect was found for these two traits, with a greater increase of dimorphism found in the predation treatment ponds. This suggests there is a possibility that predation may be a driver of predation for certain key traits.

The weak evidence of increased dimorphism in the predation treatment aligns with past research done on adult stickleback, which showed that stickleback experiencing differing predation conditions experience sex-specific selection on spine number (Reimchen & Nosil, 2004). Thus, there is a possibility that this work provides another example of the role of predation in sexually dimorphic trait diversification. Aside from stickleback, predation has been shown previously to contribute to sexual dimorphism in many species. For example, in sand fiddler crabs males with enlarged claws have been shown to experience less predation pressure

than females leading to different selection pressures (Bildstein et al., 1989). In chaffinches, higher predation risk in females is thought to promote crypsis in female tail plumage (Götmark et al., 1997). In ostracods, sexually dimorphic eyes have been noted and predation seems to be a strong maintenance factor for this specific trait (Speiser et al., 2013). Finally in toads poison glands color as well as size were sexually dimorphic due to higher predation pressure on males.

When we looked at the pattern of selection in each sex separately, we found that generally, males tended to exhibit a stronger response to predation than females. Within males, there were significant treatment effects ($P < 0.05$) for caudal peduncle depth, dorsal fin length, anal fin length, and trending ($P < 0.1$) treatment effects for eye diameter and mouth length. In contrast for females, the selective response was only significantly different between treatments for one trait, mouth length. This suggests predators may encounter and interact with the two sexes differentially. These differential interactions may lead to differences in the pattern of trait evolution for certain traits and ultimately increase dimorphism, as we found some limited evidence for above. Differences in selection between the sexes may be due to behavioral differences between males and females. However, we do not have data on diet or habitat use for the fish in the experiment, thus it is difficult to pinpoint the behavioral or ecological factors that may mediate the observed differences.

Given the differential patterns of selection, it was somewhat surprising that only two traits showed weak evidence of greater dimorphism due to predation, and we didn't find evidence of predation significantly affecting overall dimorphism. As this suggests that differential predation may not be a strong driver of overall dimorphism. However, a caveat of this study is that it considered only selection and dimorphism of juvenile stickleback. Prior work on stickleback suggests that the diet and habitat use, and levels of parasitism of juvenile males

and females are much more similar than that of adults and that as stickleback age, the differentiation between male and female ecological conditions increases (Reimchen & Nosil, 2004; Reimchen 1980; Reimchen and Nosil 2001). Males and females tend to experience significant differences between niches during adulthood. Generally, in comparison to males, females take advantage of a more limnetic-like diet and habitat usage as adults (Reimchen 1980; Reimchen and Nosil 2001; Sargent & Gebler, 1980). As a result, males and females may not have strongly different interactions with predators during the juvenile life stage. Thus, stronger effects of predation may be found if sub-adults or adults were considered (Ostlund-Nilsson et al., 2007; Bell & Foster, 1994). A future study examining patterns of dimorphism, habitat use, and predation in adult vs. juvenile samples would be very useful and insightful.

Overall, the results of the study might provide some evidence for a role of predation in the evolution of sexual dimorphism in stickleback, as we find evidence of differential patterns of selection between males and females when exposed to predators. This supports the idea that in stickleback sexual dimorphism can be driven by ecological factors rather than just sexual selection (Reimchen & Nosil 2004, Shine 1989, Slatkin 1984, Spoljaric & Reimchen, 2008), which has also been suggested for a variety of other species (Temeles et al., 2000, Shine et al., 2002, Silva et al., 2014, Woolbright, 1989). This work also might provide experimental evidence for the role of ecological factors in dimorphism, which has been rare in prior work in any taxa.

REFERENCES

- Acharya, L. (1995). Sex-biased predation on moths by insectivorous bats. *Animal Behaviour*, 49(6), 1461–1468. [https://doi.org/10.1016/0003-3472\(95\)90067-5](https://doi.org/10.1016/0003-3472(95)90067-5)
- Aguirre, W. E., Ellis, K. E., Kusenda, M., & Bell, M. A. (2008). Phenotypic variation and sexual dimorphism in anadromous threespine stickleback: Implications for postglacial adaptive radiation: Variation And Sexual Dimorphism In Anadromous Stickleback. *Biological Journal of the Linnean Society*, 95(3), 465–478. <https://doi.org/10.1111/j.1095-8312.2008.01075.x>
- Anderson, malte. (1996). Sexual selection. *Cell Press*, 11(2), 53–58. [https://doi.org/10.1016/0169-5347\(96\)81042-1](https://doi.org/10.1016/0169-5347(96)81042-1)
- Anderson, R. A., & Vitt, L. J. (1990). Sexual selection versus alternative causes of sexual dimorphism in teiid lizards. *Oecologia*, 84(2), 145–157. <https://doi.org/10.1007/BF00318265>
- Badyaev, A. V., & Martin, T. E. (2000). Sexual Dimorphism in Relation to Current Selection in the House Finch. *Evolution*, 54(3), 987–997. <https://doi.org/10.1111/j.0014-3820.2000.tb00098.x>
- Bell, M. A., & Foster, S. A. (1994). *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press,.
- Bildstein, K. L., McDowell, S. G., & Brisbin, I. L. (1989). Consequences of sexual dimorphism in sand fiddler crabs, *Uca pugilator*: Differential vulnerability to avian predation. *Animal Behaviour*, 37, 133–139. [https://doi.org/10.1016/0003-3472\(89\)90013-4](https://doi.org/10.1016/0003-3472(89)90013-4)
- Bolnick, D. I. (2003). Sexual Dimorphism And Adaptive Speciation: Two Sides Of The Same Ecological Coin. *Evolution*, 57(11), 2433–2449. <https://doi.org/10.1554/02-595>
- Darwin, C. (1874). Selection In Relation To Sex. *Descent Of Man*, 2, 397.
- Fairbairn, D. J. (1997). Allometry for Sexual Size Dimorphism: Pattern and Process in the Coevolution of Body Size in Males and Females. *Annual Review of Ecology and Systematics*, 28, 659–687.
- Ferguson, I. M., & Fairbairn, D. J. (2000). Sex-specific selection and sexual size dimorphism in the waterstrider *Aquarius remigis*. *Journal of Evolutionary Biology*, 13(2), 160–170.
- Frayer, D. W., & Wolpoff, M. H. (1985). Sexual Dimorphism. *Annual Review of Anthropology*, 14, 429–473.
- Götmark, F., Post, P., Olsson, J., & Himmelmann, D. (1997). Natural Selection and Sexual Dimorphism: Sex-Biased Sparrowhawk Predation Favours Crypsis in Female Chaffinches. *Oikos*, 80(3), 540–548. <https://doi.org/10.2307/3546627>

- Hedrick, A. V., & Temeles, E. J. (1989a). The evolution of sexual dimorphism in animals: Hypotheses and tests. *Trends in Ecology & Evolution*, 4(5), 136–138. [https://doi.org/10.1016/0169-5347\(89\)90212-7](https://doi.org/10.1016/0169-5347(89)90212-7)
- Hedrick, A. V., & Temeles, E. J. (1989b). The evolution of sexual dimorphism in animals: Hypotheses and tests. *Trends in Ecology & Evolution*, 4(5), 136–138. [https://doi.org/10.1016/0169-5347\(89\)90212-7](https://doi.org/10.1016/0169-5347(89)90212-7)
- Kitano, J., Mori, S., & Peichel, C. L. (2007). Sexual Dimorphism in the External Morphology of the Threespine Stickleback (*Gasterosteus Aculeatus*). *Copeia*, 2007(2), 336–349. [https://doi.org/10.1643/0045-8511\(2007\)7\[336:SDITEM\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2007)7[336:SDITEM]2.0.CO;2)
- Leinonen, T., Cano, J. M., & Merilä, J. (2011). Genetic basis of sexual dimorphism in the threespine stickleback *Gasterosteus aculeatus*. *Heredity*, 106(2), 218–227. <https://doi.org/10.1038/hdy.2010.104>
- Leutenegger, W., & Cheverud, J. M. (1985). Sexual Dimorphism in Primates. In W. L. Jungers (Ed.), *Size and Scaling in Primate Biology* (pp. 33–50). Springer US. https://doi.org/10.1007/978-1-4899-3647-9_3
- McGee, M. D., & Wainwright, P. C. (2013). Sexual dimorphism in the feeding mechanism of threespine stickleback. *Journal of Experimental Biology*, 216(5), 835–840. <https://doi.org/10.1242/jeb.074948>
- Mealey, Linda, S. J. C. (2000). Sex Differences: Developmental and Evolutionary Strategies. *Evolution and Human Behavior*, 22(5), 369–371. [https://doi.org/10.1016/S1090-5138\(01\)00064-2](https://doi.org/10.1016/S1090-5138(01)00064-2)
- Ostlund-Nilsson, S., Mayer, I., & Huntingford, F. A. (2007). *Biology of the three-spined stickleback*. CRC Press.
- Petrie, M., & Halliday, T. (1994). Experimental and natural changes in the peacock's (*Pavo cristatus*) train can affect mating success. *Behavioral Ecology and Sociobiology*, 35(3), 213–217. <https://doi.org/10.1007/BF00167962>
- Preziosi, R. F., & Fairbairn, D. J. (2000). Lifetime Selection on Adult Body Size and Components of Body Size in a Waterstrider: Opposing Selection and Maintenance of Sexual Size Dimorphism. *Evolution*, 54(2), 558–566. <https://doi.org/10.1111/j.0014-3820.2000.tb00058.x>
- Price, T. D. (1984). *The Evolution Of Sexual Size Dimorphism In Darwin's Finches*. 123(4).
- Reimchen, T. E. (1980). Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: An adaptation to predators? *Canadian Journal of Zoology*, 58(7), 1232–1244. <https://doi.org/10.1139/z80-173>
- Reimchen, T. E., & Nosil, P. (2001). Ecological causes of sex-biased parasitism in threespine stickleback. *Biological Journal of the Linnean Society*, 73(1), 51–63. <https://doi.org/10.1111/j.1095-8312.2001.tb01346.x>

- Reimchen, T. E., & Nosil, P. (2004). Variable Predation Regimes Predict the Evolution of Sexual Dimorphism in a Population of Threespine Stickleback. *Evolution*, 58(6), 1274–1281. <https://doi.org/10.1111/j.0014-3820.2004.tb01706.x>
- Rennison, D. J., Rudman, S. M., & Schluter, D. (2019). Genetics of adaptation: Experimental test of a biotic mechanism driving divergence in traits and genes. *Evolution Letters*, 3(5), 513–520. <https://doi.org/10.1002/evl3.135>
- Rundell, R. J., & Price, T. D. (2009). Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology & Evolution*, 24(7), 394–399. <https://doi.org/10.1016/j.tree.2009.02.007>
- Samia, D. S. M., Møller, A. P., Blumstein, D. T., Stankowich, T., & Cooper, W. E. (2015). Sex differences in lizard escape decisions vary with latitude, but not sexual dimorphism. *Proceedings of the Royal Society B: Biological Sciences*, 282(1805), 20150050. <https://doi.org/10.1098/rspb.2015.0050>
- Sá-Pinto, X., Cardia, P., & Campos, R. (2017). Sexual Selection: A Short Review on Its Causes and Outcomes, and Activities to Teach Evolution and the Nature of Science. *The American Biology Teacher*, 79(2), 135–143. <https://doi.org/10.1525/abt.2017.79.2.135>
- Sargent, R. C., & Gebler, J. B. (1980). Effects of nest site concealment on hatching success, reproductive success, and paternal behavior of the threespine stickleback, *Gasterosteus aculeatus*. *Behavioral Ecology and Sociobiology*, 7(2), 137–142. <https://doi.org/10.1007/BF00299519>
- Shine, R. (1989). Ecological Causes for the Evolution of Sexual Dimorphism: A Review of the Evidence. *The Quarterly Review of Biology*, 64(4), 419–461. <https://doi.org/10.1086/416458>
- Shine, R., Reed, R. N., Shetty, S., & Cogger, H. G. (2002). Relationships between Sexual Dimorphism and Niche Partitioning within a Clade of Sea-Snakes (Laticaudinae). *Oecologia*, 133(1), 45–53.
- Silva, J. M. da, Herrel, A., Measey, G. J., & Tolley, K. A. (2014). Sexual Dimorphism in Bite Performance Drives Morphological Variation in Chameleons. *PLOS ONE*, 9(1), e86846. <https://doi.org/10.1371/journal.pone.0086846>
- Slatkin, M. (1984). Ecological Causes of Sexual Dimorphism. *Evolution*, 38(3), 622–630. <https://doi.org/10.2307/2408711>
- Speiser, D. I., Lampe, R. I., Lovdahl, V. R., Carrillo-Zazueta, B., Rivera, A. S., & Oakley, T. H. (2013). Evasion of Predators Contributes to the Maintenance of Male Eyes in Sexually Dimorphic Euphilomedes Ostracods (Crustacea). *Integrative and Comparative Biology*, 53(1), 78–88. <https://doi.org/10.1093/icb/ict025>
- Spoljaric, M. A., & Reimchen, T. E. (2008). Habitat-dependent reduction of sexual dimorphism in geometric body shape of Haida Gwaii threespine stickleback: Reduction

In Sexual Dimorphism In Stickleback. *Biological Journal of the Linnean Society*, 95(3), 505–516. <https://doi.org/10.1111/j.1095-8312.2008.01068.x>

Temeles, E. J., Pan, I. L., Brennan, J. L., & Horwitt, J. N. (2000). Evidence for Ecological Causation of Sexual Dimorphism in a Hummingbird. *Science*, 289(5478), 441–443. <https://doi.org/10.1126/science.289.5478.441>

Vamosi, S. M. (2002). Predation sharpens the adaptive peaks: Survival trade-offs in sympatric sticklebacks. *Annales Zoologici Fennici*, 39(3), 237–248.

Vamosi, S. M., & Schluter, D. (2004). Character Shifts in the Defensive Armor of Sympatric Sticklebacks. *Evolution*, 58(2), 376–385. <https://doi.org/10.1111/j.0014-3820.2004.tb01653.x>

Woolbright, L. L. (1989). Sexual Dimorphism in *Eleutherodactylus coqui*: Selection Pressures and Growth Rates. *Herpetologica*, 45(1), 68–74.