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IMPACTS OF THE SOCIAL ENVIRONMENT ON MALE SEXUAL BEHAVIOR AND ATTENTION ALLOCATION

A THESIS APPROVED FOR THE DEPARTMENT OF BIOLOGY

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Abstract

Social communication occurs within a network and interactions between individuals rarely occur in private. The presence of observing individuals have been shown to affect the behavior of a signaling individual, however there are many aspects of a complex social environment that are still not well understood. My thesis assessed effecrs of the social environment on the mating behavior and attention allocation of male sailfin mollies *(Poecilia latipinna)*, a livebearing freshwater fish. Sailfin mollies lives in sympatry with the gynogenetic Amazon molly *(Poecilia Formosa)*, which requires the sperm of a heterospecific to begin embryogenesis. Male sailfin mollies can distinguish between conspecific females and Amazon mollies, but do receive indirect fitness benefits from copulations with Amazon mollies in cases of conspecific female mate choice copying. The use of this sexual-asexual mating system in these studies provides another interesting component of the social environment and may help elucidate ways in which Amazon mollies receive copulation opportunities. In this thesis, I addressed effects of rival male audience size on sexual activity and attention allocation toward a conspecific female or heterospecific Amazon female. Additionally, I looked at how the species of a female audience (conspecific or heterospecific) altered sexual behavior and attention division toward a conspecific female or heterospecific Amazon female.

I did not find any significant changes to male sexual behavior in the presence of a male audience of varying body size while partnered with either a conspecific of heterospecific female. However, in the presence of a female audience, I did find significant effects of stimulus female species, as males displayed more behaviors

toward conspecific stimulus females but there was no effect of the species of the audience female. This result confirms previous findings that male sailfin mollies both distinguish between and prefer conspecific females over heterospecific females but also shows that current interactions influence behavior more than possible future interactions. Due to my experimental design, my study closely replicated events that would occur in complex social situations in nature, so my results could be due in part to limits on cognitive load.

In my study of attention allocation, I found that in both cases, males direct more attention toward a stimulus female regardless of female species or male audience, but direct more attention towards conspecifics overall. In addition, the species of the female audience also significantly influenced how male attention was divided, which may be due to anticipation of future copulation opportunities. I also found that focal male size is important in attention allocation and follows predictions of perceived sperm competition risk based on relative body size. The results provided by this study show that attentional division is not determined by one factor alone, but rather several components of the social environment in which an individual interacts. Overall, my thesis highlights the importance of social context in influencing behavior and dividing an individual's attention in mating situations.

Chapter 1

Impacts of a dynamic social environment on audience effects in male sailfin mollies *(Poecilia latipinna)*

Authors: Shelby D. Burridge, Amber M. Makowicz, and Ingo Schlupp

Abstract

In environments with complex social networks, it is unlikely that interactions will occur privately between a single signaler and a single receiver. Instead, beneficial information can be gathered and utilized by observing audience individuals. In mating situations, the presence of an audience has negative repercussions for the signaling male as the audience individual may use gather information to copy the mate choice of the signaler, thus increasing the risk of sperm competition for the signaler. Audience effects result in the modification of the behavior of the signaling individual in order to minimize this risk. As these effects are widely observed in poeciliid fish species, we asked how the composition of the social environments affects male sexual behavior in the sailfin molly *(Poecilia latipinna).* Sailfin molly males are part of a sexual-asexual mating complex with the gynogenetic Amazon molly *(Poecilia formosa).* Amazon mollies reproduce clonally, but require the sperm of a heterospecific to begin embryogenesis. Thus, the sailfin-Amazon molly relationship adds another unique component to a highly social environment. Using this system, we addressed two main questions. Our first experiment investigated how focal male sexual behavior changes toward a conspecific (sailfin molly) or heterospecific (Amazon molly) female when in the presence of an audience male that is either larger in size, smaller in size, or equal to the size of the focal male. In our second experiment, we asked how a male alters his sexual behavior toward a conspecific or heterospecific female when in the presence of a female audience that is conspecific or heterospecific. We did not find any significant changes to male sexual behavior in the presence of a male audience of varying body size while partnered with either a conspecific of heterospecific female. However, in the presence of a female audience, we did find significant effects of stimulus female species, as males showed an overall greater amount of sexual activity towards conspecific females. This result confirms previous findings that male sailfin mollies both distinguish between and prefer conspecific females over heterospecific females. Conversely, we did not find an effect of audience female type on male mating behavior.

Keywords: audience effects, sperm competition, communication networks

Introduction

Displays and signals provide a pathway of communication between individuals and are typically thought to result from the coevolution of both the signaler and the receiver (Doutrelant et al. 2001). In environments where social networks are extensive, interactions between two individuals are going to be observed by more than the intended receiver (McGregor 1993; McGregor and Peake 2000). When considering such situations, it has been suggested that communication may not have evolved in simple signaler/receiver dyads but rather across more complex communication networks (McGregor and Peake 2000; Matos and Schlupp 2005).

In complex social environments, information can be gathered and utilized by individuals other than the intended target. This type of information gathering often has very little cost or risk for the bystander and but instead can be highly advantageous (McGregor 1993; Dabelsteen 2005). These eavesdroppers or audiences as they with henceforth be referred, can extract a variety of information from these observations about both the signaler and the intended receiver. Therefore, audience individuals can indirectly gain valuable information about potential mates and potential rivals.

Observing the interactions of others occurs in a variety of species and allows both males and females to assess the quality of a potential mate before expending energy on a direct interaction (Danchin et al. 2004; White 2004; Shettleworth 2010). For example, before engaging in extra pair copulations, female Great Tits *(Parus major)*, have been shown to gather information on male quality by listening to the competitive singing interaction of two rival males (Otter et al. 1999). In environments where sexual competition is high, audience individuals may gain valuable information about potential rivals before a direct confrontation ever occurs. Male Siamese fighting fish *(Betta splendens)* are highly territorial and contests over territory can result in physical injury and even death (Simpson 1968). However, by eavesdropping on the male-male interactions of potential opponents, individuals can assess the signalers fighting ability and modify their behavior towards that opponent in the future based on the gathered information (Oliveira et al. 1998). Observations made by an audience individual can also lead to mate choice copying. This occurs when an observing individual copies the mating decision of another (Dugatkin 1992; Pruett-Jones 1992; Santos et al. 2014). Sailfin molly *(Poecilia latipinna)* females prefer males that are larger in size and after observing the interaction of a conspecific female with two other males, will copy the mate choice of that female if the males do not vary greatly in body size (Witte and Ryan 1998). Females also prefer to associate with males that they have previously observed associating with high quality females, which may help the female to assess the quality of the interacting male (Hill and Ryan 2006). Females are also capable of remembering and copying the choice of another female for up to 1 day after the initial observation (Witte and Massmann 2003). This behavior has been exhibited in both laboratory studies and in wild populations (Witte and Ryan 2002). Copying the mate choice of other females provides a means to assess male quality before a direct interaction occurs and may be especially beneficial to less experienced females (Dugatkin 1992; Dugatkin and Godin 1993).

Female mate choice copying can also influence the variance in male mating success and, consequently, sexual selection (Wade and Pruett-Jones 1990). When observing females frequently copy the mate choice for a specific male, he receives a fitness benefit. Male sailfin mollies may receive a unique additional benefit from female mate choice copying. Male sailfin mollies play host to a sexual parasite, the Amazon molly *(Poecilia formosa)*, a gynogenetic hybrid that is found in mixed shoals with their host species (Hubbs and Hubbs 1932; Schlupp and Ryan 1996). Male sailfin mollies are capable of distinguishing conspecific females from Amazons and also display a strong preference towards conspecific females (Schlupp et al. 1991; Ryan et al. 1996; Gabor and Ryan 2001; Heubel et al. 2008). However, when male sailfin mollies interact with Amazons and this interaction is observed by conspecifics, conspecific females often copy the mate choice decisions of the Amazons. Thus, these interactions increase the likelihood that males will receive copulation opportunities with sailfin females (Schlupp et al. 1994; Heubel et al. 2008).

Currently, the literature is heavily weighted toward observations of female mate choice copying but several studies have detected instances of male mate choice copying (Schlupp and Ryan 1997; Witte and Ryan 2002; Frommen et al. 2009; Bierbach et al. 2011; Auld and Godin 2015). Whereas implications of female mate choice copying are relatively clear, the tradeoff between costs and benefits of male mate choice copying is a little less understood. Observing the interactions of other males has shown to be beneficial while locating females, as it reduces sampling time (Webster and Laland 2013). Additionally, copying the choice of other males may also be a means to assess the receptivity of females (Schlupp and Ryan 1997). However, in environments where sexual competition is high, males face high levels of sperm competition risk especially when by-standing males may copy the mate choice of the signaler (Bierbach et al. 2011; Jeswiet et al. 2011). Therefore, signaling males may alter their behavior in order to minimize the risk of competition and mate choice copying (Ziege et al. 2009; Bierbach et al. 2013). This alteration in behavior due to the presence of an audience is known as an "audience effect." These effects have been shown to occur in a variety of social contexts (Marler et al. 1986; McGregor and Peake 2000; Dzieweczynski et al. 2005, 2012, 2014; Matos and Schlupp 2005; Bierbach et al 2015) and in many different systems including fishes, birds and mammals (Evans and Marler 1994; Pollick et al. 2005).

The use of audience effects to counter competition has been widely studied in poeciliid fish species (Evans and Magurran 2001). Research conducted by Plath and colleagues (2008a) on the Atlantic molly *(Poecilia mexicana)* has shown that the presence of an audience male leads to a decrease in the amount of time associating with a preferred female. In another study, Atlantic molly males were also shown to direct sexual behaviors toward an initially non-preferred female, which has been interpreted as a form of deceptive behavior (Plath et al. 2008b). Male Trinidadian guppies have been observed to decrease thefrequency of courtship displays as the number of males observing the interaction increases (Auld et al. 2015). These behavioral changes may decrease the likelihood that an audience male will copy the mate choice of the signaling male and reduce their risk of sperm competition (Schlupp and Ryan 1997; Ziege et al. 2009; Jeswiet et al. 2012).

Another important aspect to consider is whether or not audience male body size will directly influence the sexual behaviors displayed by a signaling male. In a study that utilized video playback of an audience male of varying body size (smaller or larger than signaler), sailfin mollies where observed to actually increase sexual behavior towards a preferred female in the presence of an audience male that is larger in size (Makowicz et al. 2010). Although this result is different than what is expected under sperm competition risk theory, it may be due to the proximity of the audience male. Padur and colleagues (2009) found that male Atlantic mollies increase sexual activity when the male is presented outside of the immediate test tank, but do not do so when he is presented within the test tank. This difference suggests that when the audience is further away, perceived risk of sperm competition may lead to an increase in mating attempts, whereas when the audience is closer, and within immediate communication range, it is beneficial for males to conceal their mate choice to decrease the likelihood of mate choice copying (Padur et al. 2009). Although the study by Makowicz et al. (2010) did assess effects of audience male body size on signaler behavior, the audience was outside of the immediate test area. Therefore, it is still not known how the size of an observing male that is directly within communication range, affects the behavior of a signaling individual.

In this study, we investigated two different effects of the social environment on male mating behavior. Utilizing the unique sexual-asexual mating complex between sailfin mollies and Amazon mollies, we first asked whether or not the size of a rival male audience alters the way in which a male interacts with a female. By using both conspecific sailfin females and heterospecific Amazon mollies as stimuli, we can assess broader social interactions within this system. We predicted that in the presence of an audience male that is smaller in size than the focal male, focal males will increase their sexual behaviors toward a stimulus female. However, when the audience male is larger or equal in size to the focal male, the focal male will decrease his display of sexual behaviors to decrease the likelihood of mate choice copying by the audience male, a potential sperm competitor. We also predicted that focal males will display more sexual behaviors toward conspecific females than toward heterospecific females independent of audience male body size.

In our second experiment, we asked whether or not males altered their sexual behavior towards a conspecific or heterospecific female in the presence of a female audience that was also either conspecific or heterospecific. We predicted that males will increase sexual behaviors when both the stimulus and audience females are conspecifics in order to gain additional copulations through mate choice copying. However, males should not change sexual behaviors with a conspecific stimulus female when the audience female is heterospecific. Conversely, when the stimulus female is heterospecific, we predicted that males will increase their sexual behaviors toward the stimulus when the audience is a conspecific so as to increase the likelihood of future copulations with a conspecific through mate choice copying because conspecific sailfin females have been shown to copy the mate choice of heterospecific Amazons (Schlupp et al. 1994; Heubel et al. 2008). Finally, we predicted that there will not be a significant change in sexual behavior when both the stimulus and audience are heterospecific females.

By understanding how males respond to differing female stimuli while in the presence of a variable audience, we can investigate effects of a highly dynamic social environment on individual behavior. Additionally, these studies can provide us with information on how sperm competition risk is perceived and responded to, and may also help to explain the persistence of the Amazon molly in nature.

Methods

Study subjects

Subjects were wild-caught in a drainage basin in Weslaco, Texas (26° 7'13.13"N 97°57'41.08"W) and brought back to the University of Oklahoma, Norman, OK, USA. Collection trips were conducted in May 2015, July 2015, October 2015 and May 2016. Fish were housed in small mixed-sex/mixed-species groups in 37.85-L tanks under 12L/12D light conditions, with weekly 50% water changes. Fish were fed commercial flake food (TetraMin®) *ad libitum* daily, and supplemented with a mix of frozen *Daphnia* and blood worms twice weekly. Fish were allowed to acclimate to laboratory housing for a minimum of 30 days before testing began. After the 30-day acclimation period, measurements of standard length (mm) were taken for all males who were subsequently placed in individual 3.79-L isolation tanks. Female fish were then separated by species into 37.85-L tanks to await testing.

Experimental setup

In the experimental tank, Plexiglas containers were used to isolate an observing "audience" individual from a free-swimming focal male and a stimulus female. Trials were conducted in a 37.85-L tank. To prevent any distractions from the surrounding environment, three sides of the tank were covered by white plastic board while the fourth side remained uncovered so that interactions could be filmed with a Nikon D5200 24.1 MP CMOS Digital SLR camera. A clear, closed-bottomed Plexiglas container with small holes cut out to allow for chemical communication was placed in the rear center of the tank (Figure 1) to house the audience individual (a male or female individual), while two open bottomed Plexiglas containers were placed in the center of the tank to individually house the focal male and stimulus female. After a 10-minute acclimation period, the focal male and stimulus female were removed from their containers and permitted to interact. After an interaction period of 10 minutes, the camera was stopped and individuals were either placed in a holding tank to await the next trial or if all trials for that individual were complete, returned to their former housing conditions. Videos were subsequently analyzed by recording the occurrence and counts for the following male behaviors: nipping at the female's genital pore, attempted copulations (thrusting of the gonopodium) and overall time following the female (Woodhead and Armstrong 1985).

Experiment 1: Male audience

In this experiment, we assessed how audience male body size influenced focal male sexual behavior. Each trial consisted of a focal male paired with either an Amazon or sailfin molly female for stimulus, with an observing audience male that was either equal in size, smaller than, or larger than the focal male. Females were size matched to a standard length within ±3mm. Males of equal size were within ±2mm, while smaller males were at least 4mm smaller and larger males were at least 4mm larger. Eight social conditions were studied (Table 1). Each individual focal male participated in all eight trials over a 2-day period. The 2 days were split according to stimulus female species, which was chosen at random. On that day, a male would undergo the control for that female species and the three male size trials. The order of the trials was randomized within that day.

Experiment 2: Female Audience

In this experiment we assessed how audience female species influenced focal male sexual behavior. Each trial consisted of a focal male paired with either an Amazon or sailfin molly female for stimulus with an observing audience female that was either an Amazon or sailfin molly. Females were size matched to a standard length within ±3mm. Six social conditions were studied (Table 3). Each individual focal male participated in all four trials with the controls over a 2-day period. The 2 days were split according to stimulus female species, which was chosen at random. On that day, a male would undergo the control for that female species and the two audience trials. The order of the trials was randomized within that day.

Data Analysis

Experiment 1: Male audience

A total of 18 males were tested for each of the 8 treatments. To assess effects of audience male size on focal male sexual behavior, we used a MANOVA. Male identity was used as a repeated factor to account for variation between individual males while stimulus female type (sailfin or Amazon) and audience male type (control, smaller than, equal to or larger than focal) were used as fixed factors. The response variables were amount of time following a female, number of times a male nipped at the female's genital opening, and number of attempted copulations.

Experiment 2: Female audience

A total of 20 males were tested for each of the 6 treatments. To assess effects of female species and availability on attention allocation, we used a MANOVA. Male identity was used as a repeated factor to account for variation between individual males while stimulus female type (sailfin or Amazon) and audience female type (control, sailfin, or Amazon) were used as fixed factors. The response variables were amount of time following a female, number of times a male nipped at the female's genital opening, and number of attempted copulations. All data analyses were conducted using SAS Studio 3.4.

Results

Experiment 1: Male audience

For the male audience experiment, MANOVA found no overall significant effect of stimulus female type (Wilk's $\Lambda_{3,134}=0.97$, p=0.20), audience male type (Wilk's $\Lambda_{9,326.27}=0.56$ p=0.83) or their interaction (Wilk's $\Lambda_{9,326.27}=0.84$, p=0.58).

Experiment 2: Female audience

For the female audience experiment, MANOVA found a significant overall effect of stimulus female type (Wilk's $\Lambda_{3,112}=3.78$, p=0.013), but no statistically significant effect of audience female type (Wilk's $\Lambda_{6,224}=1.11$ p=0.36) or the interaction between stimulus and audience type (Wilk's $\Lambda_{6,224}=1.14$, p=0.34). When subsequent ANOVA's were run to analyze the effect of stimulus on the three dependent variables, a significant effect of stimulus was found for following ($F_{1,119}$ =10.24, p=0.002), where focal males spent more time following conspecific females in all of the treatment groups. No significant effect of audience or an interaction between stimulus and audience was found. Additionally, an ANOVA run for nipping was also significant for stimulus type ($F_{1,119}$ =11.18, p=0.001), where males nipped conspecific females more than heterospecifics females in all treatments. No significant effect was found for audience or the interaction between stimulus and audience. An ANOVA for copulation also found a significant effect of stimulus ($F_{1,119}$ =7.51, p=0.007) where males attempted to copulate with conspecific females more often than heterospecific females. No significant effect was found for audience and the interaction between stimulus and audience was also not significant.

Discussion

In our male audience experiment, we had predicted that in the presence of an audience male that was smaller in size than the focal male, a focal male would increase his sexual behavior toward a stimulus female but when the audience male was larger, the focal male would decrease his display of sexual behaviors in order to prevent mate choice copying by the audience male. These predictions were not confirmed, as we found no significant effect of audience male size. This was unexpected, as many previous studies observed changes in the sexual behavior of male poeciliids due to the presence of a rival audience (Plath et al. 2008a; Plath et al. 2008b; Padur et al. 2009; Makowicz et al. 2010; Nöbel and Witte 2013). We also predicted that focal males would display more sexual behaviors toward conspecific females than to heterospecific females independent of audience male body size and our results for this prediction were also non-significant. This result was also unanticipated as male sailfin mollies are known to prefer conspecific females (Schlupp et al. 1991; Ryan et al. 1996; Gabor and Ryan 2001). It is possible that our experimental design may have led to a decrease in observed sexual activity. Our study sought to observe many aspects of a complex social environment in a biologically realistic design that, combined, may have had negative implications for the processing ability of the focal males.

In our female audience experiment, we had predicted that males would increase sexual behaviors when both the stimulus and audience females were conspecifics in order to gain additional copulations through mate choice copying, but that males would not change sexual behaviors with a conspecific stimulus female when the audience female was heterospecific. Conversely, we had predicted that when the stimulus female was heterospecific, males would increase sexual behaviors toward the stimulus female when the audience was a conspecific so as to increase the likelihood of future copulations with a conspecific female through mate choice copying. Finally, we had predicted that there would be no significant change in sexual behavior when both the stimulus and audience were heterospecific females.

Our results show that in all treatments there was a significant effect of stimulus female species overall, as males showed more sexual behaviors toward conspecific females. Univariate analyses of variance found a significant effect of stimulus female type for following behavior, nipping behavior and copulation attempts independently as all three were exhibited to a greater degree towards conspecifics. This is not unexpected as previous studies have shown that males prefer conspecific sailfin molly females over heterospecific Amazon molly females (Ryan et al. 1996; Gabor and Ryan 2001). We did not, however, find an effect of audience female type or of the interaction between stimulus female species and audience female species. These results were unanticipated as the males are known to benefit from interacting with an Amazon female when a conspecific female observes the interaction and copies the mate choice of the heterospecific female (Schlupp et al. 1994). Our results suggest that the species of the readily accessible stimulus female is the most important factor in governing male sexual behavior. Additionally, the species of the audience female does not directly influence behaviors displayed toward a stimulus female which suggests that current interactions, rather than potential future interactions, are the main mediator of male sexual behavior.

While our results were largely non-significant, it is possible that with larger sample sizes, we would have observed greater statistical significance. Additionally, experiments were conducted in September and October of 2015, near the end of the natural breeding season of these fish which may potentially have decreased the overall amount of sexual activity displayed by focal males. Future work should address concerns of sample size and breeding season. However, we hypothesize that our results may better represent what is occurring in natural situations. While many studies confine stimulus individuals and employ a binary choice design, we wanted to achieve a balance between biological realism and a complex, yet feasible laboratory study. Males were allowed to interact with stimulus females whose own receptivity and behavior may have had effects on the signaling behavior of the focal individual. The addition of an audience individual serving to distract the focal male may have also reduced the number of behaviors observed. These factors combined may have pushed the limits of cognitive load capabilities in an organism with minimal neural capacity (Dukas and Kamil 2000; Dukas 2004).

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Appendix

Table 1 – Experimental treatments for the male audience experiment. Table displays stimulus female species and male audience body size relative to the size of the focal male. Body size was measured as standard length. Males of equal size were within a standard length of ±2mm while smaller males were at least 4mm smaller and larger males were at least 4mm larger. Females were size matched to a standard length within ±3mm.

Stimulus female species	Audience male size relative to focal male
Poecilia latipinna	Smaller (at least 4mm)
Poecilia latipinna	Larger (at least 4mm)
Poecilia latipinna	Equal (±2mm)
Poecilia latipinna	No audience (control)
Poecilia formosa	Smaller (at least 4mm)
Poecilia formosa	Larger (at least 4mm)
Poecilia formosa	Equal (±2mm)
Poecilia formosa	No audience (control)

Table 2 – Descriptive statistics for male audience experiment on male behavior according to stimulus female species and male audience body size. Stimulus was either Amazon or sailfin females. Audience males were either larger than the focal male by a standard length of at least 4mm, smaller than the focal male by at least 4mm, equal in size to the audience male (±2mm), or was the Control group where no audience was present in the audience chamber. N=18 for each group.

Stimulus Female	Audience Males	Variable	Mean	Std Dev	Minimum	Maximum
Amazon	Control	Following	19.07	38.11	0	155.93
		Nips	33.61	74.44	0	310.00
		Copulations	6.56	15.88	0	68.00
	Equal	Following	21.28	42.54	0	167.17
		Nips	32.61	63.57	0	245.00
		Copulations	8.17	16.02	0	59.00
	Larger	Following	5.62	6.04	0	19.62
		Nips	8.28	8.41	0	26.00
		Copulations	1.44	1.98	0	7.00
	Smaller	Following	15.21	24.87	0	85.65
		Nips	21.00	33.30	0	102.00
		Copulations	5.22	9.19	0	31.00
Sailfin	Control	Following	36.76	41.12	0	133.82
		Nips	58.94	61.25	0	163.00
		Copulations	11.78	15.90	0	51.00
	Equal	Following	19.51	23.88	0	85.42
		Nips	31.06	39.07	0	151.00
		Copulations	7.11	10.78	0	36.00
	Larger	Following	30.74	53.51	0	214.42
		Nips	49.78	90.36	0	377.00
		Copulations	11.44	20.38	0	82.00
	Smaller	Following	21.45	28.14	0	82.32
		Nips	36.78	45.42	0	131.00
		Copulations	7.50	11.15	0	32.00

Table 3 – Experimental treatments for the female audience experiment. Females were size matched to a standard length within ±3mm.

Stimulus female species	**Audience female species**
Poecilia latipinna	*Poecilia latipinna*
Poecilia latipinna	*Poecilia formosa*
Poecilia latipinna	No audience (control)
Poecilia formosa	*Poecilia latipinna*
Poecilia formosa	*Poecilia formosa*
Poecilia formosa	No audience (control)

Table 4 – Descriptive statistics for female audience experiment on behavior according to stimulus female species and audience female species. Stimuli were either Amazon or sailfin females. Audience was either an Amazon female, sailfin female, no fish (Control). N=20 for all groups.

Stimulus	Audience	Variable	Mean	Std Dev	Minimum	Maximum
Amazon	Amazon	Following	24.29	39.70	0	130.18
		Nips	24.15	44.25	0	165.00
		Copulations	6.55	11.39	0	38.00
	Control	Following	18.51	30.70	0	122.78
		Nips	15.80	25.72	0	119.00
		Copulations	2.35	3.88	0	17.00
	Sailfin	Following	27.87	39.82	0	120.45
		Nips	22.00	28.77	0	99.00
		Copulations	6.30	9.76	0	38.00
Sailfin	Amazon	Following	47.60	80.53	0	296.82
		Nips	43.75	70.90	0	271.00
		Copulations	6.90	12.43	0	54.00
	Control	Following	86.62	138.00	0	503.60
		Nips	93.60	130.96	0	475.00
		Copulations	14.85	19.41	0	73.00
	Sailfin	Following	80.83	103.88	0	354.00
		Nips	59.10	82.02	0	343.00
		Copulations	14.25	19.51	0	68.00

Figure Legend

Fig. 1 Diagram of experimental set up with audience housed in clear Plexiglas container (perforated for chemical and olfactory communication) in the rear center of tank, while focal male and stimulus female are permitted to swim freely after acclimation period of 10 minutes. During the male audience experiment, the audience container housed a male that was either larger, smaller or equal in size to the focal male and the stimulus female was either a conspecific sailfin or a heterospecific Amazon molly. During the female audience experiment, the audience container housed either a conspecific sailfin female or a heterospecific Amazon molly. The stimulus female was also conspecific or heterospecific.

Fig. 2 Box and whisker plots of amount of time focal males spent following stimulus females across all audience treatments. The horizontal line within each box indicates the median, boundaries of the box indicate the 1^{st} and 3^{rd} quartile, and the whiskers indicate the highest and lowest values of the results.

Fig. 3 Box and whisker plots of number of copulation attempts performed by focal males toward stimulus females across all audience male treatments. The horizontal line within each box indicates the median, boundaries of the box indicate the 1^{st} and 3^{rd} quartile, and the whiskers indicate the highest and lowest values of the results.

Fig. 4 Box and whisker plots of number of gonopore nips performed by focal males toward stimulus females across all audience male treatments. The horizontal line within each box indicates the median, boundaries of the box indicate the 1^{st} and 3^{rd} quartile, and the whiskers indicate the highest and lowest values of the results.

Fig. 5 Box and whisker plots of amount of time focal males spent following stimulus females across all audience female treatments.The horizontal line within each box indicates the median, boundaries of the box indicate the 1^{st} and 3^{rd} quartile, and the whiskers indicate the highest and lowest values of the results.

Fig. 6 Box and whisker plots of number of copulation attempts performed by focal males toward stimulus females across all audience female treatments. The horizontal line within each box indicates the median, boundaries of the box indicate the 1^{st} and 3^{rd} quartile, and the whiskers indicate the highest and lowest values of the results.

Fig. 7 Box and whisker plots of number of gonopore nips performed by focal males toward stimulus females across all audience female treatments. The horizontal line within each box indicates the median, boundaries of the box indicate the 1^{st} and 3^{rd} quartile, and the whiskers indicate the highest and lowest values of the results.

Figure 1

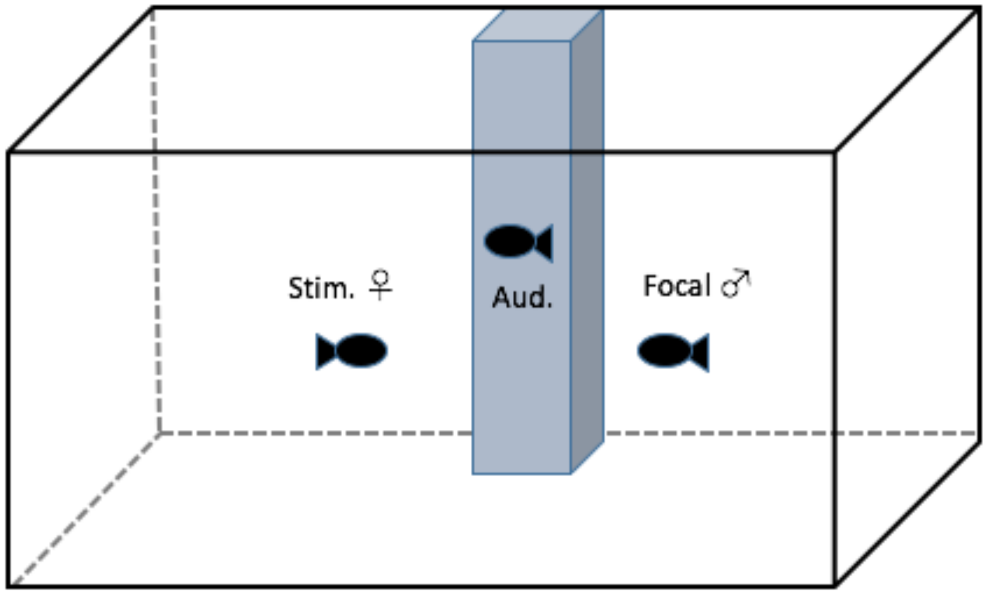


Figure 2

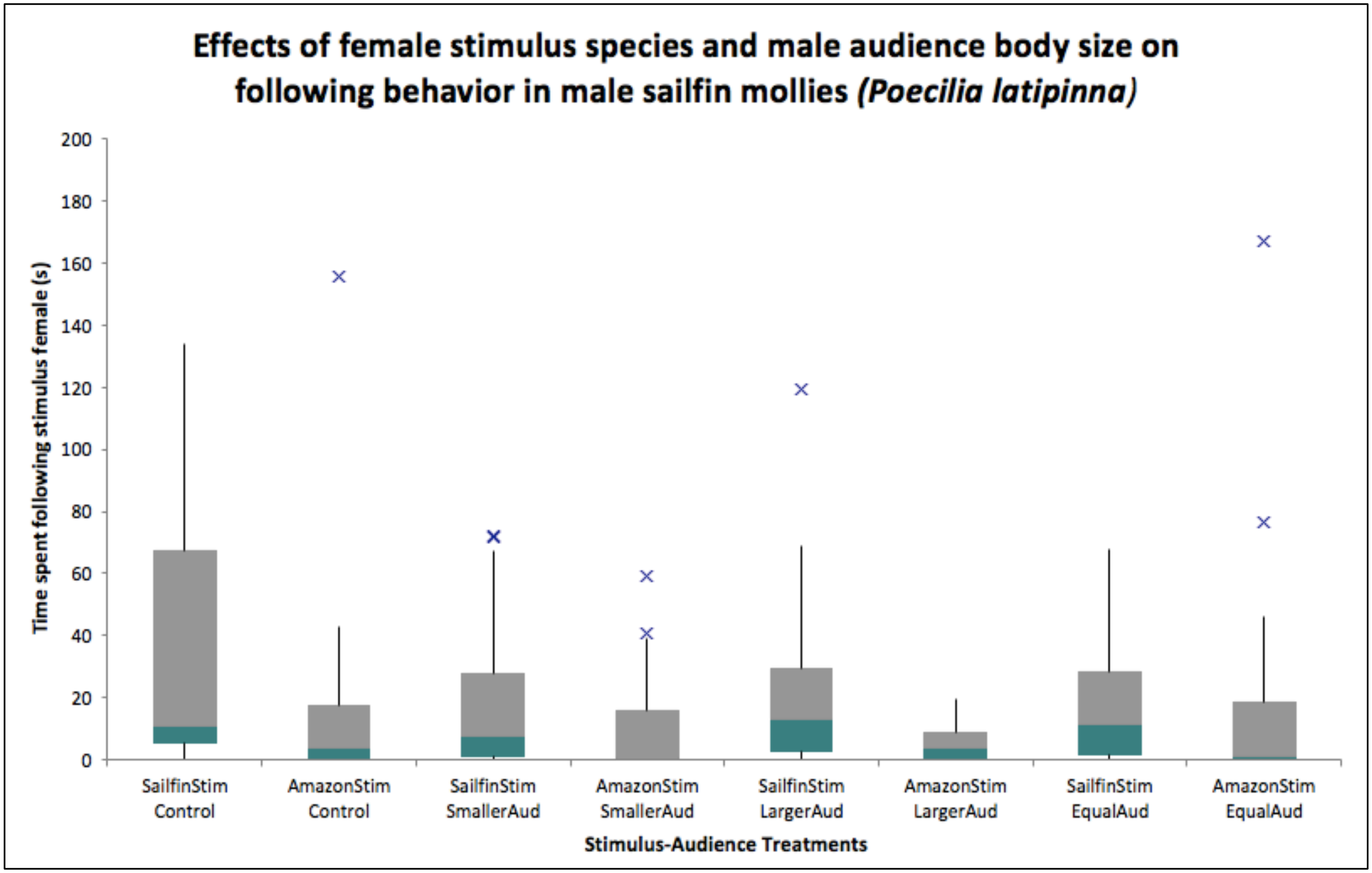
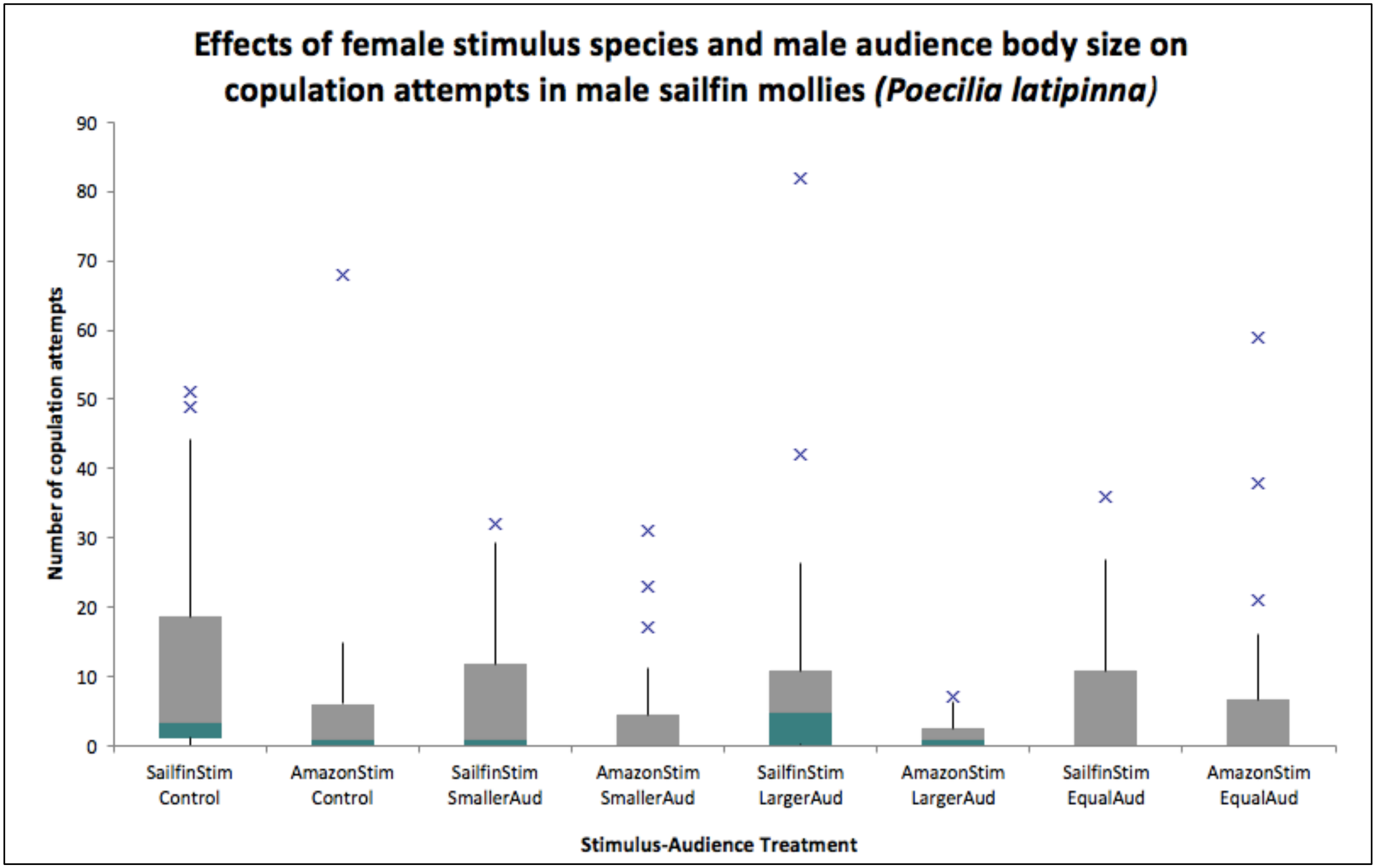

Effects of female stimulus species and male audience body size on following behavior in male sailfin mollies (Poecilia latipinna)
Time spent following stimulus female (s)
200
180
160
140
120
100
80
60
40
20
0
SailfinStim Control
AmazonStim Control
SailfinStim SmallerAud
AmazonStim SmallerAud
SailfinStim LargerAud
AmazonStim LargerAud
SailfinStim EqualAud
AmazonStim EqualAud
Stimulus-Audience Treatments

Figure 3



Effects of female stimulus species and male audience body size on copulation attempts in male sailfin mollies (Poecilia latipinna)
Number of copulation attempts
0
10
20
30
40
50
60
70
80
90
SailfinStim Control
AmazonStim Control
SailfinStim SmallerAud
AmazonStim SmallerAud
SailfinStim LargerAud
AmazonStim LargerAud
SailfinStim EqualAud
AmazonStim EqualAud
Stimulus-Audience Treatment

Figure 4

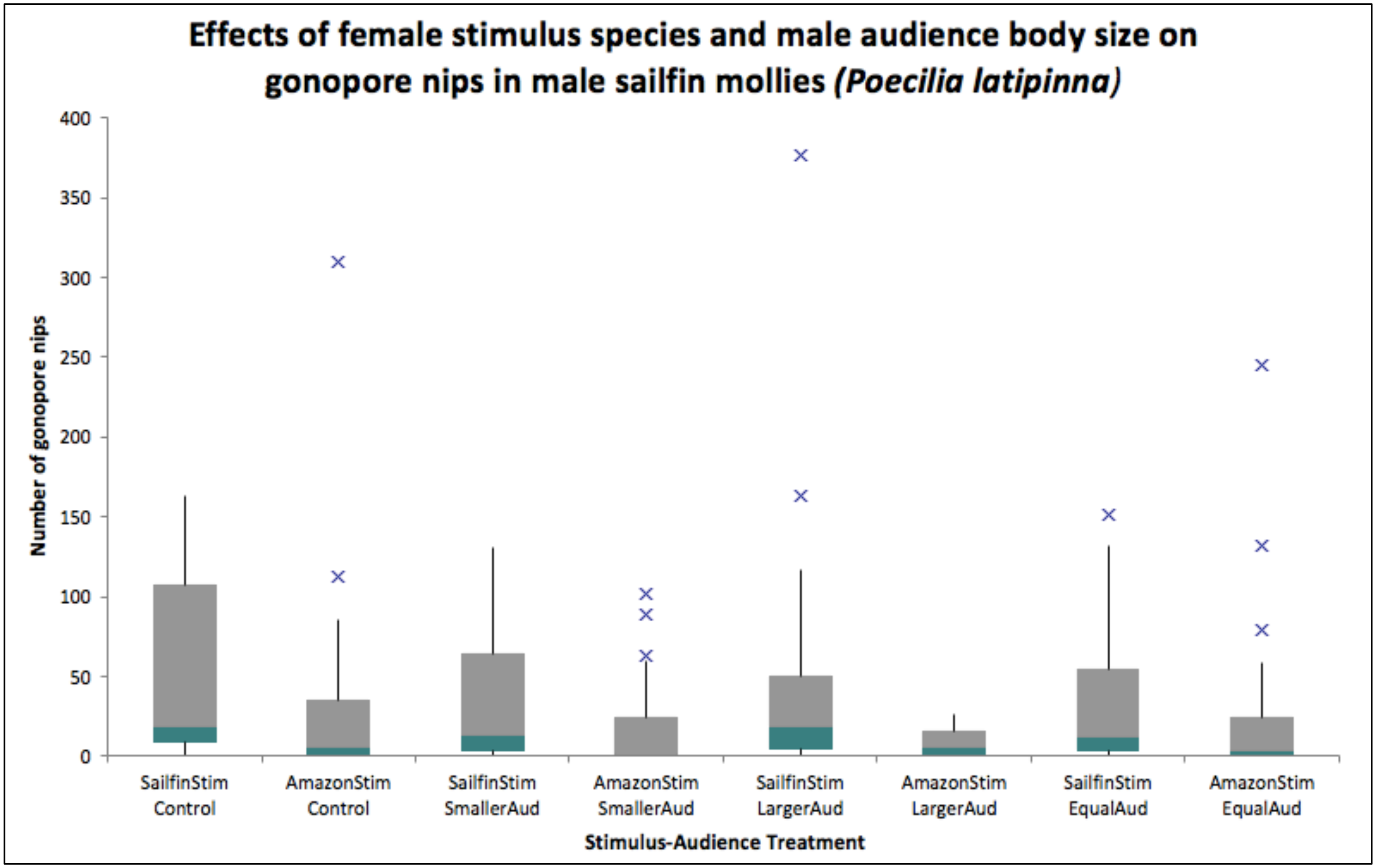
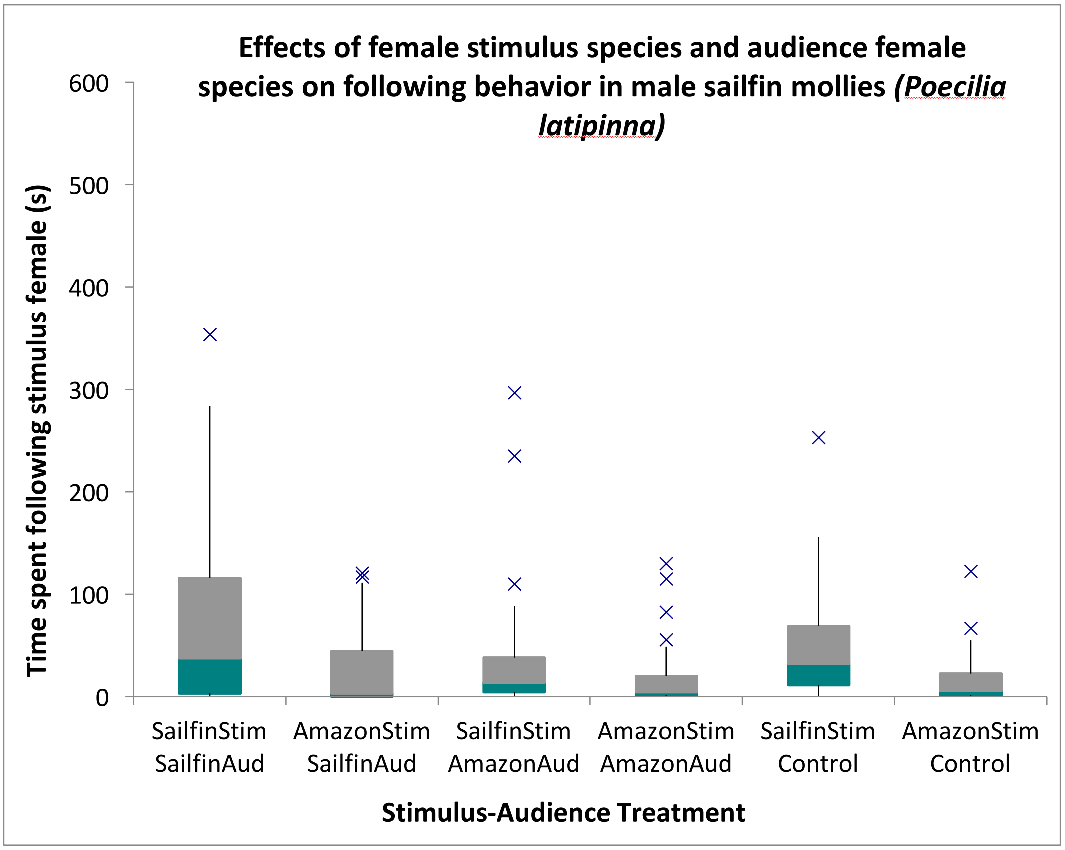

Effects of female stimulus species and male audience body size on gonopore nips in male sailfin mollies (Poecilia latipinna)
Number of gonopore nips
400
350
300
250
200
150
100
50
0
SailfinStim Control
AmazonStim Control
SailfinStim SmallerAud
AmazonStim SmallerAud
SailfinStim LargerAud
AmazonStim LargerAud
SailfinStim EqualAud
AmazonStim EqualAud
Stimulus-Audience Treatment

Figure 5



Effects of female stimulus species and audience female species on following behavior in male sailfin mollies (Poecilia latipinna)
Time spent following stimulus female (s)
600
500
400
300
200
100
0
SailfinStim SailfinAud
AmazonStim SailfinAud
SailfinStim AmazonAud
AmazonStim AmazonAud
SailfinStim Control
AmazonStim Control
Stimulus-Audience Treatment

Figure 6

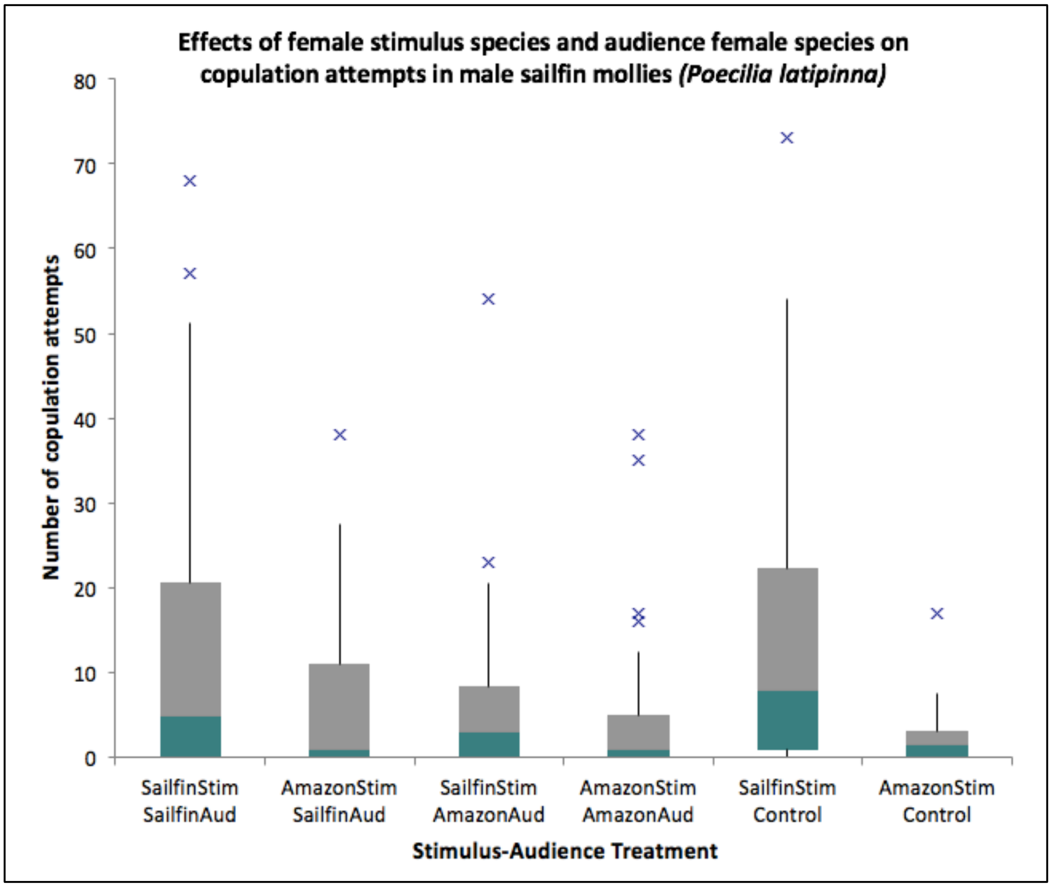
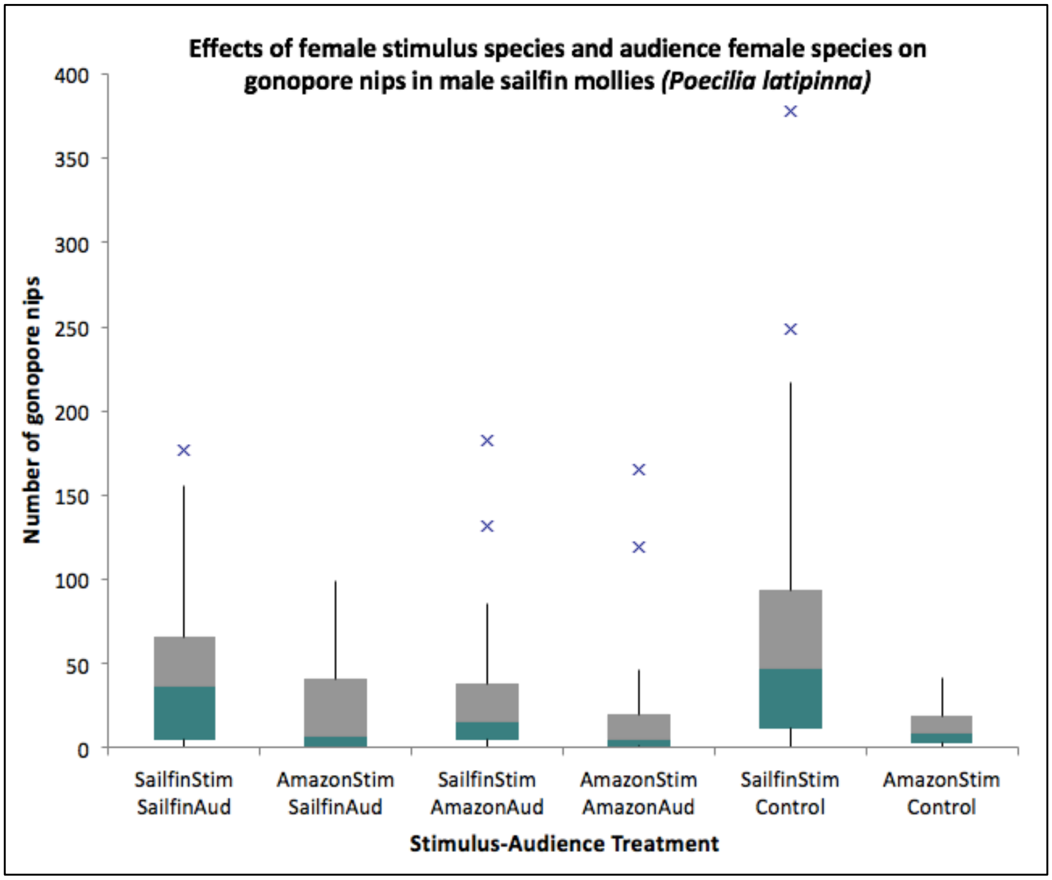

Effects of female stimulus species and audience female species on copulation attempts in male sailfin mollies (Poecilia latipinna)
Number of copulation attempts
0
10
20
30
40
50
60
70
80
SailfinStim SailfinAud
AmazonStim SailfinAud
SailfinStim AmazonAud
AmazonStim AmazonAud
SailfinStim Control
AmazonStim Control
Stimulus-Audience Treatment

Figure 7


Effects of female stimulus species and audience female species on gonopore nips in male sailfin mollies (Poecilia latipinna)
Number of gonopore nips
0
50
100
150
200
250
300
350
400
SailfinStim SailfinAud
AmazonStim SailfinAud
SailfinStim AmazonAud
AmazonStim AmazonAud
SailfinStim Control
AmazonStim Control
Stimulus-Audience Treatment

Chapter 2

Attention allocation is social context dependent

Authors: Shelby D. Burridge, Amber M. Makowicz, and Ingo Schlupp

Abstract

Attention, although limited, is one mechanism that animals employ in order to filter large amounts of information and determine what stimuli are most relevant at a particular moment. Attentional division has been well studied in tasks involving foraging, predator avoidance and predation, however divided attention has not been well studied in sexual and male-male competitive interactions. In dynamic social environments, multiple individuals may play a pivotal role in any given interaction. For example, the attention of a male may be divided between a rival, a current mate and future potential mates. How then should they best allocate this attention? In this study, we investigated impacts of the social environment on attention allocation in male sailfin mollies *(Poecilia latipinna)*. Sailfin males are host to a sexual parasite, the Amazon molly (*Poecilia formosa)*, a unisexual hybrid that arose from a natural hybridization event between sailfin mollies and the Atlantic molly *(Poecilia mexicana)*. The Amazon molly is gynogenetic, a form of parthenogenesis, requiring the sperm of a heterospecific male to begin embryogenesis. We utilized this sexual-asexual mating complex in two experiments to test attention allocation by males. First, we asked if the size of the male audience influences attention allocation when a male is paired with a heterospecific or conspecific stimulus female. Then we investigated attention allocation when a male is presented with a readily accessible stimulus female that is either conspecific or heterospecific when a conspecific or heterospecific audience is present. We found that in both cases, males direct more attention toward a stimulus female regardless of female species or male audience, but direct more attention towards conspecifics overall. By providing attention towards stimulus Amazon mollies, males may aim to receive future copulation opportunities if the audience individual copies the mate choice of the stimulus female. In addition, we found that males spend more time associating with larger males over smaller males, and when a smaller male is present as the audience, the males spend more time with the stimulus female than they do when the audience male is larger. These results suggest that smaller males pose a lower sperm competition risks than larger males and also that male-male competition is more intense in the presence of larger males. Thus, males perceive a larger male as a more relevant stimulus than a smaller male, leading to differential attention allocation. Males spent more time with the audience when the stimulus female was a heterospecific Amazon than they did when the stimulus female was a conspecific and spent more time interacting with an audience that was conspecific when compared to a heterospecific audience. The results from this study show that allocation of attention is not determined by one factor alone, but rather multiple components of the social environment in which an individual interacts.

Keywords: divided attention, limited attention, social environment, split-attention hypothesis

Introduction

Attention is one mechanism that organisms can use to screen the flood of information that is transmitted at any moment in time to determine which stimuli are most relevant at a particular moment (Shettleworth 2010). However, the ability to filter information and provide selective attention is limited. Attentional capacity is constrained by the size of an organism's brain and metabolic costs of neural tissue (Dukas 2004). These limits on attention have been shown to have negative effects on the fitness of an individual when attention has to be divided between several tasks. In Blue Jays *(Cyanocitta cristata)*, target detection rates for cryptic food items declined when the birds had to divide attention between a peripheral and central feeding location (Dukas and Kamil 2000). Detection rates continued to decline when birds were presented with an increasing number of distractor items that divided attention further. Other studies have found a balance between dividing attention towards foraging and predator vigilance. In the presence of a predator, feeding rates often decline as attention is given to predators (Milinski and Heller 1978). Conversely, division of attention towards high foraging rates may lead to an increased likelihood of succumbing to predation (Godin and Smith 1988). Predators themselves may be less successful feeders due to constraints on attention. When predators confront large groups of prey items, successful predation rates often decline. This may be due the "confusion effect." As the attention of the predator becomes divided among the individual prey items, detection rates for a single individual are reduced (Miller 1922; Landeau and Terborgh 1986; Krakauer 1995).

While there is an increasing body of literature on effects of limited and divided attention on foraging, predator avoidance capabilities, and predation success, little is known about division of attention in mating situations. In animals that live in social groups, several individuals may play a role in any given interaction. When a male is interacting with a particular female, other individuals such as rival males and other potential mates may be present that detract attention away from the current interaction (Valone 2007). In this study, we investigated how males will divide attention between a stimulus female and a variable audience to determine how the context of the social environment affects attention allocation, assuming that attentional capacity is limited. The audience individuals used in this study are confined and inaccessible but are still visible to the focal males. This approach allows the audience individual to be viewed as a possible distraction in a current mating situation.

The sailfin molly *(Poecilia latipinna)* is a species of livebearing freshwater fish that lives in dynamic social groups. Males display sexual behaviors toward preferred females that include following, nipping the female genital opening and thrusting of the gonopodium (male copulatory structure). Males also display aggressive behaviors toward rival males, biting and chasing rival males and erecting their dorsal fins in an aggressive display (Woodhead and Armstrong 1985). However, males have not been observed to develop permanent dominance hierarchies (Farr 1989) and male mating success appears to result from female choice rather than through competition between males (Ptacek 2005). Sailfin mollies provide an interesting system in that male sailfin mollies are host to a sexual parasite, the Amazon molly *(Poecilia formosa)*. The Amazon mollies are unisexual hybrid that arose from a natural hybridization event between sailfin mollies and the Atlantic molly *(Poecilia mexicana)* (Stöck et al. 2010). The Amazon molly is gynogenetic, a form of parthenogenesis, requiring the sperm of a heterospecific male to begin embryogenesis (Hubbs and Hubbs 1932). Studies have shown that male sailfin mollies are capable of distinguishing conspecific females from heterospecific Amazons and also show preference towards conspecifics (Schlupp et al. 1991; Ryan et al. 1996; Gabor and Ryan 2001). Although sperm does not contribute to offspring heredity, these copulation events may have indirect benefits to a male's fitness. Sailfin molly females have been shown to copy the mate choice of Amazon mollies when they have observed the Amazons' interaction with conspecific males. Thus interacting with Amazon mollies increases the likelihood that sailfin males will receive mating opportunities with their sexual counterparts (Schlupp et al. 1994). The use of the sexual-asexual mating system of sailfin mollies to address questions regarding effects of the social environment on attention allocation provides another interesting layer of social interaction. By assessing the scenarios in which a sailfin male allocates attention towards Amazon females, we may be able to further understand how Amazon females gain copulation events.

Using the system mentioned above, we investigated two questions. First, we asked how a focal male divides attention between a stimulus female with whom he can readily interact with and a rival audience male. We used audience males of varying body size to assess whether rival male size affects how a focal male will divide attention. In addition, we utilized both conspecific females and the heterospecific Amazon molly as the available female stimuli. Eight social conditions were studied: 1) sailfin stimulus female with smaller-sized sailfin male audience; 2) sailfin stimulus female with larger-sized sailfin male audience; 3) sailfin stimulus female with equally sized sailfin male audience; 4) sailfin stimulus female with no audience; 5) Amazon stimulus female with smaller-sized sailfin male audience; 6) Amazon stimulus female with larger-sized sailfin male audience; 7) Amazon stimulus female with equally sized sailfin male audience; and 8) Amazon stimulus female with no audience. We predicted that males would allocate more attention overall towards a stimulus female than towards an audience male, as stimulus females were readily accessible while the confined audience males served to detract attention away from the stimulus female. We also predicted that when comparing attention provided toward a conspecific versus a heterospecific female, conspecific females would receive a greater overall amount of focal male attention. Since male mating success is largely determined by female choice (Ptacek 2005) and females prefer larger males over smaller males (Marler and Ryan 1997; Witte and Ryan 1998), we predicted that audience males equal in size would detract the greatest amount of the focal male's attention away from the stimulus female since they were the most closely matched competitor to the focal male. However, audience males smaller in body size would not detract much attention away from a stimulus female since they did not pose a significant competition risk. Finally, we predicted that in the presence of an audience male that is larger in body size compared to the focal male, the focal male will allocate less attention towards a stimulus female in order to conceal his preference toward that female and reduce the likelihood that the audience male will copy his mate choice (Bierbach et al. 2011; Bierbach et al. 2013). The treatments without audience males were used to determine the amount of attention given toward a stimulus female when no audience was present to divide the attention of the focal male.

In our second study, we assessed how a male will divide attention between a readily accessible stimulus female and an inaccessible but visible audience female. This study also utilized conspecific and heterospecific females as stimuli and also used both species as potential audience individuals. Six social conditions were simulated in which a focal male was observed were created: 1) sailfin stimulus female with sailfin audience female; 2) sailfin stimulus female with Amazon audience female; 3) sailfin female with no audience; 4) Amazon stimulus female with sailfin audience female; 5) Amazon stimulus female with Amazon audience female; and 6) Amazon stimulus female with no audience. We again predicted that males would allocate more attention overall towards a stimulus female than towards an audience female, as stimulus females were readily accessible while the confined audience females served to detract attention away from the stimulus female. We also predicted males would allocate more overall attention toward conspecific stimulus females than heterospecific stimulus females. Male attention towards a stimulus female was predicted to be reduced when the audience female is conspecific as males may receive future mating opportunities by providing attention toward the conspecific audience. Finally, we would expect to see a reduction in attention allocation toward both a stimulus female and an audience female when they are both heterospecific.

Methods

Study subjects

Subjects were wild-caught in a drainage basin in Weslaco, Texas (26° 7'13.13"N 97°57'41.08"W) and brought back to the University of Oklahoma, Norman, OK, USA. Collection trips were conducted in May 2015, July 2015, October 2015 and May 2016. Fish were housed in small mixed-sex/mixed-species groups in 37.85-L tanks under 12L/12D light conditions, with weekly 50% water changes. Fish were fed commercial flake food (TetraMin®) *ad libitum* daily, and supplemented with a mix of frozen *Daphnia* and blood worms twice weekly. Fish were allowed to acclimate to laboratory housing for a minimum of 30 days before testing began. After the 30-day acclimation period, measurements of standard length (mm) were taken for all males who were subsequently placed in individual 3.79-L isolation tanks. Female fish were then separated by species into 37.85-L tanks to await testing.

Experimental setup

In the experimental tank, Plexiglas containers were used to isolate an observing "audience" individual from a free-swimming focal male and a stimulus female. Trials were conducted in a 37.85-L tank. To prevent any distractions from the surrounding environment, three sides of the tank were covered by white plastic board while the fourth side remained uncovered so that interactions could be filmed with a Nikon D5200 24.1 MP CMOS Digital SLR camera. A clear, closed-bottomed Plexiglas container with small holes cut out to allow for chemical communication was placed in the rear center of the tank (Figure 1) to house the audience individual (a male or female individual), while two open-bottomed Plexiglas containers were placed in the center of the tank to individually house the focal male and stimulus female. After a 10-minute acclimation period, the focal male and stimulus female were removed from their containers and permitted to interact for 10 minutes. Videos were then analyzed by recording the male's association times (the amount of time a male spent interacting with either the stimulus female or the audience individual).

Experimentl 1: Male audience

In a male audience experiment, we assessed how audience male body size influenced focal male attention by comparing association times (s) with multiple audiences. Each trial consisted of a focal male paired with either an Amazon or sailfin molly female for stimulus with an observing audience male that was either equal in size, smaller than, or larger than the focal individual. Females were size matched to a standard length within ±3mm. Males of equal size were within ±2mm while smaller males were at least 4mm smaller and larger males were at least 4mm larger. Eight social conditions were studied (Table 1). Each individual focal male participated in all eight trials over a two-day period. The two days were split according to stimulus female species, which was chosen at random. On that day, a male would undergo the control for that female species and the three male size trials, which were randomized within that day.

Experiment 2: Female audience

In the female audience experiment, we assessed how female species and female availability influenced focal male attention by measuring association times with multiple audiences. Each trial consisted of a focal male paired with either an Amazon or sailfin molly stimulus female with an observing audience individual that was either an Amazon or sailfin molly. Females were size-matched to a standard length within ±3mm. Six social conditions were studied (Table 3). The same males were used for this experiment as were used for the male audience experiment, however each male was given a one-week rest between experiments. Each individual focal male participated in all eight trials over a two-day period. The two days were split according to stimulus female species, which was chosen at random. On that day, a male would undergo the control for that female species and the two female audience trials, which were randomized within that day.

Data Analysis

Experiment 1: Male audience

A total of 18 males were tested in each of the 8 treatments. To assess effects of audience male size on attention allocation, we ran two mixed model two-way repeated measures ANOVAs. In the first mixed model, male identity was used as a fixed factor to account for variation between individual males while stimulus female type (sailfin or Amazon) and audience male type (control, smaller than, equal to or larger than focal) were used as fixed factors. The response variable was association time (s) with the stimulus female. In the second mixed model, the random and fixed factors remained the same, while the response variable was association time (s) with the audience individual.

Experiment 2: Female audience

A total of 20 males were tested in each of the 6 treatments. To assess effects of female species and availability on attention allocation, we ran two mixed model two-way repeated measures ANOVAs. In the first mixed model, male identity was used as a fixed factor to account for variation between individual males while stimulus female type (sailfin or Amazon) and audience female type (control, sailfin or Amazon) were used as fixed factors. The response variable was association time with the stimulus female. In the second mixed model, the random and fixed factors remained the same, while the response variable was association time with the audience individual. All data analysis was conducted using SAS Studio 3.4.

Results

Experiment 1: Male audience

For the male audience experiment, in the first mixed model ANOVA where the response variable was association time with the stimulus female, we found a significant effect of stimulus female type ($F_{1,17}=9.54$, $p=0.007$) where males spent more time with a stimulus female when she was conspecific rather than heterospecific (Figure 2). Additionally, there was a significant effect of audience male type ($F_{3,51}=28.77$, $p<0.0001$), but no interaction effect between the two factors was detected ($F_{3,51}=0.72$, $p=0.55$). A Tukey's Post Hoc analysis showed that all three categories of male audience body size influenced the amount of time a male spent associating with a stimulus female when compared to the control of no audience (Table 4). However, there was not a significant difference between the stimulus association time when the audience male was equal in size compared to larger in size or when the audience male was equal in size compared to smaller in size (Table 5). Time associating with the stimulus female was also not significantly different in presence of an audience male that is larger than the focal male when compared to an audience that is smaller than the focal male ($T_{3,51}$=-2.61, p=0.056) (Figure 2).

In the second mixed model ANOVA where the response variable was association time with the audience male, there was no significant effect of stimulus female type ($F_{1,17}$ =4.21, p=0.056). We also found a significant effect of audience male type ($F_{2,34}$ =6.00, p=0.006). This analysis also showed no significant interaction effect between the two factors ($F_{2,34}$ =1.01, p=0.37). A Tukey's Post Hoc test showed no significant variation in association time with the audience when the audience male was equal in size compared to larger in size ($T_{2,34}$=-2.42, p=0.054). There was no significant variation in focal male association time with the audience male when the audience male was equal in size to the focal male when compared to an audience male that is smaller in size than the focal male ($T_{2,34}$=0.94, p=0.62) but significant variation was observed between the treatments of larger and smaller body size audiences. ($T_{2,34}$=3.36, p=0.005), where males spent a greater amount of time interacting with the larger male audience compared to the small (Table 6) (Figure 2).

Experiment 2: Female audience

For the female audience experiment, in the first mixed model ANOVA where the response variable was association time with the stimulus female, we found significant effect of stimulus female type ($F_{1,19}$ =8.98, p=0.007) as males preferred to associate with a conspecific stimulus female (Figure 3). Additionally, there was also a significant effect of audience female type ($F_{2,38}$ =75.22, p<0.0001), however a Tukey's Post Hoc analysis showed that significance was only seen between each type of audience female when compared to the lack of audience and was not significant when comparing a conspecific to heterospecific audience (Table 7). No interaction effect between the two factors was detected ($F_{2,38}$ =1.56, p=0.22).

In the second mixed model ANOVA where the response variable was association time with the audience female, we found no significant effect of stimulus female type ($F_{1,19}$ =0.47, p=0.50) however there was a significant effect of audience female type ($F_{1,1}$ =7.02, p=0.016), where males preferred to associate with the audience more when the audience was a conspecific (Figure 3). This analysis also showed no significant interaction effect between the two factors ($F_{1,19}$ =3.15, p=0.09).

Discussion

Our results show that stimulus female type can impact association time with the stimulus female as sailfinmales prefer to interact with sailfin females rather than Amazon females. This result is not unexpected as it has been documented in previous studies that sailfin males prefer conspecific females over heterospecifics (Ryan et al. 1996; Gabor and Ryan 2001; Heubel et al. 2008). In all of the combinations of stimulus female type and audience male body size, males spent overall more time associating with a stimulus female than with an audience male. This may imply that it is more beneficial to the fitness of an individual male to allocate his attention toward a possible mate than to compete with a potential rival. This result is not unexpected, given that females within the system prefer larger males over smaller males, and that female choice rather than male competition leads to mating opportunities (Marler and Ryan 1997; Witte and Ryan 1998; Ptacek 2005). Had the rival and the stimulus male been able to physically interact rather than only being able to interact visually, these results may have been different.

Additionally, we saw significant variation in time spent associating with a stimulus female according to audience male type. Although not significant, males appeared to allocate more time toward the stimulus female when the audience male was smaller than the focal male compared to when the audience male was larger in size than the focal male. Focal males did spend significantly more time associating with a larger audience male than they did with a smaller audience male regardless of female stimulus species. Although not statistically significant, a similar result appeared to occur in trial that utilized a male equal in size compared to a male that was larger in size. This result may be due in part to a lower sperm competition risk posed by a smaller male or a male close to the size of the focal male and an increase in male-male competition pressure by a larger male (Marler and Ryan 1997; Witte and Ryan 1998). However, the result may also imply that males perceive a larger male as a more relevant stimulud than a smaller male, leading to differential division of attention.

Finally, although the results were not quite statistically significant, in the study of male audience, association time with the male audience appeared to be influenced by stimulus female type. Males spent more time with the audience male when the stimulus female was a heterospecific Amazon molly than they did when the stimulus female was a conspecific sailfin molly. Since Amazon mollies do not provide a direct benefit to male fitness (Hubbs and Hubbs 1932; Schlupp et al. 1994), male attention was divided more than when a sailfin female is presented as a stimulus.

In the study of female audience, we again found that males spent an overall greater amount of time associating with a stimulus female over an audience and that males prefer to divide more attention towards associating with a conspecific rather than a heterospecific (Ryan et al. 1996; Gabor and Ryan 2001). There was however, no significant effect of audience female type on the amount of attention given toward a stimulus female. Since the stimulus females are more readily accessible than the audience females (who were enclosed in a chamber), males may direct attention towards the stimulus female regardless of audience species in order to gain immediate copulations and to increase the possibility that an audience female will copy the mate choice of the stimulus female in future reproductive encounters. When associating with an audience, stimulus female type did not influence directing attention towards an audience female of either species, however the species of the audience female did significantly affect this division of attention. Males spent significantly more time interacting with an audience that was conspecific when compared to a heterospecific audience. This was, again, expected.

The results provided by this study show that division of attention is not determined by one factor alone, but rather the components of the social environment in which the organism is interacting. Social context mediates allocation of attention so that an individual gives the greatest amount of attention to the most relevant stimuli at a given moment, whether that is toward a rival male, an accessible female, or a possible future mate. The use of a partially open field experiment allowed a male to directly interact with a stimulus female, while setting the confined audience as a distraction of attention rather than a choice for direct interaction.

This study is the first to address division of attention based on sexual and competitive interactions. In the future, it would be interesting to add additional layers of stimuli that an individual must assess and divide their attention between. One such layer may be the presence of two female stimuli while an audience male is present. Another study could increase the number of audience males to determine whether or not a higher density of rivals shifts attention away from a mating opportunity. Our study shows that division of attention is multifaceted and context dependent, However, a great deal more work can be done to elucidate a broader range of social implications and the limits of being able to divide attention during sexual and competitive interactions.

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Appendix

Table 1 – Experimental treatments for the male audience experiment. Body size was measured as standard length. Males of equal size were within a standard length of ±2mm while smaller males were at least 4mm smaller and larger males were at least 4mm larger. Females were size-matched to a standard length within ±3mm.

Stimulus female species	**Audience male size relative to focal male**
Poecilia latipinna	Smaller (at least 4mm)
Poecilia latipinna	Larger (at least 4mm)
Poecilia latipinna	Equal (±2mm)
Poecilia latipinna	No audience (control)
Poecilia formosa	Smaller (at least 4mm)
Poecilia formosa	Larger (at least 4mm)
Poecilia formosa	Equal (±2mm)
Poecilia formosa	No audience (control)

Table 2 – Descriptive statistics for male audience experiment of attention according to stimulus female and male audience body size. Stimuli were either Amazon or sailfin females and audience males were either larger than the focal male by a standard length of at least 4mm, smaller than the focal male by at least 4mm, equal in size to the audience male (±2mm), or was the Control group where no audience was present in the audience chamber. N = 18 for all groups.

Stimulus	Audience	Time spent with	Mean	Std Dev	Minimum	Maximum
Amazon	Control	Stimulus	475.07	115.80	132.44	600.00
		Audience	--	--	--	--
	Equal-sized male	Stimulus	310.77	122.89	87.52	535.12
		Audience	127.90	64.21	33.34	276.93
	Larger male	Stimulus	242.00	90.37	82.20	404.08
		Audience	195.47	105.95	59.35	438.29
	Smaller male	Stimulus	326.59	87.45	137.99	439.88
		Audience	118.38	74.30	20.04	273.76
Sailfin	Control	Stimulus	517.95	87.82	227.45	592.34
		Audience	--	--	--	--
	Equal-sized male	Stimulus	335.76	119.76	57.50	553.31
		Audience	119.29	68.40	21.35	248.85
	Larger male	Stimulus	335.07	111.47	66.27	527.25
		Audience	137.68	77.17	25.98	327.79
	Smaller male	Stimulus	376.18	98.82	158.40	502.82
		Audience	95.44	67.92	15.72	266.92

Table 3 – Experimental treatments for the female audience experiment. Table displays stimulus female species and female audience species. Females were size-matched to a standard length within ±3mm.

Stimulus female species	**Audience female species**
Poecilia latipinna	*Poecilia latipinna*
Poecilia latipinna	*Poecilia formosa*
Poecilia latipinna	No audience (control)
Poecilia formosa	*Poecilia latipinna*
Poecilia formosa	*Poecilia formosa*
Poecilia formosa	No audience (control)

Table 4 – Descriptive statistics for female audience experiment of attention according to stimulus female species and audience female species. Stimuli were either Amazon or sailfin females. Audience was either an Amazon female, sailfin female, no fish (Control). N=20 for all groups.

Stimulus	Audience	Time spent with	Mean	Std Dev	Minimum	Maximum
Amazon	Control	Stimulus	504.64	71.31	339.20	600.00
		Audience	--	--	--	--
	Amazon	Stimulus	276.21	107.20	43.11	425.86
		Audience	113.02	63.99	7.76	243.79
	Sailfin	Stimulus	205.50	76.16	38.74	329.76
		Audience	224.23	125.24	30.99	519.75
Sailfin	Control	Stimulus	531.58	102.47	252.97	660.46
		Audience	--	--	--	--
	Amazon	Stimulus	315.47	131.42	110.84	527.84
		Audience	140.43	116.41	4.75	460.13
	Sailfin	Stimulus	308.02	128.83	118.59	527.84
		Audience	162.45	141.01	19.79	458.47

Table 5 – Results for the Tukey Post Hoc test for the mixed model ANOVA for male audience where the response variable was association time with stimulus female. Stimuli were either Amazon or sailfin females and audience males were either larger than the focal male by a standard length of at least 4mm, smaller than the focal male by at least 4mm, equal in size to the audience male (±2mm), or was the Control group where no audience was present in the audience chamber. N = 18 for all groups.

Differences of Least Squares Means											
Effect	**Stimulus**	**Audience**	**Stimulus**	**Audience**	**Estimate**	**Std Error**	**DF**	**t Value**	**Pr>|t|**	**Adjustment**	**Adj P**
Stimulus	Amazon		Sailfin		-52.63	17.04	17	-3.09	0.007	Tukey	0.007
Audience		Control		Equal-sized male	173.24	24.10	51	7.19	<.0001	Tukey	<.0001
Audience		Control		Larger male	207.97	24.10	51	8.63	<.0001	Tukey	<.0001
Audience		Control		Smaller male	145.12	24.10	51	6.02	<.0001	Tukey	<.0001
Audience		Equal-sized male		Larger male	34.73	24.10	51	1.44	0.156	Tukey	0.480
Audience		Equal-sized male		Smaller male	-28.12	24.10	51	-1.17	0.249	Tukey	0.650
Audience		Larger male		Smaller male	-62.85	24.10	51	-2.61	0.012	Tukey	0.056

Table 6 - Results for the Tukey Post Hoc test for the mixed model ANOVA for male audience where the response variable was association time with audience male Stimuli were either Amazon or sailfin females and audience males were either larger than the focal male by a standard length of at least 4mm, smaller than the focal male by at least 4mm, equal in size to the audience male (±2mm), or was the Control group where no audience was present in the audience chamber. N = 18 for all groups.

Differences of Least Squares Means											
Effect	**Stimulus**	**Audience**	**Stimulus**	**Audience**	**Estimate**	**Std Error**	**DF**	**t Value**	**Pr>|t|**	**Adjustment**	**Adj P**
Stimulus	Amazon		Sailfin		29.78	14.51	17	2.05	0.006	Tukey	0.056
Audience		Equal-sized male		Larger male	-42.99	17.77	34	-2.42	0.021	Tukey	0.054
Audience		Equal-sized male		Smaller male	16.68	17.77	34	0.94	0.355	Tukey	0.620
Audience		Larger male		Smaller male	59.67	17.77	34	3.36	0.002	Tukey	0.005

Table 7 – Results for the Tukey Post Hoc test for the mixed model ANOVA for female audience where the response variable was association time with stimulus female. Stimuli were either Amazon or sailfin females. Audience was either an Amazon female, sailfin female, no fish (Control). N=20 for all groups.

Differences of Least Squares Means											
Effect	**Stimulus**	**Audience**	**Stimulus**	**Audience**	**Estimate**	**Std Error**	**DF**	**t Value**	**Pr>|t|**	**Adjustment**	**Adj P**
Stimulus	Amazon		Sailfin		-56.25	18.77	19	-3.00	0.007	Tukey	0.007
Audience		Amazon		Control	-222.27	22.99	38	-9.67	< .0001	Tukey	< .0001
Audience		Amazon		Sailfin	39.08	22.99	38	1.70	0.097	Tukey	0.218
Audience		Control		Sailfin	261.35	22.99	38	11.37	< .0001	Tukey	< .0001

Figure Legend

Fig. 1 Diagram of experimental set up with audience housed in clear Plexiglas container (perforated for chemical and olfactory communication) in the rear center of tank, while focal male and stimulus female are permitted to swim freely after an acclimation period of ten minutes.

Fig. 2 Box and whisker plots of amount of time focal male spent associating with stimulus females in the presence of a male audience that varied in body size. The horizontal line within each box indicates the median, boundaries of the box indicate the 1^{st} and 3^{rd} quartile, and the whiskers indicate the highest and lowest values of the results.

Fig. 3 Box and whisker plots of amount of time focal male spent associating with audience male of varying body size when in the presence of a stimulus female across treatments. The horizontal line within each box indicates the median, boundaries of the box indicate the 1^{st} and 3^{rd} quartile, and the whiskers indicate the highest and lowest values of the results.

Fig. 4 Box and whisker plots of amount of time focal male spent associating with stimulus females when an audience female is present across treatments. The horizontal line within each box indicates the median, boundaries of the box indicate the 1^{st} and 3^{rd} quartile, and the whiskers indicate the highest and lowest values of the results.

Fig. 5 Box and whisker plots of amount of time focal male spent associating audience females in the presence of a stimulus female across treatments. The horizontal line within each box indicates the median, boundaries of the box indicate the 1^{st} and 3^{rd} quartile, and the whiskers indicate the highest and lowest values of the results.

Figure 1

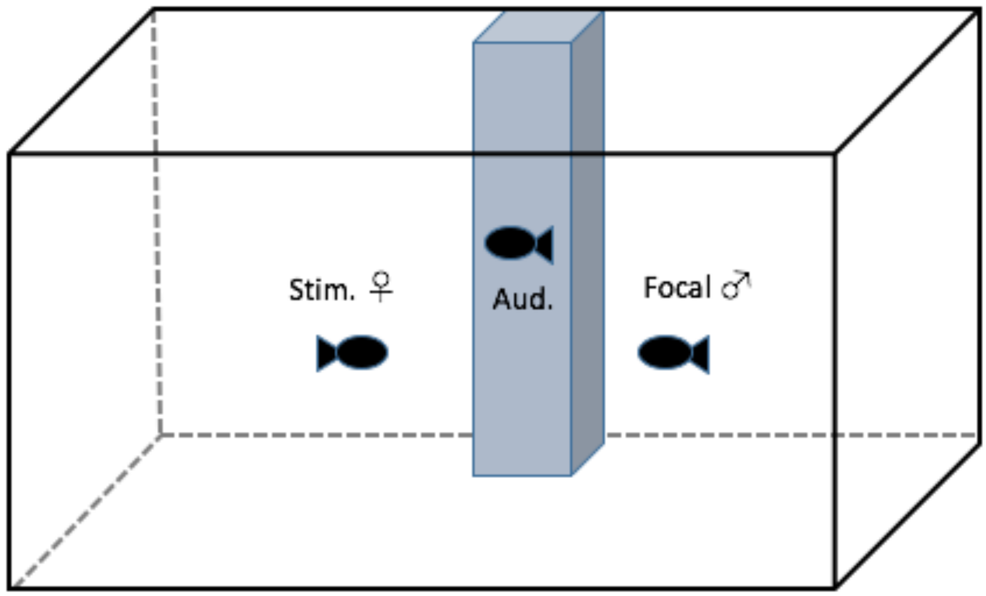


Figure 2

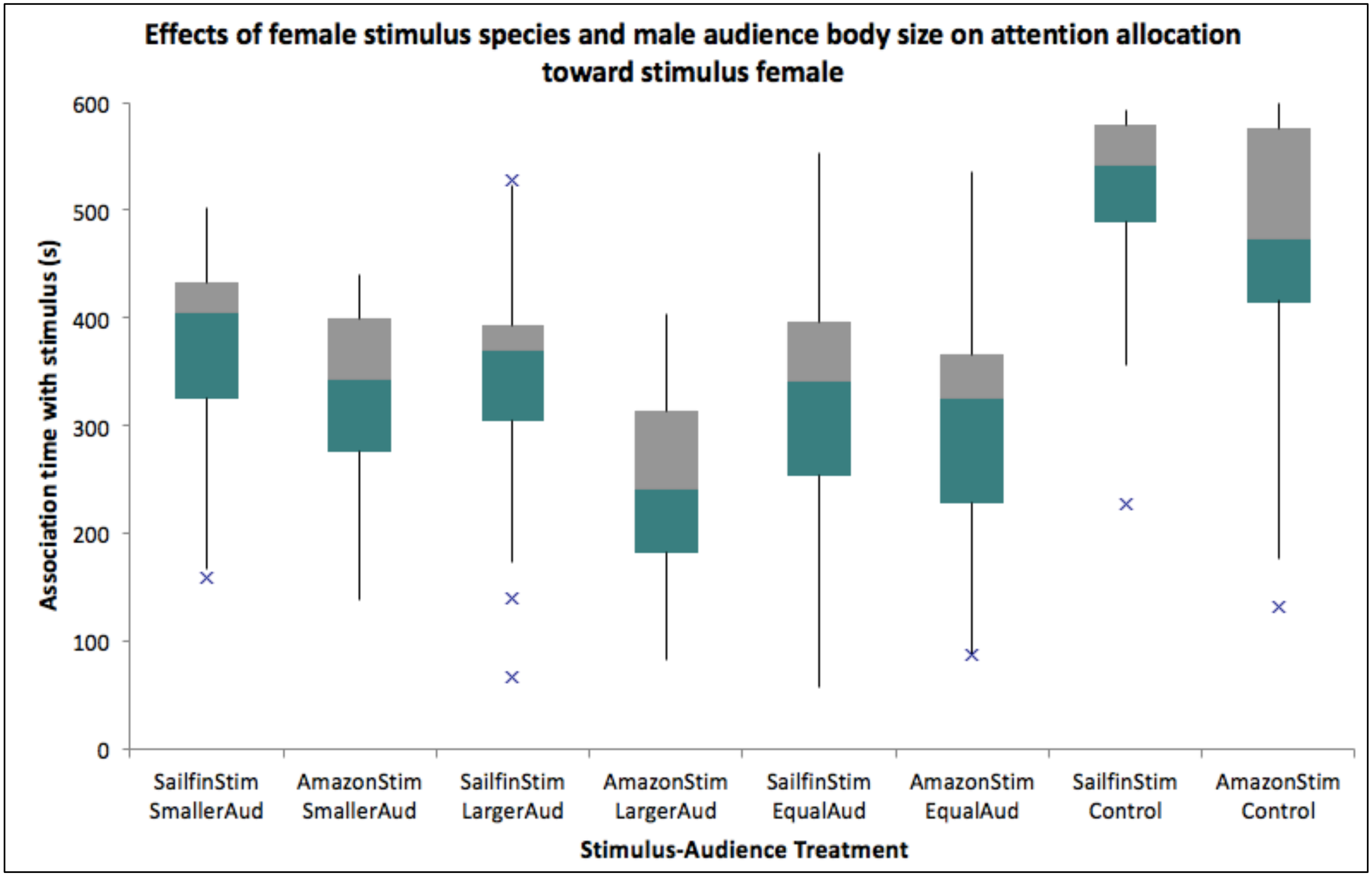

Effects of female stimulus species and male audience body size on attention allocation toward stimulus female
Association time with stimulus (s)
600
500
400
300
200
100
0
SailfinStim SmallerAud
AmazonStim SmallerAud
SailfinStim LargerAud
AmazonStim LargerAud
SailfinStim EqualAud
AmazonStim EqualAud
SailfinStim Control
AmazonStim Control
Stimulus-Audience Treatment

Figure 3

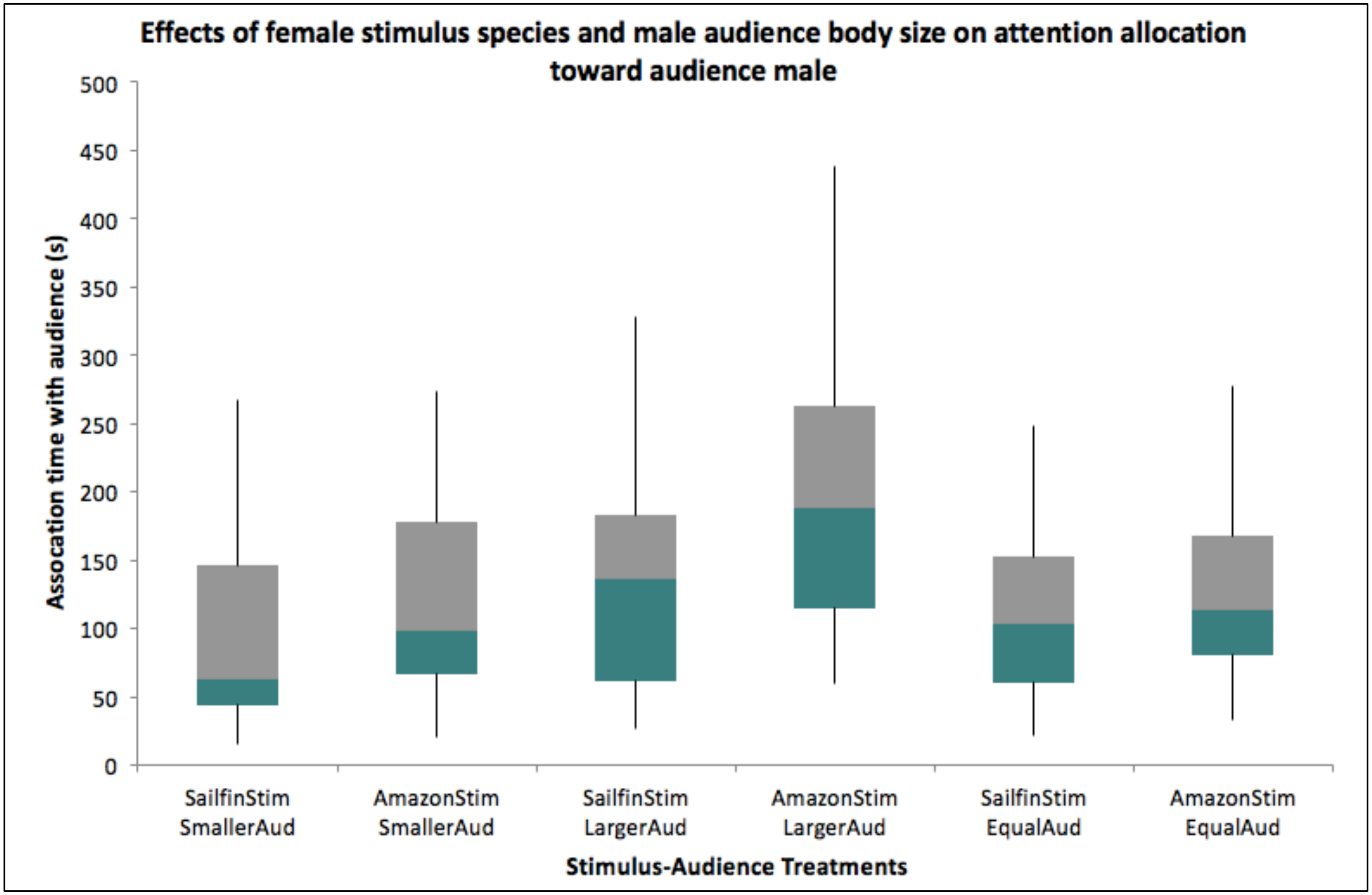
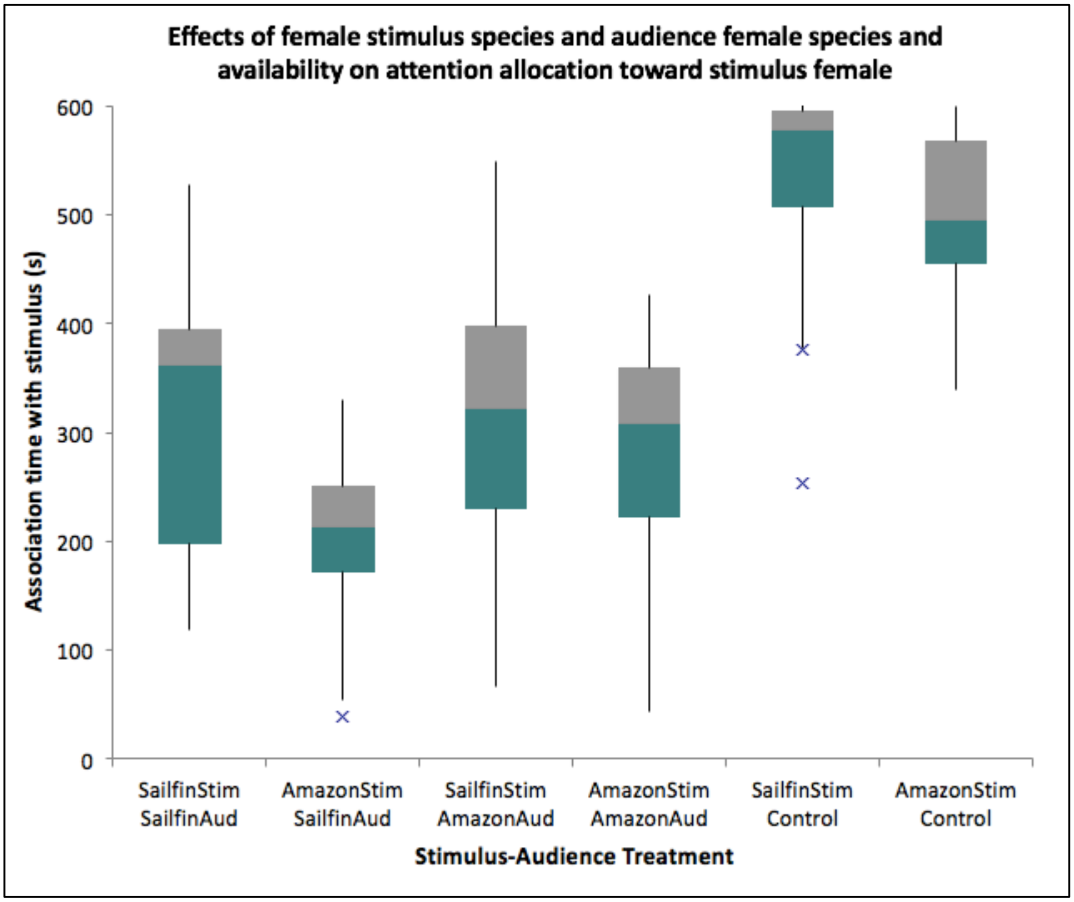
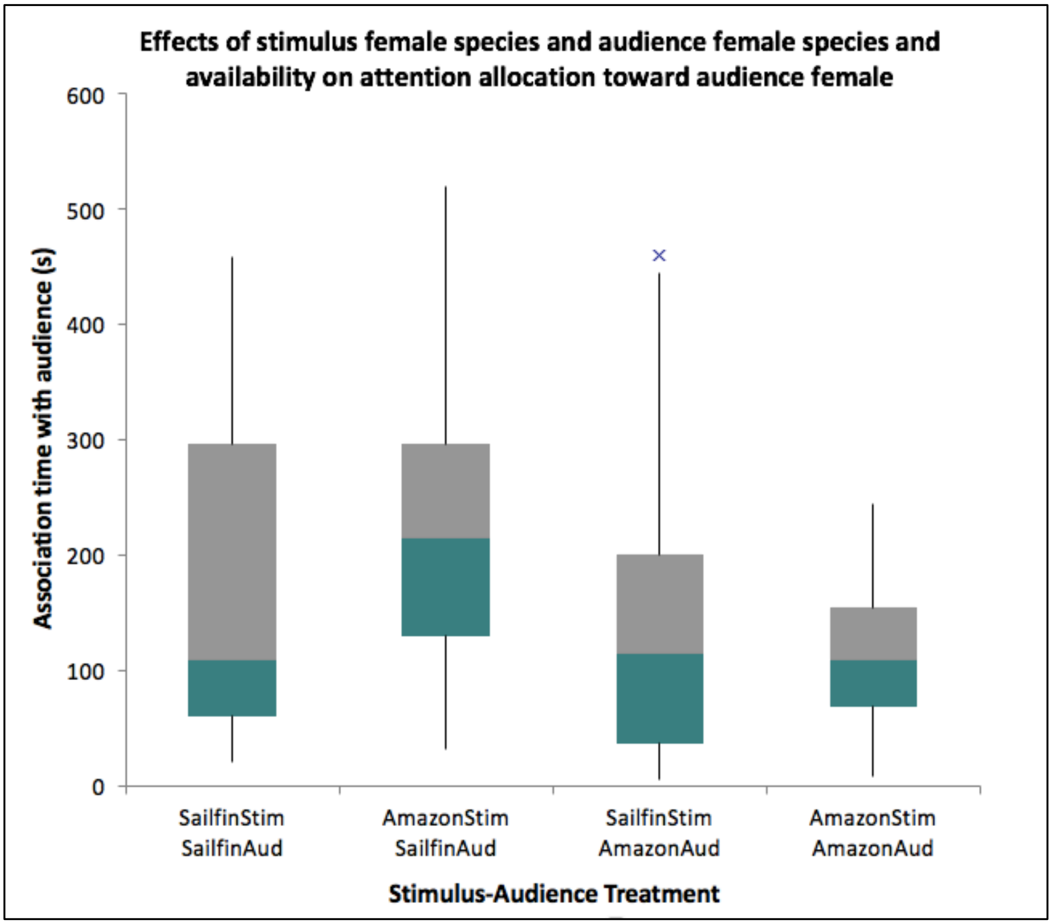

Effects of female stimulus species and male audience body size on attention allocation toward audience male
Assocation time with audience (s)
500
450
400
350
300
250
200
150
100
50
0
SailfinStim SmallerAud
AmazonStim SmallerAud
SailfinStim LargerAud
AmazonStim LargerAud
SailfinStim EqualAud
AmazonStim EqualAud
Stimulus-Audience Treatments

Figure 4



Effects of female stimulus species and audience female species and availability on attention allocation toward stimulus female
Association time with stimulus (s)
0
100
200
300
400
500
600
SailfinStim SailfinAud
AmazonStim SailfinAud
SailfinStim AmazonAud
AmazonStim AmazonAud
SailfinStim Control
AmazonStim Control
Stimulus-Audience Treatment

Figure 5


Effects of stimulus female species and audience female species and availability on attention allocation toward audience female
Association time with audience (s)
600
500
400
300
200
100
0
SailfinStim SailfinAud
AmazonStim SailfinAud
SailfinStim AmazonAud
AmazonStim AmazonAud
Stimulus-Audience Treatment