# Third wheel, but still a big deal: how mating fish respond to a conspecific audience

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## Introduction

- Communication between mating partners occurs in social networks where multiple signalers and receivers may be present during mating and competition[1]
- **Audience effect:** the presence of eavesdroppers elicits a behavioral change in focal animals[1] (Fig. 1)
- Audience effects on a mating pair may be different depending on audience identity
- Selection should favor behavioral plasticity in mating response to audience identity
- *Girardinus metallicus* is a Cuban endemic, livebearing fish (Poeciliidae) that has discrete male types[2] (Fig. 2):
  - **Black morph males** have black coloration on their ventral surface and perform a courtship display
  - **Plain morph males** sneak copulations without female cooperation
- Few studies have examined audience effects in **polymorphic** species[3] (those with distinct male types)

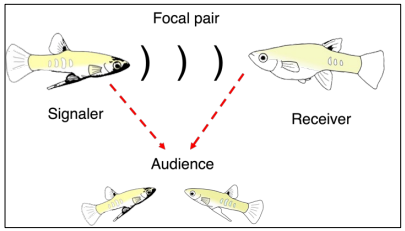


**Figure 1.** Figure illustrating a social network with a mating pair. Black morph male is the signaler and female is the receiver. Audience are conspecifics being perceived by mating pair. Not to scale.

**Figure 2.** *Girardinus metallicus:* Plain morph male (top), black morph male (middle), and female (bottom). Photo: M. Tobler

**Figure 3.** A focal pair. The black morph male is performing a courtship display to the female. Photo: A. Wilcox.

## Hypothesis and Prediction

**H**: The behavior of focal male and female *G. metallicus* will differ depending on the audience identity.

**P**: In a mating pair:

1) Focal females will peck at female audiences more than male audiences because females provide a safe, shoaling opportunity.
2) Males will peck at audience females because they are potential mates, and will peck at audience males because they are potential competitors.

## Methods

### Animal Husbandry

- All fish were housed in stock-specific, mixed-sex and mixed-morph groups and fed twice per day using flake food, occasionally supplemented with frozen brine shrimp (*Artemia sp.*)
- Tanks were maintained at 25 ± 0.5 °C on a 12:12 h light:dark schedule and contained naturally colored gravel and plants

### Behavioral Trials

- Data were collected from video recordings from of a previous study of audience effects on focal fish behavior[3]
- A focal pair was one black morph male and one female (Fig. 3)
- Trials consisted of N = 54 focal pairs
- Each focal pair was placed together in a test tank with gravel, a plastic plant for cover, and a clear cylinder for containing the audience (Fig. 4)
- Each focal pair was exposed to a randomized order of each treatment group, in 10-minute behavior trials (Table 1)

**Table 1.** Audience treatments. There were N = 15 of each treatment.

| Audience Treatments |
|---|
| Female |
| Black Morph Male |
| Plain Morph Male |
| Empty (control) |

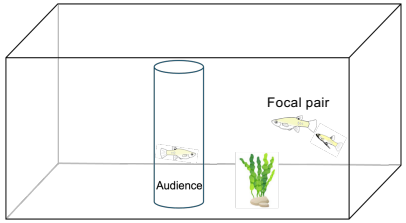


**Figure 4.** Diagram of behavioral trials. Fish not to scale.

- Exposure to each treatment was separated by a 10-minute acclimation
- We observed behaviors in Table 2 using preprogrammed keystrokes in Behavioral Observation Research Interactive Software (BORIS)

**Table 2.** Ethogram of behaviors observed during trials.

| Behavior | Description |
|---|---|
| Pecks at audience fish () | • Peck at a region of the tube where the audience fish is located. |
| Copulation attempts with contact (mating success) | • Focal male performs copulation attempt that makes contact with female genital opening. |

- Immediately after trials, all focal and audience fish were sedated using tricaine methanesulfonate (MS-222) to measure standard length

### Data Analysis

- All data were analyzed in using R v 4.0.3 in RStudio v 1.3.1093
- We generated a generalized linear mixed model (GLMM) with audience treatment, focal sex, and focal sex × audience treatment as main effects of pecks at audience
- We generated a second GLMM with male audience treatments only

## Results

### Focal Fish Behavior Towards Audience

- Audience treatment affected pecking behavior ($X^2 = 32.50$, $p < 0.0001$)
- However, there was a focal sex × audience treatment interaction (Fig. 4, $X^2 = 10.95$, $p = 0.004$):
  - Focal females pecked equally at either male audience, but the *most* at female audiences
  - Focal males pecked equally at black morph and female audiences, but the *least* at plain morph audiences

### Focal Fish Behavior Towards Each Other

- Greater mating success with black morph audience than plain morph audience (Fig. 5, $X^2 = 7.56$, $p = 0.0006$)

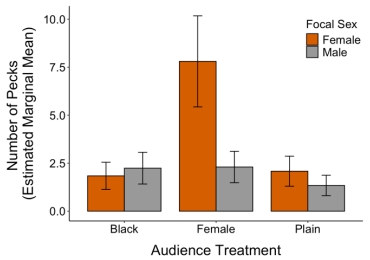


**Figure 5.** Estimated marginal means of number of pecks at audience × audience treatments for focal males and focal females.

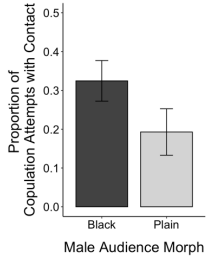


**Figure 6.** Different proportions of copulation attempts with contact performed by focal male between male audience morphs.

## Discussion

- **This is the first study, to our knowledge, that demonstrates an audience effect in response to audience morph in a sexually polymorphic species**
- Focal fish recognized audience fish, and pecked at the tube where the audience was, which likely represents attempts to interact with that fish
- Focal females may have interacted with female audiences more in an attempt to shoal and avoid mating harassment[5]
- Focal males may have attempted to aggressively compete with audience males and mate with audience females.
- Focal males interacted more with and had higher mating success with a black morph audience than a plain morph audience
- This could be explained by males exhibiting higher mating effort or by females being distracted more in the presence of a black morph audience (because black morphs harass females more than plain morphs)
- Audience effects highlight the importance of the social network and using multiple audience types in behavioral ecology (because mating individuals adjust their behavior differently depending on the composition of the social environment)

## References

[1]R.J. & Schlupp, I. (2005). *Performing in front of an audience: signallers and the social environment.* In McGregor, P.K., ed. Animal Communication Networks (pp. 63-83). Cambridge University Press. [2]Kolluru, G. R., Bertram, S. M., China, E. H., Dunmeyer, C. V. & Graves, J. S. *Mating behavior and its morphological correlates in two color morphs of Girardinus metallicus (Pisces: Poeciliidae), a species previously thought not to exhibit courtship display. Behav. Process.* **106**, 44–52 (2014). [3]West, S. M., Johnson, W. A., Wortmann, H.A., Kimura, L., Stepanian, D., Kolluru, G.R. submitted 2025. *Audience effects in a sexually polymorphic species: audience morph generates plasticity in mating behavior.* [4]Padur, L., Streit, B., Plath, M., Öztürk, Ö., Tiedemann, R., & Wedekind, J. (2009). *Do audience effects lead to relaxed male sexual harassment?* Behaviour, 146(12), 1739-1758. [5]Dadda, M. (2015). *Female social response to male sexual harassment in poeciliid fish: a comparison of six species.* Frontiers in psychology, 6: 1453.

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