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Alisa Wallace and Kelsey Crutchfield-Peters

ABSTRACT

According to the principle of allocation, energy is limited and must be allocated by an organism amongst its life processes. The balance between growth and reproduction is of particular interest due to its implications for a species' life history and evolutionary success. We examined this trade-off through a behavioral lens by determining how hunger level and conspecific sex impacted a male guppy's choice between food and a courtship opportunity. Using twelve hungry and well-fed males, we set up a ten-gallon tank with food available on one end and another guppy, either male or female, on the other. An individual focal male was placed in the middle and the proportion of time he spent in each zone of the tank was recorded over a period of five minutes. By conducting three separate 2-way ANOVA's, we found that hunger level did not significantly impact the males' choice for food or social interaction and that there was a unanimous preference for feeding among both hunger levels and conspecific sexes, as was expected. This preference to eat, however, was stronger in focal males exposed to a female than in those exposed to a male. We therefore concluded that feeding, and thus the growth and survival benefits that ensue from it, is a higher priority than procreative efforts. However, the incentives for reproduction and the social dynamics of courtship for this species are more complex than initially presumed, and it is likely that a combination of both male and female attributes contribute to the observed patterns.

INTRODUCTION

The phrase "survival of the fittest" is keystone theme to evolution, and indicates that only the strongest organisms will survive and reproduce. Fitness, however, requires energy, and all organisms will therefore seek to maximize their energy levels. This occurrence ultimately relates back to the principle of allocation, which states that energy is limited and must be allocated by organisms amongst various life processes. The balance between growth and reproduction is a central facet of this theme due to its implications for a species' life history and evolutionary success. Much work has been done examining physiological energy allocations (Reznick 1983, Reznick and Yang 1993) and developing models (Heino and Kaitala 2001, McCauley et al. 1990), but behavior is simply a different lens from which to answer the same questions. With greater organism complexity, behavior become an increasingly relevant factor that dictates the survival and evolution of both an individual species and, indirectly, broader ecological communities.

Guppies (*Poecilia reticulata*) are an ideal model organism for studying the behavioral dynamics of the tradeoff between growth and reproduction. Their behavior is relatively simple, yet still connects directly to energy partitioning without presenting an overwhelming number of confounding variables that could complicate interpretations of energy allocation. At the same time, it is complex enough to provide a foundation for understanding similar relationships in more advanced organisms. Guppies are also easy animals to implement experimental treatments on and keep in a laboratory setting.

Sexual selection in guppies has received much study largely due to the elaborate courtship and mating techniques the males display, and it is this key feature that makes them so exemplary for behavioral observations. The vast amount of literature on the subject is an indication of how

many factors can influence the reproductive processes, but investigating the effects of resource availability on sexual behavior provides the most direct link to inferring energy allocation strategies.

It is already known that the costs and benefits of the choice to feed or mate depend largely on the environment (Abrahams 1993). For instance, males from populations with more abundant resources are able to devote more energy towards reproduction, whereas males from populations without such resources are forced to spend more time foraging, in order to ensure survival (Kolloru and Grether 2004). Exploitation can indirectly affect this allocation as well. An increase in predation results in less foraging opportunities, which in turn decreases the amount of courtship displays from males (Fraser et. al 2004). The same trend is seen in males infected with parasites, which causes the male to be in poorer condition and requires more time spent foraging to survive (Kolloru et al. 2008). On the flip side, males with abundant food sources area able to grow faster (Fraser et al. 2004). Body size positively correlates to fecundity (Reznick 1983), so an indirect relationship between food availability and successful reproduction does exist.

Many questions, however, have yet to be answered. In populations with varying resources, the discovered differences in energy allocation are a result of genetic differences (Kolloru and Grether 2004), but no work has yet been done on how environmental stressors affect the behavioral choice points concerning foraging and courtship in the short-term. "Short-term" is used here in an evolutionary context, that is, referring to environmental factors that have not yet caused any genetic divergence. While a short-term focus does not provide information on a species' evolution, it can grant insight into organisms' initial behavioral adaptations to changing environments. With this short-term perspective, it remains unknown how direct changes in resource availability (as opposed to the indirect effects of exploitation) will influence the

dynamics between reproductive and foraging behaviors. Additionally, it is known that a male guppy will choose to eat first and then mate when presented with a clear choice (Abrahams 1993). However, that occurred when all males were in similar physical condition. The effects of both physical condition and behavioral choices are necessary to understand the broader relationship between the environment and energy allocation strategies.

Our experiment sought to determine through a behavioral lens whether the allocation of energy between foraging and reproduction differed between domesticated male guppies that were well-fed and those that were hungry. As a secondary priority, we also determined whether this choice differed based on the social opportunity provided, i.e. in the presence of either a male or female conspecific. Hunger level was used as a representation of growth and overall condition, as well as to provide additional relevance to the choice point between eating and courting. Addressing the influences of conspecific sex allowed us a better context for understanding the many factors that contribute to sexual behavior in guppies and therefore the choices made.

Given that a male has greater incentive to feed than mate while hungry (Siems and Sikes 1998) and reduced feeding opportunities result in less time spent courting (Fraser et. al 2004, Kolloru et al. 2008), we predicted that, when given the choice to forage or court, hungry males would allocate more of their time to eating than would well-fed males. Additionally, we predicted that males of both hunger groups would unanimously spend more time with the female conspecific than the male, due this species' lack of a breeding season (Farr 1975) and the greater reproductive benefits a female offers compared to that of another male.

METHODS

Maintenance

We used 12 male guppies, obtained from the local pet shop, for our testing. These were haphazardly divided into two treatment groups, hungry and well fed, consisting of six fish each. After the first round of data collection, treatment group assignments were switched, so the hungry became well fed and vice versa. All fish were given one week to acclimate to their treatment condition, both prior to initial testing and at the time of switching.

Well-fed males were fed approximately one flake of generic flake food per fish once a day, whereas hungry males were fed the same amount every other day. The two treatment groups were housed in separate ten-gallon tanks to ensure differences in food intake. In addition to our testing males, we had two extra males and three females, to test for shoaling or courtship activity. These were housed in a separate ten-gallon tank out of sight from the other housing tanks, so focal males would not become acclimated to the sight of a female. The stimulus males and females were separated by a divider and fed the same amount and frequency as the well-fed group.

All tanks, including the two testing tanks, were 10 gallons and filled with DI water along with one tablespoon of Jungle Aquarium Salt and one cup of water from a preexisting fish tank to promote positive bacteria growth. Water was allowed to acclimate for one week prior to the introduction of fish, and housing and testing tanks were kept at the same temperature.

Testing Protocol

We conducted a simple choice test paradigm for our focal males by offering them a choice between a feeding opportunity and a courtship opportunity with a female. Each fish was also tested with another male as the stimulus to determine whether any interaction with the females was the result of sexual or shoaling attraction. Filters were removed from the test tanks so each end was equal, and then divided into three 15 cm zones: food, neutral, and stimulus. To reduce distractions, three sides of the tank were wrapped with dark paper. The female or conspecific male was kept at the end of the stimulus zone behind a clear, porous divider in the additional five cm of space. Testing males were then placed in clear plastic tubes in the middle of the tank and allowed to acclimate for five minutes. Right before testing began, food was added to the feeding zone and secured with a small strip of plastic on the surface of the water so it would not float to the rest of the tank. All testing took place in lieu of a male's regular feeding time to ensure incentive to eat. After acclimation, the tube was removed and timing began. The male's location was recorded over a course of five minutes to produce the total number of seconds spent in each zone.

For ease of testing, each treatment group was further divided into subgroups, A and B, with three fish per subgroup. On a given test day, one subgroup was tested with a female and the other tested with the conspecific male to reduce any possible temporal biases.

Data Analysis

The times (in seconds) spent in the feeding and conspecific zones were converted to proportions. For feeding, two proportions were calculated. To allow a direct comparison, the first was time spent feeding out of time spent only in the feeding and conspecific zone (i.e. when

a choice was made). The second proportion, though, was time spent feeding out of the total trial time of 300 seconds, including time in the neutral zone (i.e. regardless of whether a choice was made). The proportion of time spent with the conspecific was also calculated out of the total trial time. The values for feeding when a choice was made were log $(x + 1)$ transformed. Three 2-Way ANOVAs were run in R Commander, one for each proportion, with hunger state and conspecific sex as our explanatory variables.

RESULTS

Proportion of time spent feeding out of time in choice zones

When including only time spent in a choice zone in the proportion, proportion of time spent feeding was not significantly different between hungry and well-fed focal males (2-Way ANOVA, $F_{1,44} = 0.02$, $p = 0.890$; Figure 1), but there was a marginally significant difference based on conspecific sex, with focal males spending slightly more time feeding in the presence of a female ($F_{1,44} = 4.02$, $p = 0.051$). The effect of hunger level on proportion of time spent feeding was not influenced by stimulus type ($F_{1,44} = 0.57$, $p = 0.448$). All groups, regardless of stimulus type, spent more time (proportion > 0.5) feeding than with the conspecific (Figure 1).

Proportion of time spent feeding out of total time

When incorporating total testing time into the proportion, proportion of time spent feeding was not significantly different between hungry and well-fed focal males (2-Way ANOVA, $F_{1,44} = 1.51$, $p = 0.167$; Figure 2), nor did it differ with conspecific sex ($F_{1,44} = 1.98$, $p = 0.226$). The effect of hunger level on proportion of time spent feeding was not influenced by conspecific type ($F_{1,44} = 0.14$, $p = 0.705$).

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Proportion of time with conspecific

The proportion of time spent with the conspecific was not significantly different between well-fed and hungry focal males (2-Way ANOVA, $F_{1,44} = 0.03$, $p = 0.859$; Figure 3). However, males of both hunger levels spent 2-3 times more time in the stimulus zone when the conspecific was a male ($F_{1,44} = 4.52$, $p = 0.039$). The effect of conspecific sex on proportion of time spent socializing was not influenced by hunger level ($F_{1,44} = 0.67$, $p = 0.416$).

DISCUSSION

Effect of hunger level on choice

With this experiment, we sought to determine whether the choice between a foraging opportunity and a courtship opportunity differed between well-fed and hungry males. Because hungry males have a greater incentive to feed than do well-fed males, we hypothesized that hungry males would allocate more time to feeding than well-fed males, and likewise, well-fed males would spend more time with the conspecific. Our data did not support this prediction. There were no significant differences in the proportion of time a focal male spent feeding between hungry and well-fed males (Figure 1). These results complement Abraham's (1993) work and allow us to conclude that feeding is a higher priority than courtship, regardless of a male's short-term fitness level. Theoretically, a male that is able to feed more would have a higher level of fitness and could afford to forgo feeding in favor of a reproductive opportunity, but clearly the higher inducement to feed while hungry does not necessarily mean a well-fed male has less motivation to feed. This finding also contrasts with the outcomes of Kolloru and

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Grether's (2004) study, because individuals from the same genetic population do not have different behavioral energy allocation strategies with varying levels of short-term fitness.

The guppy's mating tactics are a probable explanation. Males frequently employ flashy courtship displays, aggressive chasing of the female and, in the absence of receptivity from the female, sneak copulations (Farr 1975). Additionally, intraspecifc competition is often present, which increases the intensity and frequency of displays (Farr 1976). The high energetic costs associated with a reproductive effort likely gives even well-fed males a reason to try to increase their energy state as much as possible.

In addition to allowing a male to better endure the rigors of courtship, females also prefer larger males (Plath et al. 2005). Feeding more frequently positively corresponds to more growth (Reznick 1983), so there are indirect reproductive benefits in choosing to feed as well. All of these factors that lead to higher fitness ultimately result in greater reproductive success for the male, suggesting that an individual benefits most by sacrificing an immediate mating opportunity in favor of long-term success. Such a conclusion, however, needs to be supported by more evidence. For future work, it would be advantageous to directly examine the factors that influence the reproductive life-history tactics of the guppy.

Effect of conspecific sex on choice

The secondary aim of our experiment was to determine whether the choice to feed or socialize differed in the presence of a female or male conspecific. We predicted that sexual attraction to the female would cause focal males to associate with a female more than a male, but our results don't support this hypothesis and suggest that the dynamics of social and reproductive interactions in this species are more complicated than anticipated. Focal males actually showed

a stronger preference to feed in the presence of a female or, to put in other words, interacted more with a male conspecific.

There are two alternative ways to interpret these results. The first is to assume that the greater proportion of time spent feeding ensues from a neutrality or aversion to the females, and that the conspecific males are correspondingly more favorable. Guppies are social fish and live in mixed-sex schools in the wild (Farr 1975). Shoaling instincts therefore seem the mostly likely explanation for male-male attraction, but if our results were to truly represent such a response, there would be no preference for either conspecific type. Our two methods of analysis provided slightly contradictory results, with one indicating a strong discrepancy based on conspecific type (Figure 3) and the other showing only a marginal difference (Figure 1). Taken collectively, however, we can conclude with confidence that focal males did in fact interact with other males more than with females. The question remains, then, as to what accounts for the preference.

Our methods are one possibility. Guppies do not have a breeding season, and the males are ready to reproduce at any time (Farr 1975), so they would approach a female with the goal of mating. Due to males' employment of sneak copulation techniques, a female does not need to be receptive to courtship to be inseminated (Farr 1975). She does, however, need to be accessible. The porous dividers allowed only visual and chemical exchanges, not physical interaction, and could have acted as a deterrent, especially for males that rely on more aggressive tactics. Feeding would therefore become a more beneficial option when a female is unavailable. With conspecific males, however, the attraction is presumed to be based upon shoaling, and mere proximity to the other fish would fulfill the purpose of the social interaction. It would be interesting to learn how the choice would change if a male was allowed to directly interact with a female.

Based on our personal observations, males were usually aware of the divider, swimming into it and around it frequently. Others, however, never crossed the threshold to the conspecific zone and began eating immediately. It is therefore plausible that those males were never accurately aware of the choice before them, suggesting that either the he was not receiving any cues or the female was not sending any to begin with. The former seems unlikely given how long the females were in the tank, but a variety of confounding variables are present, such as the relative chemical cues of the food, the absence of a filter (and thus less current to spread the pheromones), and the individual female. For a conclusive answer, more information is needed on the use of chemical cues in this species. Based on what is known, though, the females may not have been releasing pheromones (or at least those conveying sexual availability). Unlike the males, females are only ready to breed about once a month, as well as right after the birth of a brood (Farr 1975). These reproductive cycles are not synced or mediated by environmental cues, so guppies in the wild live in dense populations to ensure a receptive female is always available (Farr 1975). Only three females were used in our trials, and their personal reproductive statuses were completely unknown. A direct comparison on the effects of physiologically receptive and unreceptive females on males' mating tactics would help to provide additional context for our results.

The alternative interpretation of our results has a different focal point. Rather than viewing the male conspecific as somehow more desirable than the female, it instead suggests that the presence of a female actually stimulated a male to feed. This view falls in line with Abraham's (1993) work, and relates back to the previously discussed topic of the energetic costs of reproduction. It has been established that feeding takes initial priority over reproduction, as well as the fact that well-fed males are more likely to be successful in their mating attempts, so it

is logical to hypothesize that feeding could be a means of preparing for courtship, as triggered by the presence of a female. However, the guppies were not observed after they finished eating. To determine the true cause of the males' choice, a similar test to this one could be conducted, but with emphasis on the actions of the guppies post-eating. If they proceeded to engage in courtship, as Abraham's (1993) results would suggest, but did not socialize with the conspecific males, then it would support the idea that, when food is readily available, males choose to feed in order to increase their short-term energy states prior to mating.

As is, both possibilities are likely, and the most realistic standpoint probably incorporates all of the discussed factors to some extent. Nonetheless, our work builds on the large amount of research focusing on the behavior and sexual selection in guppies while providing a foundation for future research and offering a connection to the broader theme of energy allocation.

LITERATURE CITED

Abrahams, M. V. 1993. The trade-off between foraging and courting in male guppies.
        Animal Behavior **45**: 673-681.

Farr, J. A. 1975. The role of predation in the evolution of social behavior of natural populations
        of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). Evolution **29**: 151-158.

Farr, J. A. 1976. Social facilitation of male sexual behavior, intrasexual competition, and sexual
        selection in the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). Evolution **30**: 707-717.

Fraser, D. F., Gilliam, J. F., Akkara, J. T., Albanese, B. W., and S. B. Snider. 2004.
        Night feeding by guppies under predation release: effects on growth and daytime
        courtship. Ecology **85**: 312-319.

Heino, M., and V. Kaitala. 2001. Evolution of resource allocation between growth and
        reproduction in animals with intermediate growth. Journal of Evolutionary Biology **12**:
        423-429.

Kolluru, G. R., and G. F. Grether. 2004. The effects of resource availability on alternative
        mating tactics in guppies (*Poecilia reticulata*). Behavioral Ecology **16**: 294-300.

Kolloru, G. R., Grether G. F., Dunlop E. D., and S. H. South. 2008. Food availability and parasite infection influence mating tactics in guppies (*Poecilia reticulata*). Behavioral Ecology **20**: 131-137.

McCauley, E., Murdoch, W. W., Nisbet, R. M., and W. S. C. Gurney. 1990. The physiological ecology of *Daphnia*: development of a model of growth and reproduction. Ecology **71**: 703-715.

Plath, M., Heubel, K. U., García de León, F. J., and I. Schlupp. 2005. Cave molly females (*Poecilia mexicana*, Poeciliidae, Teleostei) like well-fed males. Behavioral Ecology and Sociobiology **58**: 144-151.

Reznick, D. 1983. The structure of guppy life histories: the tradeoff between growth and reproduction. Ecology **64**: 862-873.

Reznick, D. and A. P. Yang. 1993. The influence of fluctuating resources on life history: patterns of allocation and plasticity in female guppies. Ecology **74**: 2011-2019.

Siems, D. P., and R. S. Sikes. 1998. Tradeoffs between growth and reproduction in response to temporal variation in food supply. Environmental Biology of Fishes **53**: 219-229.
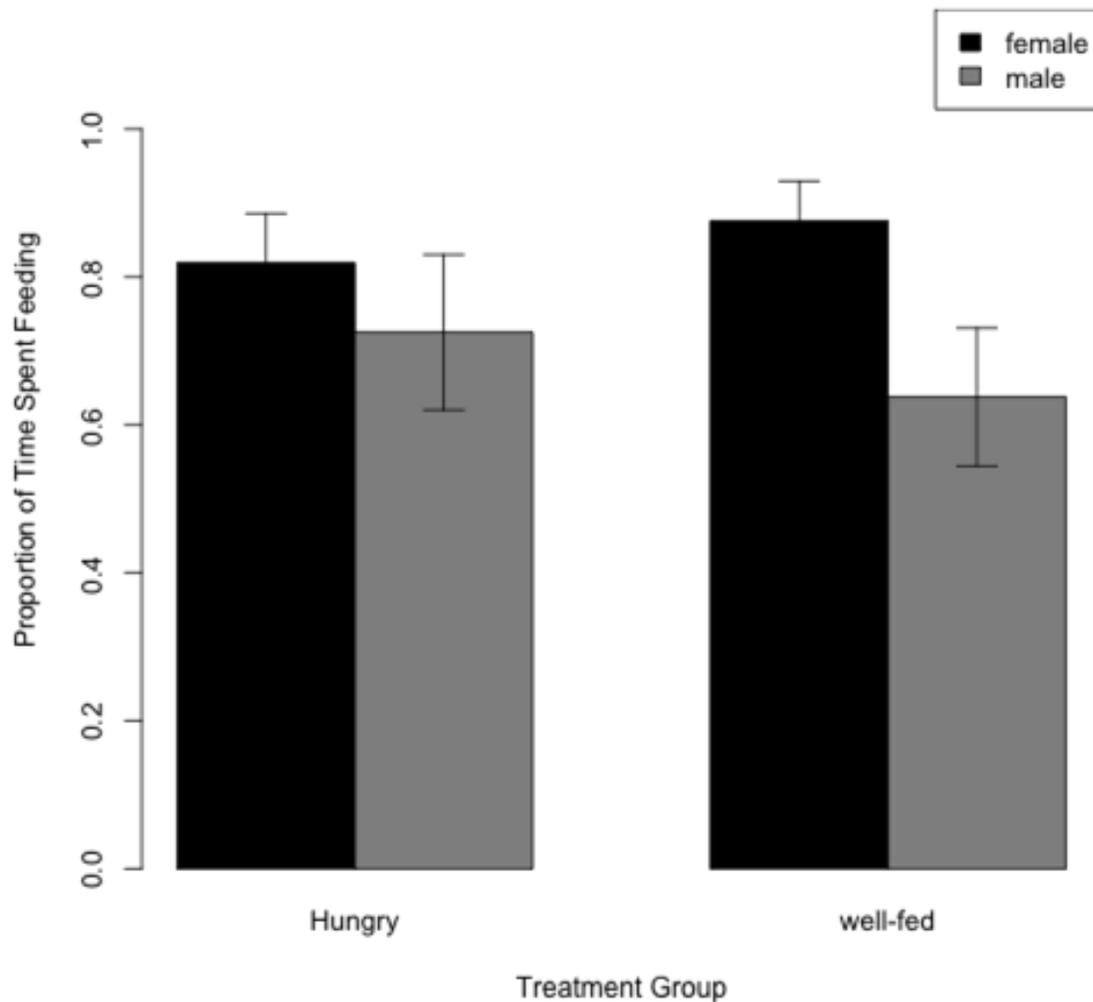
Figure 1. Effects of hunger level and stimulus type on mean (± SE) proportion of time spent feeding in male guppies when focal males made a choice. For 12 guppies, there were no significant differences in the proportion of time spent feeding between hungry and well-fed males ($p = 0.890$), nor between males exposed to either female or male stimuli ($p = 0.051$). The effect of hunger level on time spent feeding was not influenced by stimulus type ($p = 0.448$). Because all means are above 0.5, there is an overall preference to feed amongst all groups.
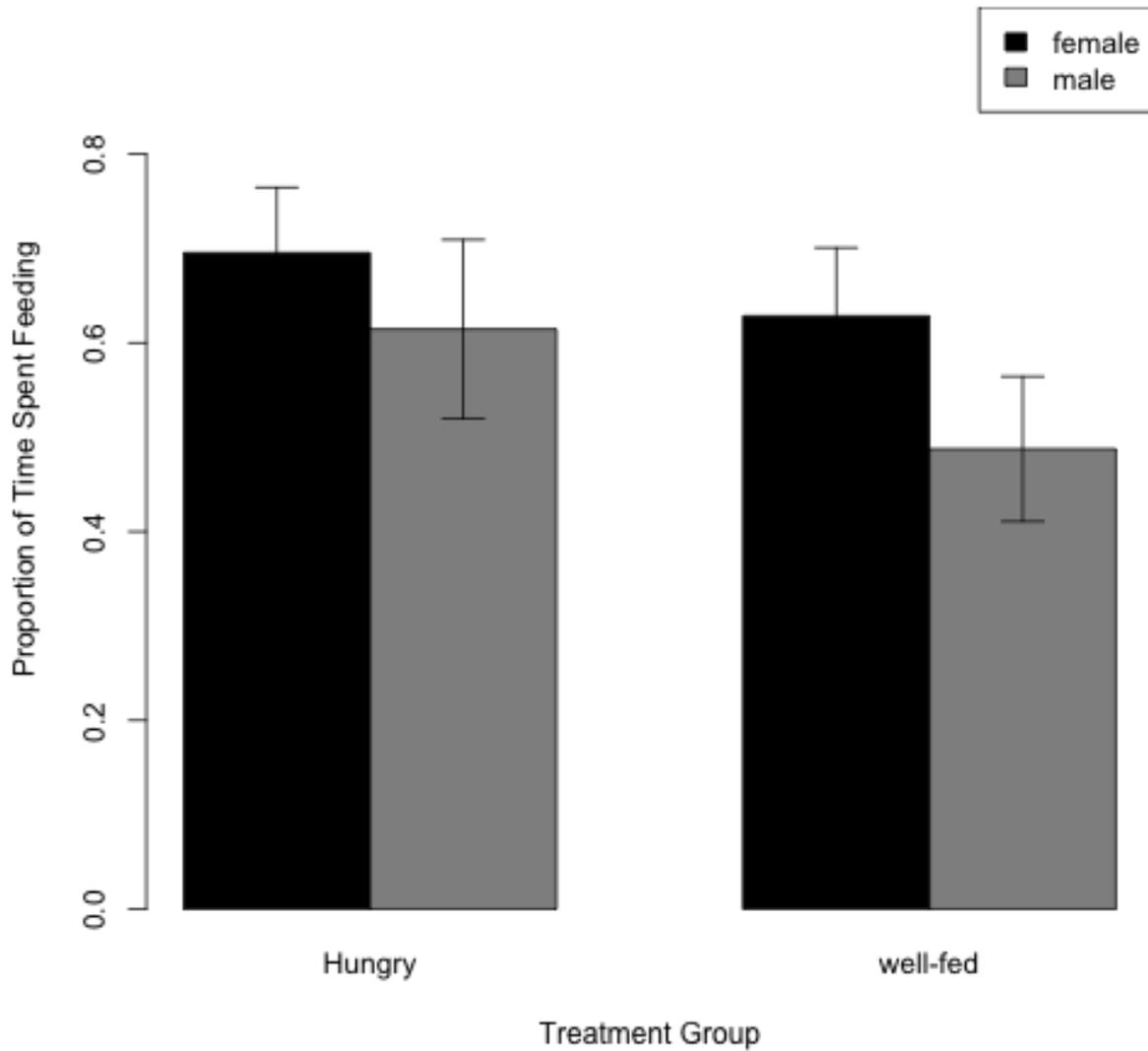
Figure 2. Effect of hunger level and conspecific sex on mean (± SE) proportion of time spent feeding, regardless of whether a choice was made. For 12 guppies, there were no significant differences in time spent feeding between well-fed and hungry males (p = 0.167), nor were there any differences based on conspecific type (p = 0.226). The effect of hunger level on time spent feeding was not influenced by conspecific sex (p = 0.705).
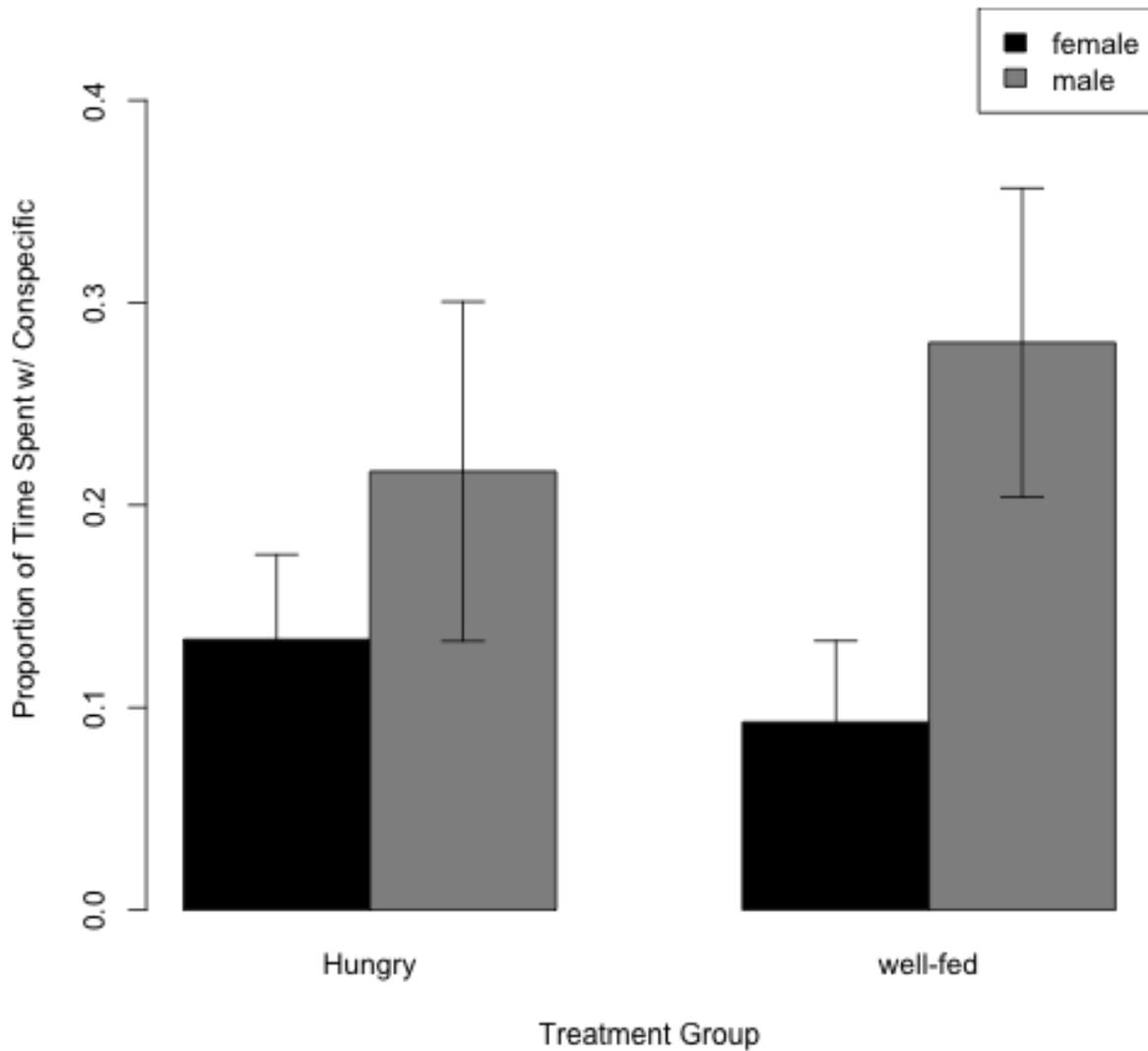
Figure 3. Effect of hunger level and conspecific sex on mean (± SE) proportion of time spent with conspecific, regardless of whether a choice was made. There were no significant differences in proportion of time spent with conspecific between hungry and well-fed males (p = 0.859), but focal males socialized 2-3 times more when the conspecific was male (p = 0.039). The effect of conspecific sex on proportion of time spent with the conspecific was not influenced by hunger level (p = 0.416).

R COMMANDER OUTPUTS

Proportion of time spent feeding out of two choice zones

Anova Table (Type II tests)

Response: log.prop.feed
             Sum Sq Df F value Pr(>F)
Stimulus   0.027604 1 4.0200 0.05114 .
treatment   0.000132 1 0.0193 0.89028
Stimulus:treatment 0.004026 1 0.5863 0.44795
Residuals   0.302136 44

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> tapply(GuppyDataFeeding$log.prop.feed,
+   list(Stimulus=GuppyDataFeeding$Stimulus,
+   treatment=GuppyDataFeeding$treatment), mean, na.rm=TRUE) # means
      treatment
Stimulus   Hungry well-fed
  control 0.2263244 0.2046893
  female 0.2559701 0.2709672

> tapply(GuppyDataFeeding$log.prop.feed,
+   list(Stimulus=GuppyDataFeeding$Stimulus,
+   treatment=GuppyDataFeeding$treatment), sd, na.rm=TRUE) # std. deviations
      treatment
Stimulus   Hungry   well-fed
  control 0.10460966 0.10077421
  female 0.06424334 0.04733959

> tapply(GuppyDataFeeding$log.prop.feed,
+   list(Stimulus=GuppyDataFeeding$Stimulus,
+   treatment=GuppyDataFeeding$treatment), function(x) sum(!is.na(x))) # counts
      treatment
Stimulus Hungry well-fed
  control   12   12
  female   12   12

R COMMANDER OUTPUTS CONT.

Proportion of time feeding including time in neutral zone

Anova Table (Type II tests)

Response: proportion

	Sum Sq	Df	F value	Pr(>F)
stimulus	0.1474	1	1.9755	0.1669
treatment	0.1128	1	1.5114	0.2255
stimulus:treatment	0.0108	1	0.1447	0.7054
Residuals	3.2832	44		

> tapply(GuppyFeedingWN$proportion, list(stimulus=GuppyFeedingWN$stimulus,
+   treatment=GuppyFeedingWN$treatment), mean, na.rm=TRUE) # means
         treatment
stimulus   Hungry   well-fed
  female 0.6952778 0.6283333
  male   0.6144444 0.4875000

> tapply(GuppyFeedingWN$proportion, list(stimulus=GuppyFeedingWN$stimulus,
+   treatment=GuppyFeedingWN$treatment), sd, na.rm=TRUE) # std. deviations
         treatment
stimulus   Hungry   well-fed
  female 0.2397071 0.2503553
  male   0.3282624 0.2656653

> tapply(GuppyFeedingWN$proportion, list(stimulus=GuppyFeedingWN$stimulus,
+   treatment=GuppyFeedingWN$treatment), function(x) sum(!is.na(x))) # counts
        treatment
stimulus Hungry well-fed
  female   12   12
  male   12   12

R COMMANDER OUTPUTS CONT.

Proportion of time spent in conspecific zone

Anova Table (Type II tests)

Response: proportion
```
              Sum Sq Df F value  Pr(>F)
stimulus        0.21960  1  4.5151 0.03925 *
treatment       0.00156  1  0.0320 0.85884
stimulus:treatment 0.03273  1  0.6729 0.41648
Residuals       2.14002 44
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

> tapply(GuppyStimulusWN$proportion, list(stimulus=GuppyStimulusWN$stimulus,
+   treatment=GuppyStimulusWN$treatment), mean, na.rm=TRUE) # means
```
      treatment
stimulus   Hungry   well-fed
  female 0.1336111 0.09277778
  male   0.2166667 0.28027778
```

> tapply(GuppyStimulusWN$proportion, list(stimulus=GuppyStimulusWN$stimulus,
+   treatment=GuppyStimulusWN$treatment), sd, na.rm=TRUE) # std. deviations
```
      treatment
stimulus   Hungry   well-fed
  female 0.145272 0.1392101
  male   0.290329 0.2641451
```

> tapply(GuppyStimulusWN$proportion, list(stimulus=GuppyStimulusWN$stimulus,
+   treatment=GuppyStimulusWN$treatment), function(x) sum(!is.na(x))) # counts
```
      treatment
stimulus Hungry well-fed
  female    12      12
  male      12      12
```