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Offspring retrieval behaviors after brood loss in the convict cichlid

Heather Witherspoon Govert
Eastern Kentucky University

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by

Heather Govert

Thesis Approved:

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Member, Advisory Committee

Dean, Graduate School

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Signature Heather Gott

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by

Heather Govert

Bachelor of Science
Kennesaw State University
Kennesaw, Georgia
2003

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Abstract

Parents provide care for their young in several different ways. One of these is searching for and retrieving any young that may become displaced from their nest. In monogamous biparental species, parents may share care of their young, including retrieval efforts. Convict cichlids (*Amatitlania siquia*) are an example of a monogamous biparental fish that show shared parental care of young. In the wild, these cichlids defend nests and search for missing young in response to complete or catastrophic brood loss. This retrieval behavior is important for both parents because parents, especially the males, have been observed to abandon their mate and brood when it does not appear to be successful.

Because brood loss in a natural setting would most likely result in partial brood loss due to predation, instances of partial brood losses were manipulated in a laboratory setting by progressively removing young to see if the male and female convict cichlid parents would alter their behavior based on how many young were missing from the nest. The behaviors of the female and male parents were compared. The female was also retested as a single or "abandoned" parent to determine if her behaviors were altered by her pairing status. As the amount of brood loss increased, the parents spent an increasing amount of time searching the tank for their displaced young. The paired females and males searched the tank differently, making different numbers of trips and spending a differing amount of time in each area of the tank. However, paired and "abandoned" females did not differ in their offspring search behavior suggesting that that females could be maximally invested in their young (i.e., putting a maximum amount of effort

into their broods) regardless of whether or not they have a male present to assist them. In addition, there was not a significant difference between the retrieval success of the paired parents and the "abandoned" females regardless of the amount of brood loss to which each was subjected. Although males perform a variety of parental duties necessary for successful care of the offspring, the offspring searching and retrieval data suggest that the male is not as helpful in retrieval of young; he might contribute different behaviors to the care of the young. Potentially, because females are maximally invested, males could be assessing the success of females in regard to an increase in the brood size before deciding whether abandonment is a higher fitness payoff option.

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Introduction

In terms of reproductive fitness, parental care provides benefits (increasing survival rates of current offspring; Westneat and Sherman 2002), but also has costs, including the time and energy needed to feed and defend the young. During each breeding attempt, therefore, parents must weigh the costs and benefits of continuing to provide care for the current brood relative to those of abandoning and, instead, investing in a future breeding attempt (Daan and Tinbergen 1997). Factors that can influence this decision include the risk of parents starving if they stay in the nest, the ability of the young to care for themselves if abandoned, and the likelihood of future successful broods (Webb et al. 2002).

An abandoning parent may be able to find another mate and initiate another breeding attempt, thus increasing its fitness. For example, abandoning a mate and brood has been observed to be a male-dominated phenomenon in some fishes (Keenleyside and Mackereth 1992). For example, when the number of offspring in a brood is reduced due to predation, convict cichlid (*Amatitlania siquia*) parents, specifically males, may find it advantageous to abandon their current mate, find a new mate, and create a new brood (Wisenden 1994b). This abandonment places additional parenting pressures on the parent left behind. For example, when young become separated from nests, paired parents can share responsibility in looking for missing young and guarding any remaining young (Snesker and Itzkowitz 2009). When young are missing from the nest of an abandoned parent, that parent has to choose whether to look for missing young or guard the remaining young. Abandoning can occur in this situation if parents do not retrieve enough offspring to make continuing to care for a brood worthwhile. Convict cichlids exhibit a division of labor or role specialization between the parents (Itzkowitz et al. 2001). Females spend more time in the nest than males during times of offspring retrieval (Snesker and Itzkowitz 2009; Snesker and Itskowitz 2014).

During a field study in a Costa Rican stream, Wisenden et al. (2008) examined how male and female convict cichlids dealt with the removal of offspring from their nests. Brood loss was manipulated to be "catastrophic" (i.e., all offspring removed).

After the loss, both males and females continued to defend their territories and search for missing young. Females spent more time in nests and attacking predators than did males. However, as Wisenden et al. (2008) pointed out, in a natural, non-manipulated setting, predation would be most likely to cause a partial brood loss instead of a catastrophic loss. Thus, additional experiments involving different levels of brood reduction might help determine if searching behavior, and the likelihood of abandonment by one or both parents, changes with different levels of brood loss. There are no published studies on how instances of partial brood loss affect parental behaviors in the convict cichlids.

My objectives were to (1) determine if there is a difference in searching behaviors of paired and abandoned females when the brood has been removed from the nest, and (2) to determine if the searching behavior of convict cichlids changes as the number of young in brood changes. I tested paired females then tested each again as an abandoned female to see if the presence of the male had an effect on her retrieval efficiency. If her efficiency (i.e., percentage of young retrieved) was equally high when paired it would imply that she is maximally invested in her young regardless of whether or not she has a male partner to assist her. My first hypothesis was that the female would be maximally invested in her young regardless of whether or not she had a male partner to assist her. The behaviors of paired females were also compared to the behaviors of paired males to see how the sexes behaved differently when their young were missing. My second hypothesis was that female behavior would change based on the number of missing young; specifically I predicted that the amount of searching behavior would increase as the number of missing young increased. Not only has partial brood loss not been studied in the convict cichlid, but there are no published studies on partial brood loss and retrieval behaviors in fish.

Methods

This study was conducted from July 2009 to February 2011. Male and female convict cichlids were housed in separate stock tanks and the experimental fish chosen from these stock tanks. To measure parental behaviors, pairs of convict cichlids were first established using methods similar to those used by Itzkowitz et al. (2002). The pairing process began by setting up a 190-liter tank partitioned into two sections separated by a clear divider that spanned the entire width of the tank. One section was approximately 75 cm long (Figure 1[1]) and housed a male and female termed the "paired" male and female. The second section was approximately 15 cm wide and hosted a conspecific male, or intruder. The purpose of the intruder was to draw attacks from the pairing male and female to reduce within-pair aggression and facilitate pair-bond formation (Itzkowitz and Draud 1992).

Before testing, the tank was marked externally with tape dividing the tank into five 18-cm sections so the location of the breeding pair could be recorded. A clay flower pot used as the nest site was placed in the section of tank farthest from the intruder); this section was referred to as the "nest area." The other sections from left to right were named: "near," "middle near," "middle far," and "far away" (Figure 2). The "intruder" area from initial set-up became the "far away" area once the nest was complete and the intruder and partition removed as described later.

All fish were monitored daily. To mimic the size preferences shown by convict cichlids in the wild (Wisenden 1995), pairs were established with females being 5-15 mm shorter than males. After a pair laid and fertilized eggs, the intruder was removed. Eggs typically hatched after three or four days and entered into the six-day wriggler stage where young had tails, but were still immobile. All testing for this project took place on days two and four of this wriggler stage.

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[1] See Appendix

Reduction Treatment with Paired Females - On day two of the wriggler stage, the clear partition was replaced with an opaque partition temporarily blocking the far away section. Then, using a disposable pipette, wrigglers were removed from the tank and counted. After counting, I returned 0%, 25%, 50%, 75%, or 100% of the original brood to the far section, with the remainder returned to the flower pot nest. I tested 70 pairs, with 14 pairs in each reduction treatment. The 0% reductions were considered controls. All wrigglers, regardless of the amount of reduction of the group, were removed from the tank for 15 minutes for counting. After returning wrigglers to the tank, the opaque partition was removed and parental behaviors were recorded for 30 minutes using a video camera. After 30 minutes, all wrigglers in the flower pot were counted as a measure of searching success of the parents (male and female combined). Retrieval was considered successful if at least 67% of young were retrieved.

All behaviors were recorded using the computer software Observer (Noldus). For both males and females, I quantified the number of visits to each section of the tank, duration of each visit, number of bouts, number of pecks at the substrate, number of tending visits, latency, number of bites, and the number of tail-beats (see Table 1 for definitions of these behaviors).

Reduction Treatment with Abandoned Females - Following the same methods as above, the behavior of females was tested without the presence of the paired male. To establish "abandoned" females, males were removed from the tank immediately after testing on day two of the wriggler stage and placed into a separate stock tank to ensure no male was re-used in a future trial. On day 4 of the wriggler stage, wrigglers were again temporarily removed, counted, and placed into the tank and the female was then video-taped. The percent of wrigglers reduced on day four was the same percent reduction used on day two for that female. This allowed for comparison of the female's performance at a certain reduction to be based on whether she was paired or abandoned. After testing on day four of the wriggler period, all wrigglers were permanently removed from the tank and placed in a stock tank. Following completion of each test, each female was eventually returned to a specified stock tank for "used paired females" so no female was reused in the experiment.

Statistical Analysis

The responses of paired females were compared using a five-by-five within-subject design whereby all five levels of reduction were used for each female. A similar within-subject design was also used for paired males and abandoned females. Comparisons between the paired females, paired males, and abandoned females for certain behaviors at each amount of reduction comprised a five-by-five-by-three between-subject design. These behaviors included number of visits to an area of the tank, amount of time spent there, number of pecks, number of tending visits, and latency. A repeated measures ANOVA was performed, i.e., results were recorded for each adult under each level of reduction. Comparisons between paired females and males for the number of bites and number of tail-beats at each amount of reduction were made using a five-by-five-by-two ANOVA design. A comparison between paired females and abandoned females for certain behaviors comprised a five-by-five-by-two ANOVA design. Microsoft Excel and *Statistica*® were used to analyze data. An f test was used to determine that the data met parametric assumptions. Post-hoc Tukey tests were used to further explore significant differences found in the overall ANOVA design. Planned comparisons were used when comparing the paired females' to males' number of pecks, tending visits, and latency because differences were expected for those comparisons. Planned comparisons were also used when comparing the paired to abandoned females' number of pecks, tending visits, and latency due to the differences expected. A planned comparison was also performed to compare the retrieval success of the paired parents and the abandoned females at each level of reduction because a difference in retrieval success as brood reduction increased was expected. Values are provided as means ± 1 SD.

Results

The average number of offspring per brood (N =70) was 180 ± 102, and did not differ among the different reduction groups ($F_{4, 52} = 0.4$, $p > 0.05$). Male convict cichlids averaged 81.3± 12.4 mm in length, and females 71.0 ± 11.6 mm. The length of males and females did not differ among reduction groups ($F_{4, 63} = 0.6$, $p > 0.05$).

Several behaviors did not differ ($P>0.05$) among groups in any comparisons, including bouts, total number of bites, and number of tail-beats. Other behaviors that were significantly different among some groups or had significant interactions among groups will be described in more detail.

I: Effect of reduction treatment

A. Number and duration of visits - The reduction treatments influenced the number of times paired females, paired males, and abandoned females visited each area of the tank (Table 2, Figure 3) as well as the duration of each of those visits (Table 2, Figure 4). As more young were removed, the number of trips to the far away area increased, as did the number of trips to the near, middle near, and middle far areas because the fish were swimming through those areas to get to and from the far away area. As the amount of reduction increased, fish spent less time in the nest area and more time in the far away area.

B. Retrieval success - I found no overall effect of reduction treatment on retrieval success ($F_{1, 52} = 1.0$, $p = 0.38$; Figure 5). The retrieval success was not affected by the reduction treatment in the no reduction and partial reduction groups. Retrieval success in the complete reduction group was lower, but overall it was not significantly lower.

C. Aggression toward mate - The reduction treatment did not affect either the number of bites inflicted on mates ($F_{1, 65} = 1.2$, $p > 0.005$; figure 6) or the number of tail-beats inflicted on mates ($F_{4, 65} = 1.0$, $p > 0.05$). The number of tail beats inflicted upon a mate did not alter significantly between the sexes regardless of the amount of reduction. The number of bites inflicted upon a mate also did not differ between the sexes regardless of reduction amount with one exception, a couple of females in the 75% reduction group were aggressive than the others giving the appearance of more aggression at this level of reduction.

II. Effect of sex

A. Number of visits - I found an interaction between sex and reduction treatment on the number of visits made to each area of tanks. The main effect of sex was significant ($F_{1, 65} = 6.2$, $p < 0.05$), with males typically making more visits than females to most areas away from the nest, but that difference varied among reduction groups, and was not evident in the 100% reduction group where abandoned females made more visits away from the nest to all areas of the tank than males ($F_{8, 206.62} = 0.7$, $p = 0.90$; Figure 3).

B. Duration of visits - There was an interaction between the effect of sex and the reduction treatments on overall duration of time spent in each area of the tank by the paired female when compared to the paired male. The paired female spent more time in the nest area than the paired male in the all of the reduction groups, but the paired female spent less time in all other areas of the tank than the paired male ($F_{40, 206.6} = 0.7$, $p = 0.90$) (Figure 4).

C. Number of pecks - There was an interaction between the reduction treatment and sex on the total number of pecks performed ($F_{8, 26} = 12.5$, $p<0.05$, Table 3). Males always had fewer pecks than females in the nest area at all reduction treatments ($F_{8, 126} = 2.5$, $p = 0.014$), but in the far area, females only outpaced males in the 100% reduction area where pecks were highest for abandoned females ($F_{8, 126} = 2.2$, $p = 0.029$ Table 3,Figure 7).

D. Tending - Overall, paired and abandoned females visited nests more than males, but the interaction of sex and reduction treatment was significant ($F_{8, 126} = 1.9$, $p = 0.059$, Table 3, Figure 8). In the 0% reduction and all partial reduction treatments the paired and abandoned females made significantly more visits to nests than paired males. In the 100% reduction group, however, the pattern was the same but the difference was not significant (Figure 8).

E. Latency - The sex and reduction treatment showed an interaction on the latency of the paired females and males ($F_{8,126} = 1.0$, $p = 0.47$). There was a main effect of sex where the paired males searched the far away area sooner than the paired females

(Figure 9). This effect was strongest in the 25% reduction group where the paired males reached the far away area to search it much faster than the paired females did. This drastic difference was not seen at the other levels of brood reduction (Table 3).

F. Biting - Although there was an overall difference between males and females in the number of bites inflicted upon a mate ($F_{4, 63} = 4.1$, $p = 0.005$), the difference was only significant in the 75% reduction group (Table 3). In that group, females averaged over 15 bites inflicted on their mate, whereas males inflicted fewer than five bites on their mate (Figure 6).

III: Effect of pairing status on females

A. Number and duration of visits - There was not a main effect of pairing status (paired versus abandoned female) on the overall number of visits made to other areas of the tank ($F_{1, 65} = 2.2$, $p > 0.05$; Figure 3). There was, however, an effect of pairing status on the duration of visits ($F_{8, 520} = 19.6$, $p < 0.05$). The duration of visits away from the nest were generally higher for abandoned females than for paired females (Figure 4), but differences in duration varied among the reduction treatments (interaction) as described previously.

B. Retrieval success - Pairing status had no effect on retrieval success between the paired parents and the abandoned females ($F_{2,40} = 1.1$, $p = 0.38$, Table 4, Figure 5). There were no significant differences in retrieval success between the paired parents and the abandoned females in the 0%, 25%, 50%, and 75% reduction groups.

C. Number of pecks - There was not an effect of pairing status on the total number of pecks performed by the paired females and the abandoned females (Table 4, Figure 7). Pairing status did, however, have an effect on the number of pecks performed by females in the 100% reduction group only (Table 4), where the number of pecks in the far away area for the abandoned females was higher than for the paired females ($F_{2, 126} = 2.2$, $p = 0.029$, Figure 7). A closer look at the number of pecks performed in the nest area revealed a significant effect of pairing status between the paired and abandoned females ($F_{8, 126} = 2.5$, $p = 0.014$, Table 4, Figure 7). The only difference between paired and abandoned females at the nest was in the 25% reduction treatment group (Table 4, Figure 7), where the paired females pecked more than abandoned females.

D. Tending - There was an effect of pairing status on the number of tending visits between the paired and abandoned females (Table 4, Figure 8). Overall, paired females made significantly more tending visits than the abandoned females.

E. Latency - There was no effect of pairing status on the latencies of the paired and abandoned females ($F_{(2, 130)} = 1.0$, $p = 0.47$, Table 4, Figure 9).

Discussion

Although the size of the fish and the number of young that they produced varied, these variations were not significant and thus did not affect the behaviors of the fish. A study on small mouth bass concluded that male size only affected the survivability of a brood in the later stages of the nesting cycle, a stage not reached in this experiment (Suski and Ridgway 2007). The same study suggested that smallmouth bass males guarding larger broods were less likely to abandon their broods because they invested more energy into brood defense than males with smaller broods (Suski and Ridgway 2007). Brood defense was not an issue in my study. While the size of the fish and the number of young produced varied, each reduction group showed the same variations.

I. Effect of reduction treatment

A. Paired females - Paired females made more visits to all areas of the tank, especially the nest area, as the amount of brood reduction increased, suggesting they alter their behavior based on the proportion of displaced young. Females spent more time in the nest area than in any other area of the tank regardless of the reduction treatment. This could indicate how important the current brood is to the paired female and that they place a high priority on guarding and tending to whatever young are in the nest. As the amount of reduction increased, the females did spend less time in the nest area because they were spending more time searching the tank for missing young. This is confirmed in that as the amount of brood reduction increased, females spent more time in the far away area retrieving young. Even with 100% brood loss, paired females spent significantly more time in the nest area than in the far away area where the young were placed, possibly indicating that the paired females are guarding that empty nest at first. Wisenden et al. (2008) also saw that paired females would defend and guard an empty nest in instances of 100% brood loss. Jennions and Polakow (2001) saw that female cichlids rarely left the nest spending their time guarding and caring for their young. My results confirmed Wisenden's results that paired females placed importance on the empty nest, as that was where their young had been before being displaced, and where any young would be cared for should they be found and returned.

B. Paired Male - There were significant differences in the number of visits the paired males made to each area of the tank within each reduction treatment; this could indicate that an increase in brood loss may affect how males search for young. Paired males spent significantly more time in the nest area of the tank than they did in the other areas of the tank regardless of their reduction treatment. This could indicate that the males may place more importance on any young remaining in their nest (Snesker and Itzowitz 2014) than searching for any displaced young. The paired males in the 0% and all partial reductions all spent significantly more time in the far away area (where displaced young were located) than the males in the 100% reduction treatment. They may be choosing to spend more time either guarding the empty nest (Wisenden et al. 2008) or just staying in that area of the tank since there was nowhere else in the tank where they could go should they want to abandon. This is seen in that the paired males did spend more time in the nest area than they did in the far away area (Figure 4). This also confirms results in Wisenden et al.'s (2008) study where the males were subjected to a 100% reduction treatment and the majority spent their time guarding their nest. This was especially true for males who had a significantly larger brood before the catastrophic loss (Wisenden et al. 2008). However, because the amount of time spent searching in the far away area was less than the time spent in other areas of the tank this could indicate that males who experience 100% brood loss may show a tendency to abandon their mate and nest and instead search for another mating opportunity. Previous studies have shown that males in some species of fish may tend to abandon mates and seek other mating opportunities after instances of brood loss (Jennions and Polakow 2001; Keenleyside and Mackereth 1992; Zuckerman et al. 2014).

C. Abandoned females - The number of visits made by the abandoned female to each section of the tank appears to have been affected by the reduction treatment that each female was subjected to, but there was not a significant difference. Although there are no significant differences between the numbers of visits made to the nest and far away areas, there are differences in the numbers of visits made to each of the middle areas of the tank compared to the ends of the tank. This is due to the fact that in order for the fish to reach the far away area to retrieve young it had to swim through those middle areas of the tanks twice (once going in each direction). This would cause the number of visits to those areas to be higher when the abandoned female is searching for and retrieving her displaced young. The number of visits made to the middle areas of the tank were counted to see if the fish would search them differently based on their position relative to the nest and where the missing young were placed, as seen in Snesker and Itzkowitz (2009), however this did not appear to be the case. This could be further verified by looking at the amount of time that the abandoned females were spending in each section of the tank since very little time was spent in the middle areas of the tank per visit.

As the amount of brood loss increased, abandoned females spent significantly less time in the nest area. This confirms that the abandoned females were just swimming through these areas to get from their nest to the far away area to retrieve displaced young and then back to the nest. The bouts made by the abandoned females confirm the emphasis that each placed on each area of the tank and also confirms the results shown by the duration of time spent in each section of the tank.

D. Aggression - The number of bites and tail beats that the paired females and males inflicted upon their mates was not affected by an increase in brood loss. This could indicate that aggression between a female and male may be due to factors other than the proportion of young that they may or may not be missing. In the lab, one factor that may encourage aggression between mates could include presence of a predator (Itzkowitz et al. 2000). The presence of a predator could alter the cichlid's retrieval behavior and other parental care behaviors (Itzkowitz et al. 2003; Richter et al. 2005; Snesker and Itzkowitz 2014).

E. Retrieval success - Overall, the amount of the reduction treatment did not affect how successfully the pairs or the abandoned females retrieved young, at least within the reduction treatments of 0%, 25%, 50%, and 75%. A 100% brood loss did affect how successfully missing young were retrieved. Cichlids subjected to a 100% brood loss either successfully retrieved their young or did not retrieve them at all. Successful retrieval of young was defined as the majority, at least two-thirds, of young being retrieved to the nest. In this study, some cichlids retrieved their young while others did not and abandoned them. There was no significant difference in the number of parents that did retrieve their young versus those who did not. This possibility of abandonment of a partner and brood could indicate that the cichlid is putting the likelihood that a future brood would be more successful as more likely than the current brood has of being successful. Wisenden et al. (2008) found that most males would not abandon a brood after 100% brood loss. These differences could be due to several factors including setting for the study and length of testing. Wisenden's (2008) study was conducted in a natural setting allowing the male to leave the nest to seek other mating opportunities and still come back to the nest. My study was conducted in a laboratory setting which confined the pair to a single tank. In this situation the male does not have the ability to look and see if abandoning is a good option before doing so. The length of testing for my study was also longer than his to allow ample time for parents to find and retrieve their young. Longer observations may have revealed behaviors that his study did not.

II. Effect of sex

A. Number of visits - As the amount of brood loss increased there were differences in the number of visits made to each area of the tank, with more visits being made to the far away area and less visits made to the nest area. This would suggest that as the amount of brood loss increased, the paired females and males showed changing strategies for searching for their displaced young. Although males made more trips throughout the tank overall, paired females showed an increase in searching the far away area. The females searched the area where the missing wrigglers were known to be whereas the males underwent a slower, more wide spread search. This difference could indicate that the females search for young in a more systematically than males do. These differences could also indicate a male's tendency to abandon his partner and young (Jennions and Polakow 2001; Keenleyside and Mackereth 1992). Whereas the paired female is more devoted to finding displaced young and the male is searching more, but he could be searching for other opportunities than this brood has presented (Wisenden et al. 2008). This difference could be due to the fact that, in the wild, males can mate more than once per season (Wisenden 1994a), whereas females typically only mate once a season (Wisenden 1994a). This difference may lead females to place more value on each brood because each brood is more critical to the female's overall reproductive success (Wisenden 1994a). This was seen in the 100% brood loss group where abandoned females were more successful at retrieving young than the paired parents.

B. Duration of visits - Paired females in the 0%, 25%, and 50% reduction treatments spent significantly more time in the nest area than their mates. Paired females in the 75% and 100% reduction groups did not spend a significantly different amount of time in the nest area. These differences in the lower amounts of reduction could indicate that the paired female was doing more of the guarding of the nest and tending to any remaining young in the nest than her male partner. When more young (75%) were displaced from the nest, paired females tended to leave the nest more to search for young. Paired females in the 25% reduction treatment also spent significantly less time in the far away section than their male partner did. This could indicate that even though a relatively small proportion of her young are missing, paired females invest more energy in caring for the remaining young than males. The fact that there did not appear to be a significant difference in the amount of time spent in all areas by the paired females and males of the 100% reduction treatment could indicate that they are either searching together for their missing young or are both abandoning their young in hopes that a future brood may be more successful. This could also indicate that the paired female may make her decision of whether or not to abandon her brood based on the behaviors of her mate. Snesker and Itzkowitz (2009) proposed that with retrieval behaviors convict cichlids retrieval patterns are more of a division of labor than a difference in sex roles. This was seen in retrieval success of the 100% reduction group where if the male did not retrieve the young, the female did not either.

C. Number of Pecks - The paired females of the 0% reduction treatment performed significantly more total pecks than their male partners did. When looking closer at the number of pecks that were actually performed in the nest area only, we see that paired female actually pecked in the nest area significantly more times than her male partner did at 0%, 25%, 50%, and 75% reduction treatments (no significant difference in the 100% reduction treatment). This could indicate that at no brood loss or partial brood loss the females are pecking around the nest to care for and tend to the remaining young more than their male partners are. This difference between the paired females and males provides us with an example of a division of labor that is found in convict cichlids. The female is putting more effort into caring for the young in the nest area than the male is (Snesker and Itzkowitz 2009). There was not a significant difference between the number of pecks performed by the paired females and males in the far away area. This indicates that no matter what the amount of brood loss the paired females are searching for and retrieving their young just as much as their male partners are, further indicating that when it comes to retrieval of young the amount of brood loss a pair faces does not change their searching and retrieval. It also indicates that the paired females retrieve young in a similar manner as their male partners (Snesker and Itzkowitz 2009).

D. Tending - The paired females entered the nest to tend to the young significantly more than the paired males did within the 0%, 25%, 50%, and 75% reduction treatments. This could indicate that at no brood loss or any level of partial brood loss the female is providing more direct care to the young. In the 100% reduction treatment group there was not a significant difference in the number of tending visits made by the paired females and males. This could indicate that in these instances both parents will go into the nest to see that their young are not there and also to return any displaced young that they have found (Snesker and Itzkowitz 2014). Both parents will enter the nest a similar amount of times.

E. Latency - The paired male searched the far away area for their missing young sooner than their female partners did. However, this difference was really only significant in the pairs that were missing 25% of their young. This could indicate that when only a small proportion of the young (25%) are displaced from the nest the paired female may tend to guard the remaining young more than she would spend time searching for missing young. Once the amount of brood loss is increased to at least 50% the female may spend more time away from the nest to find those displaced young.

F. Biting - The only time there was a significant difference between the numbers of bites inflicted by the paired females versus males was within the 75% reduction treatment when female bites were more than 3 times that of males. At this level of brood loss, the increase in aggression by females could be due to the fact that more young are missing and may signal the male to search more for their young, but more research would need to be done to verify this suggestion.

III. Effect of pairing status on females

A. Number of visits - Females visited each section of the tank equally whether or not she had a male to help her. This may suggest that females put a maximum amount of effort into searching the tank for missing young regardless of the presence or absence of a male partner (Jennions and Polakow 2001).

B. Duration of visits - There were very few differences between the paired and abandoned females in regard to the amount of time that they spent in each area of the tank. The only difference seen was that in the 75% and 100% reduction groups, the females spent more time in the nest area when they were paired versus when they had been abandoned as well as the females spent more time in the far away area once they had been abandoned. Neither of these differences was significant. This would suggest that the females are maximally invested in their young regardless of whether or not they have a male partner present to help them search for missing young. Jennions and Polakow (2001) found that female cichlids did not change their parental care efforts regardless of any brood reductions they were subjected to based on whether or not they had been abandoned by their male partner.

C. Retrieval success - There was not a significant difference in the retrieval success of the paired parents and the abandoned females at each amount of brood reduction. This could indicate that in this controlled setting the female may successfully retrieve any missing young without help from the male.

D. Number of pecks - In instances of no brood loss or partial brood loss there was not a significant difference between the total number of pecks performed by the females when paired and when abandoned. This could indicate that at these levels of brood loss the females search for and pick up any found young at a maximal level regardless of whether or not she has a male partner to assist her. At 100% brood loss the abandoned females peck at the substrate significantly more times than the paired females do. This could indicate that she recognizes that she no longer has a male partner to help her regardless of the amount of brood loss, and when faced with such a catastrophic level of brood loss she may peck more at the substrate to retrieve her young. This ‘recognition’ by the abandoned female may only have been seen at 100% brood loss because that was the only group with poor retrieval success for the paired parents.

When looking closer at the number of pecks performed in the nest area the status of the female did not impact how many times she pecked at the substrate. The only time there was a difference was within the 25% reduction treatment. Here the paired females pecked at the substrate more than the abandoned females did. This difference could be due to the female recognizing that some of her young are missing and she is searching around the nest for them while her partner searches other areas of the tank. At such a small amount of brood loss, the female may rely on the male to more of the searching in farther away areas while she searches closer (Snesker and Itzkowitz 2009). Another explanation for this difference between the paired and abandoned females could be due to the way the wrigglers floated back into the nest at the beginning of the testing, this improved with experience in returning the wrigglers to the nest. Since the wrigglers were hard to control when returning them to the tank they could have landed more outside of the nest than inside, leading the female to have to peck around the nest area of the tank more vigorously.

When looking at the number of pecks that were performed in the far away area of the tank, the status of the female did not appear to have an effect within the no brood loss and all partial brood loss treatments. This could indicate that the females put a maximal amount of effort when pecking at the far away area to retrieve their displaced young at these levels of brood loss. In instances of 100% brood loss the pairing status did affect the number of pecks performed in the far away area by the females. In this group, the abandoned females pecked at the substrate significantly more than they did when they had a male partner. This could indicate that a catastrophic brood loss may cause a paired female to depend on their male partner for successfully retrieving the displaced young (Snesker and Itzkowitz 2009; Wisenden et al. 2008). When the female recognizes that she does not have a male to help her retrieve she steps up the amount of effort that she puts into retrieving. This could indicate that the female’s behavior is cued on whether or not she has a male to help her.

E. Number of tending visits - The number of visits made by the females inside the nest to tend to young was not affected by her pairing status. The exception to this was the number of tending visits made by the females within the 25% reduction treatment. In this group the paired females made significantly more trips into the nest than they did as an abandoned female. This difference could be due to the young having to be retrieved from the nest area and placed into the nest as discussed in an earlier paragraph.

F. Latency - The latency, or amount of time that it took the female to search the far away area for displaced young, was not affected by the females pairing status. This could indicate that a desire to find any displaced young and look for them is not effected by whether or not a male partner is present. The female searched for missing young regardless of male presence. The speed at which she searches is also not affected by the presence or absence of a male, the female's searching time remains consistent regardless of pairing status. This could be due to the possibility that some males do abandon their broods (Keenleyside and Mackereth 1992). The females could be searching the same regardless of whether or not she has a male because she does not know if he is as maximally invested in the brood as she is, or if he might abandon her and the brood.

Conclusions

As the amount of brood loss increased, the amount of time that the parents spent searching also increased, thus my second hypothesis that the female would alter her behavior based on the number of young missing and that searching behavior would increase was supported. This indicates that convict cichlids may possess the ability to recognize how many young they had initially, as well as recognize when those young become missing from the nest. This ability to recognize that they have young missing and the ability to place importance on those missing young may lead the parent or parents to actively search for and retrieve their young.

The female appears to be maximally invested in her young and her searching and retrieval behavior is similar whether or not she has a male to help her, thus supporting my first hypothesis correct as well. In the 100% reduction group, the abandoned females pecked more than the paired females possibly due to the lack of assistance from a partner. The presence of a male partner aided the paired female in searching and returning young to the nest, thus the paired female was still maximally invested in her young, but she had less young to peck for because the male was helping her. This could be further tested in a natural setting to see if the female becomes influenced by environmental factors and the possible presence of predators both as a paired and an abandoned parent.

The paired male appears to vary his searching strategy based on the amount of brood loss that he faces. At no brood loss or only a small amount of brood loss, he tends to stay closer to the nest with very little searching. At higher amounts of partial brood loss (50% and 75%), he tends to search more aggressively. In instances of a complete brood loss, he either searches very successfully or shows a tendency to abandon. This tendency to abandon could indicate his recognition of reduced fitness resulting from this brood and a decision to invest in a future brood in the hopes that it will be more successful (Jennions and Polakow 2001; Wisenden 1994a; Wisenden et al. 2008).

Aggression did not appear to have a significant effect on the searching and retrieval of young in this study, an exception to this was seen at 75% brood loss, here the females bit at their male partners possibly to spur them into helping search for a retrieve missing young. This could be due to the laboratory setting; it is possible that in a natural setting 75% brood loss may be the point where males begin to show abandonment. Testing retrieval of a brood at different levels of partial brood loss in a natural setting might be a better indicator of aggression between parents during brood loss.

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Appendix

Tables

Table 1. Definition of behaviors quantified during the project.

Number of visits	The total number of times a fish visited each area of the tank, regardless of the amount of time spent there.
Duration of visit	The total amount of time a fish spent in each area of the tank.
Bouts	The average amount of time that a fish devoted to an area of the tank during one visit. This is an indicator of the importance a fish places on that area of the tank.
Pecks	The total number pecks at the substrate in a particular area to pick up or search for wrigglers.
Tending	Each time a fish entered the flower pot nest to return wrigglers to the nest to drop off or tend to any wrigglers. A fish returning to the nest to drop off young or tend to wrigglers in the nest can be seen in the videos when the fish enters the nest and goes to the very back of the nest.
Latency	The amount of time that passes before a fish begins to search the far away section for the missing young.
Bites	The number of bites inflicted on the fish's mate, used as a measure of aggression.
Tail-beats	The number of tail-beats inflicted on the fish's mate, used as a measure of aggression (observed by Santangelo).

Table 2. The overall effect of all reduction treatments on each recorded behavior. These comparisons were performed using post hoc Tukey tests; significant ($P<0.05$) comparisons are marked with '*'.

Behavior	Paired Females	Paired Males	Abandoned Females
Number of visits	$F_{4, 260} = 68.995$*	$F_{4, 260} = 58.302$*	$F_{4, 260} = 133.05$*
Interaction between area of tank visited and number of visits	$F_{16, 260} = 2.592$	$F_{16, 260} = 2.239$*	$F_{4, 260} = 9.557$*
Duration of visit	$F_{4, 260} = 359.69$*	$F_{4, 260} = 70.516$*	$F_{4, 260} = 467.04$*
Interaction between area of tank visited and duration of visit	$F_{16, 260} = 4.616$*	$F_{16, 260} = 1.321$	$F_{16, 260} = 16.311$*

Table 3. Results of Tukey tests and planned comparisons showing the effect of sex within all levels of reduction treatment on total number of pecks, number of pecks in the nest area, number of pecks in the far away area, number of tending visits, latency, and biting. Significant interactions (*P*<0.05) are marked with '*'.

Reduction treatment	Total pecks	Pecks in nest area	Pecks in far away area	Tending visits	Latency	Biting
All levels	$F_{8, 26}$ = 12.466*	$F_{2, 126}$ = 61.124*	$F_{2, 126}$ = 1.852	$F_{2, 130}$=117.299*	$F_{2, 130}$ = 6.334*	$F_{1, 65}$= 0.120
0%	$F_{8, 26}$ = 5.5306*	$F_{2, 126}$ = 15.933*	$F_{2, 126}$ = 0.001	$F_{2, 130}$ = 30.899*	$F_{2, 130}$ = 1.086	$F_{1, 65}$ = 0.143
25%	$F_{8, 26}$ = 3.612	$F_{2, 126}$ = 35.450*	$F_{2, 126}$ = 3.874	$F_{2, 130}$ = 48.238*	$F_{2, 130}$ = 6.778*	$F_{1, 65}$ = 1.808
50%	$F_{8, 26}$ = 0.168	$F_{2, 126}$ = 12.762*	$F_{2, 126}$ = 1.167	$F_{2, 130}$ = 24.580*	$F_{2, 130}$ = 1.775	$F_{1, 65}$ = 0.072
75%	$F_{8, 26}$ = 1.964	$F_{2, 126}$ = 8.351*	$F_{2, 126}$ = 0.367	$F_{2, 130}$ = 27.660*	$F_{2, 130}$ = 0.064	$F_{1, 65}$ = 12.513*
100%	$F_{8, 26}$ = 1.0102	$F_{2, 126}$ = 0.832	$F_{2, 126}$ = 0.290	$F_{2, 130}$ = 2.363	$F_{2, 130}$ = 0.113	$F_{1, 65}$ = 1.802

Table 4. Results of Tukey tests and planned comparisons showing the effect of pairing status within all levels of reduction treatment on retrieval success, total number of pecks, number of pecks in the nest area, number of pecks in the far away area, tending, and latency. Significant interactions (*P*<0.05) are marked with '*'.

Reduction Treatment	Retrieval success	Total pecks	Pecks in the nest area	Pecks in the far away area	Tending	Latency
All levels	$F_{1, 52}$ = 0.983	$F_{8, 26}$ = 0.9912	$F_{2, 126}$ = 11.296*	$F_{2, 126}$ = 11.754*	$F_{2, 130}$ = 8.137*	$F_{2, 130}$ = 1.298
0%	$F_{1, 52}$ = 0.014	$F_{8, 26}$ = 0.3071	$F_{2, 126}$ = 0.245	$F_{2, 126}$ = 0.024	$F_{2, 130}$ = 0.095	$F_{2, 130}$ = 0.023
25%	$F_{1, 52}$ = 0.009	$F_{8, 26}$ = 2.084	$F_{2, 126}$ = 13.325*	$F_{2, 126}$ = 1.511	$F_{2, 130}$ = 0.014	$F_{2, 130}$ = 0.482
50%	$F_{1, 52}$ = 0.266	$F_{8, 26}$ = 0.053	$F_{2, 126}$ = 1.988	$F_{2, 126}$ = 3.042	$F_{2, 130}$ = 2.835	$F_{2, 130}$ = 0.0197
75%	$F_{1, 52}$ = 2.591	$F_{8, 26}$ = 0.027	$F_{2, 126}$ = 2.871	$F_{2, 126}$ = 2.674	$F_{2, 130}$ = 3.274	$F_{2, 130}$ = 0.011
100%	$F_{1, 52}$ = 0.082	$F_{8, 26}$ = 14.300*	$F_{2, 126}$ = 0.032	$F_{2, 126}$ = 12.196*	$F_{2, 130}$ = 0.015	$F_{2, 130}$ = 2.984

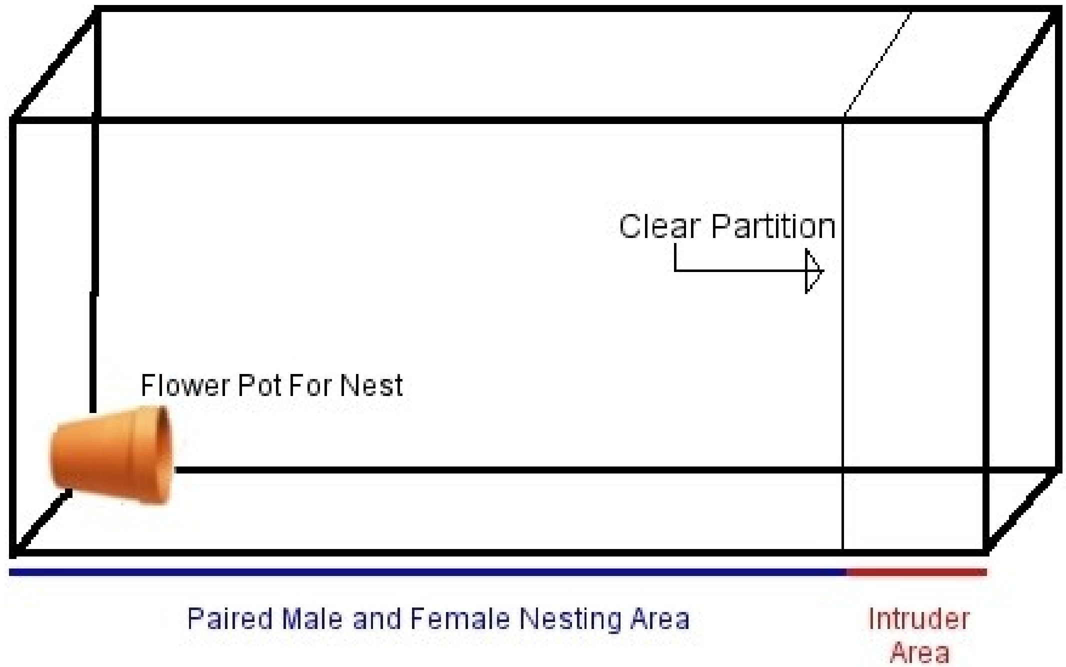

Figure 1:

A diagram of the experimental tank during initial setup for mating. Not drawn to scale.

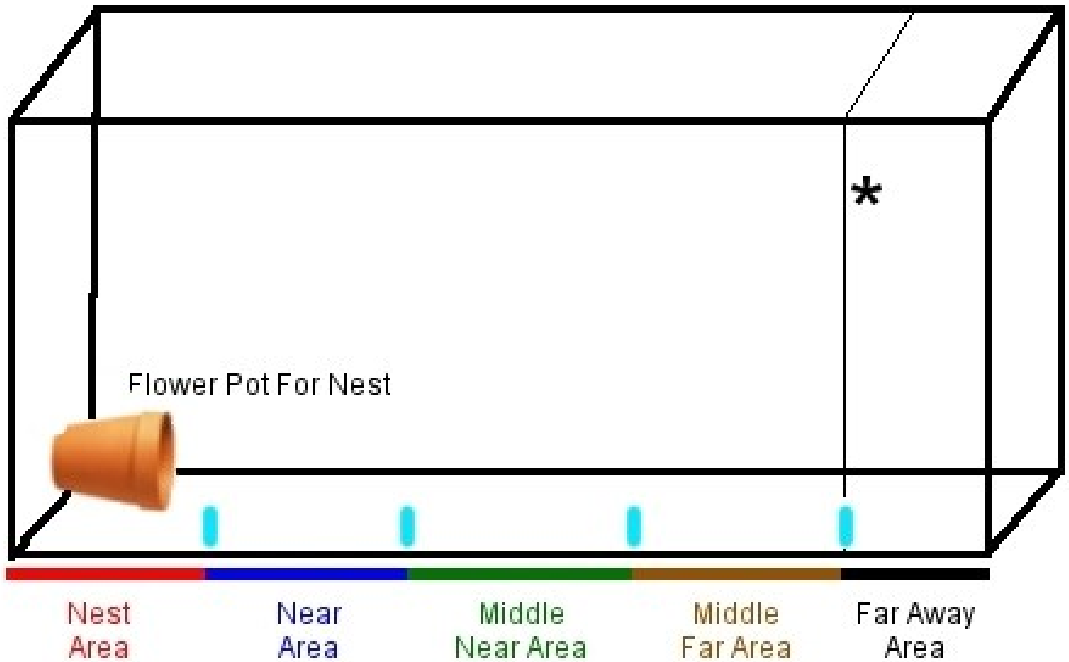

Figure 2:

A diagram of the experimental tank during testing. The small light blue dividing lines between the sections mark where the tape was placed at the bottom of the front of the tank externally to visually divide the tank into five sections. The dividing line between the middle far and far away sections (marked with '*') is where the opaque partition was temporarily placed during the setup of the testing. Not drawn to scale.

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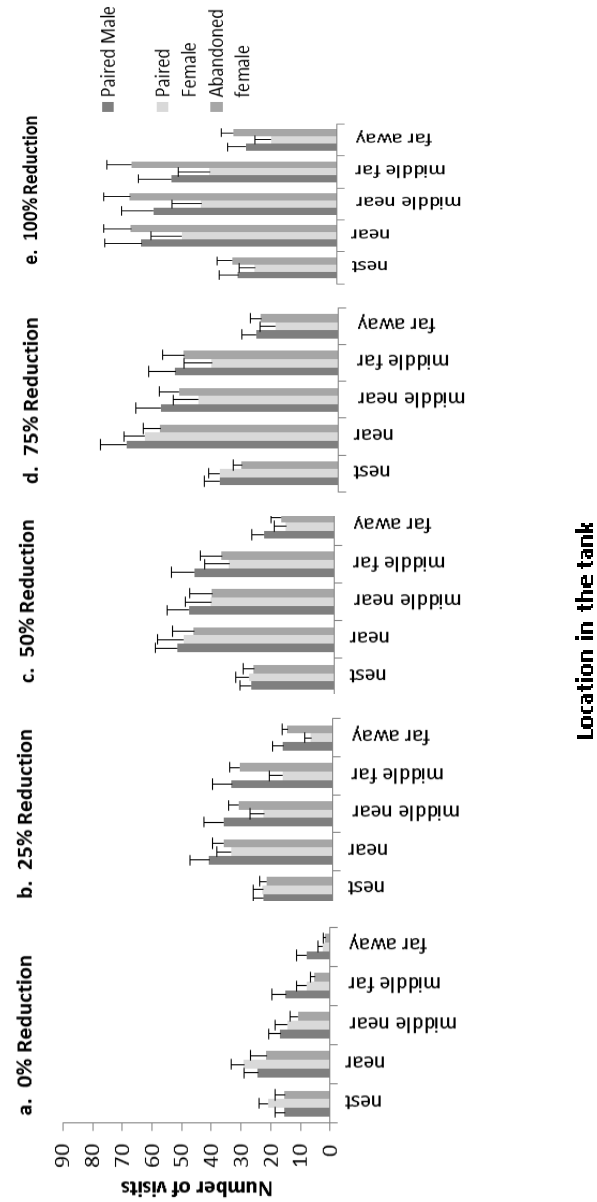

Figure 3

The number of visits made to each area of the tank by the paired males, paired females, and abandoned females for each brood reduction treatment.

$F_{(40, 206.6)} = 0.70908$, $p = 0.90223$ Vertical bars represent standard error bars.

F and p values represent an ANOVA looking at each level of brood reduction, area of the nest, and sex and status.

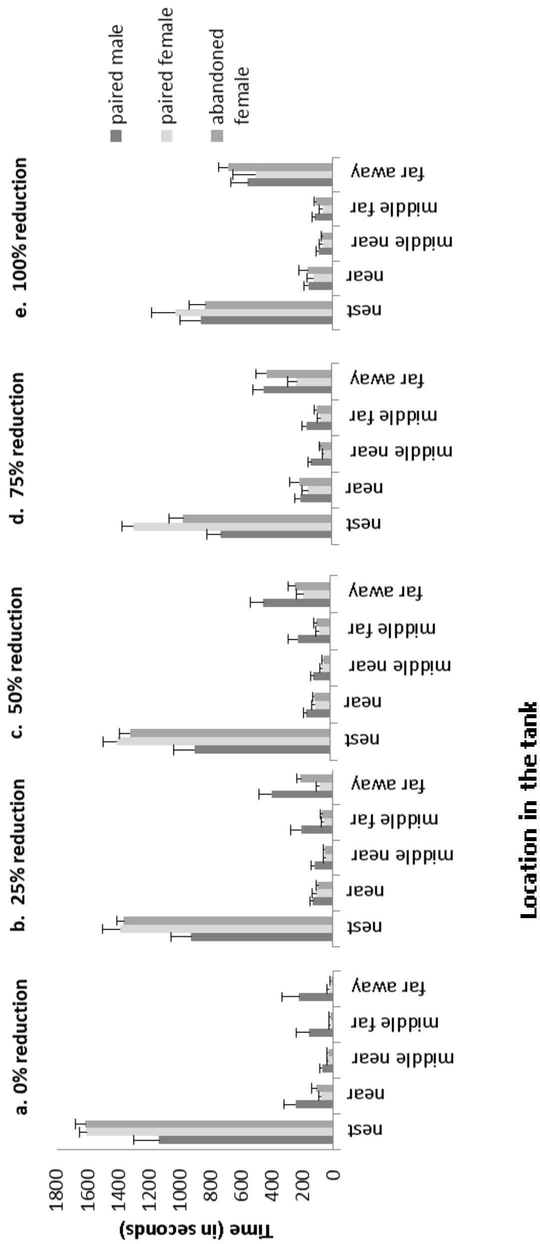

Figure 4.

The total duration of all visits made to each area of the tank by the paired males, paired females, and abandoned females for each brood reduction treatment.

$F_{(40,\ 206.6)}$ = 1.1479, p = 0.26534 Vertical bars represent standard error bars.

F and p values represent an ANOVA looking at each level of brood reduction, area of the nest, and sex and status

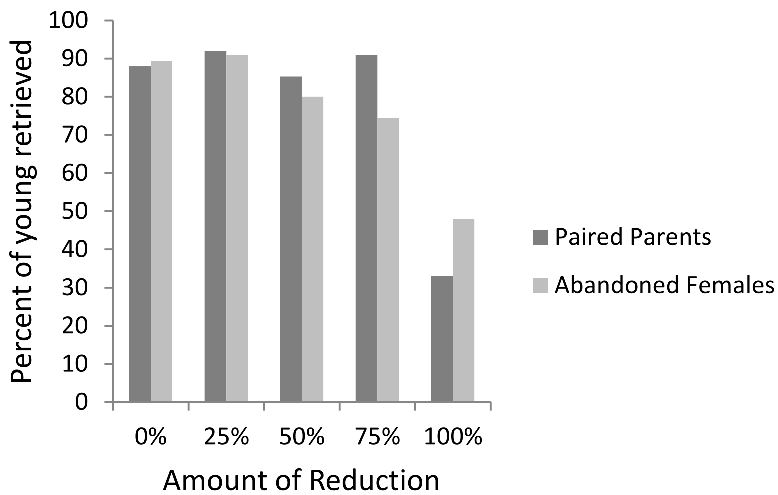

Figure 5.

The percentage of young retrieved by the paired parents and abandoned females for each brood reduction treatment.

$F_{(4,40)}$ = 1.0694, p = 0.38435 Vertical bars represent standard error bars.

F and p values represent an ANOVA looking at each level of brood reduction and paired status.

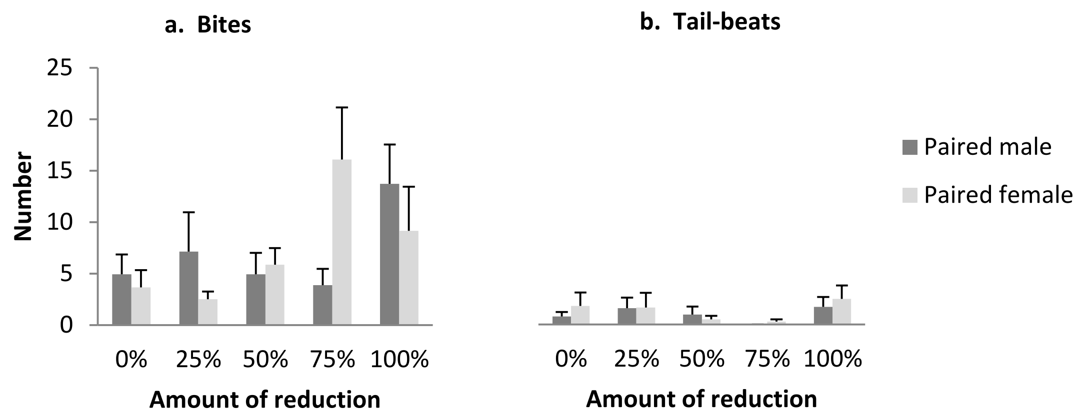

Figure 6.

The amount of aggression (as measured by bites and tail-beats inflicted upon a mate) displayed by the paired males and paired females for each brood reduction treatment.

Vertical bars represent standard error bars.

a. Bites: $F_{(4, 63)} = 4.094$, $p = 0.005$

b. Tail beats: $F_{(4, 63)} = 1.0758$, $p = 0.376$

F and p values represent an ANOVA looking at each level of brood reduction, type of aggression and sex.

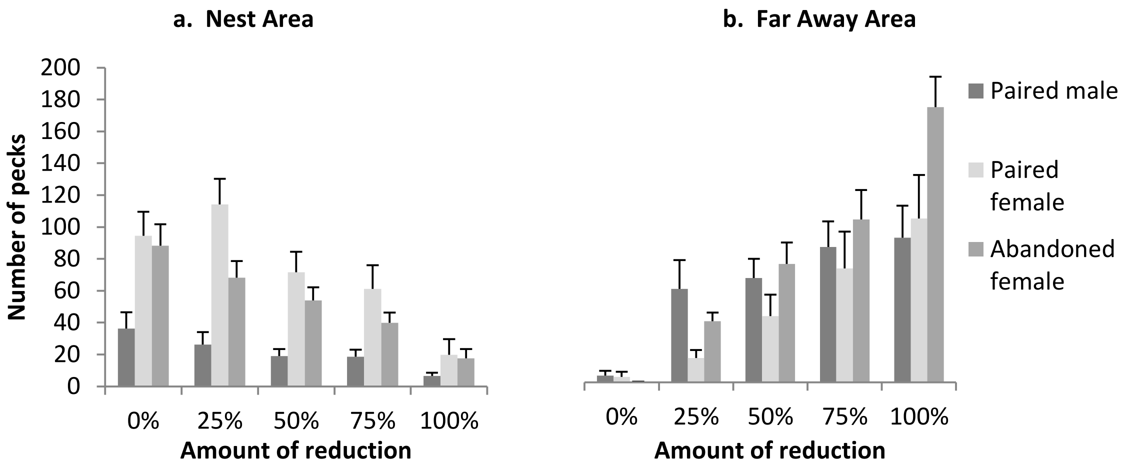

Figure 7

The number of pecks made to the substrate in the nest and far away areas of the tank by the paired males, paired females and abandoned females for each brood reduction treatment.

Vertical bars represent standard error bars.

a. Nest Area: $F_{(8, 126)} = 2.5129$, $p = 0.0144$

b. Far Away Area: $F_{(8, 126)} = 2.2321$, $p = 0.02918$

F and p values represent an ANOVA looking at each level of brood reduction, area of the nest, and sex and status.

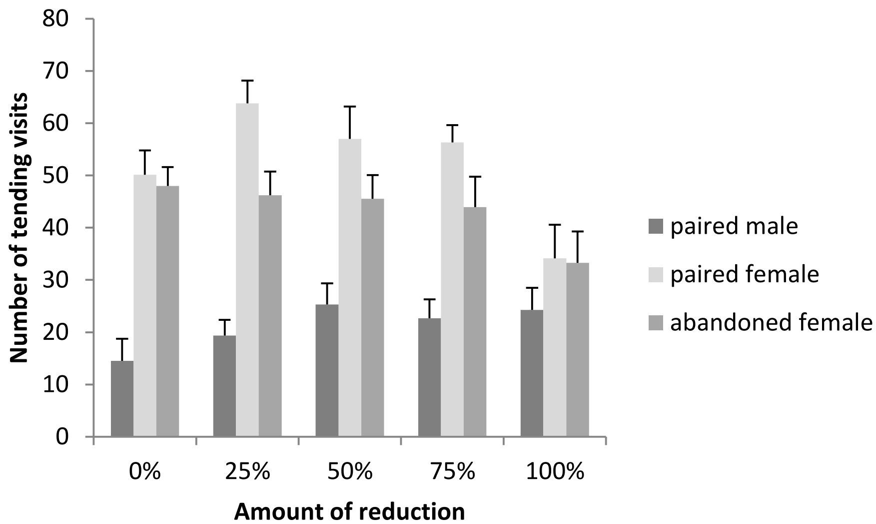

Figure 8.

The number of tending visits made to the nest by the paired males, paired females, and abandoned females for each brood reduction treatment.

$F_{(8, 126)} = 1.9472$, $p = 0.05855$ Vertical bars represent standard error bars.

F and p values represent an ANOVA looking at each level of brood reduction, and sex and status.

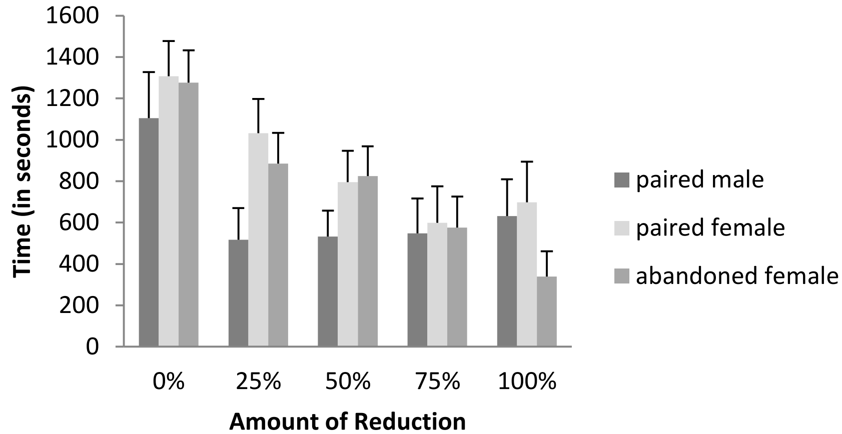

Figure 9.

The latency (amount of time passed until the far away area was searched) for the paired males, paired females, and abandoned females for each brood reduction treatment.

$F_{(8,126)}$ = 0.95563, p = 0.47382 Vertical bars represent standard error bars.

F and p values represent an ANOVA looking at each level of brood reduction, and sex and status.