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### **Ménage à Trois in the Atlantic Brief Squid (*Lolliguncula brevis*): Prior Presence Affects Mate Choice**

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(*LOLLIGUNCULA BREVIS*)

Ménage à Trois in the Atlantic Brief Squid (*Lolliguncula brevis*):

Prior Presence Affects Mate Choice

By Rachel A. Schlessinger

Submitted in partial fulfillment  
of the requirements for the degree of  
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May 6<sup>th</sup>, 2016

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Date

Peter Moller, Ph.D

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Signature of First Reader

May 6<sup>th</sup>, 2016

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Date

Karin Akre, Ph.D

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Signature of Second Reader

**Abstract**

*Lolliguncula brevis* given prior presence experienced a significantly greater number of contacts with opposite sex squid than rivals. Males given prior presence also spent significantly more time in proximity to females than rivals. This suggests that for both female and male Atlantic brief squid, having prior presence influences mating behavior.

*Key words:* prior presence, acclimation time, mate preference, sexual dimorphism, *Lolliguncula brevis*

### **Acknowledgements**

I would like to start by thanking Dr. Karin Akre for getting me started on this project and sharing the data from her work on *Lolliguncula brevis* with me. Without her support, I would not have been able to begin working with cephalopods, and her expert guidance throughout this project has been essential to the completion of this thesis. I would also like to thank the faculty at Hunter College who have been my dedicated teachers over the past years. Special thanks to Dr. Martin Chodorow for always going above and beyond to help my fellow students and me. Lastly, this project would not have been possible without the help of my thesis advisor, Dr. Peter Moller. I am beyond grateful for the countless hours and sound advice he graciously offered throughout this project. His patience and wisdom has been invaluable to me in the completion of this study.

Ménage à Trois in the Atlantic Brief Squid (*Lolliguncula brevis*):

Prior Presence Affects Mate Preference

The Atlantic brief squid, *Lolliguncula brevis*, is a small species of squid that lives in turbid waters near the coast of the Western Atlantic Ocean (Hanlon, Hixon, & Huley, 1983). These squid have “short rounded bodies, large rounded fins, third arms with heavy keels, and often reside in shallow, complex, temporally variable environments,” (Bartol, Patterson, & Mann, p. 3656). They are adept at maneuvering in their environment and can swim in either an arms-first (typically used when swimming slowly or hunting prey) or a tail-first orientation (used when swimming at higher speeds). The Atlantic brief squid, however, is generally a slower moving squid than other species (Bartol, Patterson, & Mann). Like other species of the Order Teuthida, *L. brevis* are “social, schooling, inquisitive, creatures that actively react to everything in their environment” (Hanlon, Hixon, & Huey, 1983, p. 658).

The vast majority of cephalopod species, including the Atlantic brief squid, are short lived, generally taking one to two years to complete their life cycle, and have semelparous reproduction. The known exceptions are species that live in cold, polar waters, and larger species like the giant squid or giant octopus (Hanlon & Messenger, 1996). Like most cephalopod species, the Atlantic brief squid appears to be promiscuous and no clear mate preference has been observed. Hanlon & Messenger (1996) observed that *L. brevis* “has a spermatophoric ‘pad’ located on the inner mantle (near the left gill) where males attach spermatophores during copulation” (p.95). Dr. Akre noted that some females actually have two spermatophoric pads, one on each side, and that while *L. brevis* usually mates in the parallel position, they have occasionally been observed

mating in the head to head position as well. Female *L. brevis* die soon after mating, but males of the species do not appear to show the same deterioration post-mating that females do (Akre, Ryan, & Johnsen, under review). In addition, they are the “only known cephalopod to typically inhabit low-salinity estuaries,” (Bartol, Patterson & Mann, p. 3656). Because of this, *L. brevis* is a useful species for laboratory experiments, as it is able to tolerate wide fluctuations in temperature and salinity. It also has a high survival rate under proper captive conditions. Furthermore, due to the turbidity of the waters it is found in, *L. brevis*, is difficult to study in situ (Hanlon, Hixon, & Huley, 1983) and therefore may be better observed in a laboratory setting.

*L. brevis* forms schools and while minimal research has been conducted on squid schooling behavior, a lot of research has been done on the schooling behavior of fish (Sugimoto and Ikeda, 2012). When fish form schools it is primarily as a defense against predators; however, there are drawbacks to being a member of a school, such as decreased resources for individuals (Griffiths & Magurran, 1998). There is evidence that schooling fish can recognize conspecifics and are choosy about which individuals they associate with, preferring to school with familiar conspecifics (Griffiths, 2003). “There is a considerable body of evidence to show that many fish species recognize and preferentially associate with familiar schoolmates or neighboring territory holders,” (p. 257). There is some question as to how true this research holds in a wild setting, since in the wild, the number of conspecifics is much greater and the environment is more complex. There may also be a difference between the sexes when it comes to schooling behavior. For example, in the Trinidadian guppy, females form the core of schools while males trade off between the safety provided by schooling and the increased opportunities

for mating when not part of a school (Griffiths & Magurran, 1998). Further research is needed on the schooling behavior of squid, but it is likely that similar pressures influence schooling cephalopods and that these highly intelligent animals may be able to recognize familiar individuals and behave differently around them.

As Hanlon & Messenger (1996) point out, “all evidence to date suggests that cephalopods rely on mechanoreceptors, chemoreceptors, and photoreceptors to guide their lives” (p. 12). Cephalopods are unique, however, in their use of chromatophores to create visual displays and signaling components. Like other species of cephalopods, *L. brevis* is able to exert neuromuscular control over its chromatophores and does so, among other things, as a method of intraspecific signaling. Cephalopods rely on “postural, locomotor, textural and above all, chromatic signals to communicate with conspecifics and others” (p. 124). That being said, partly due to the intelligent nature of cephalopods, there is a great diversity of intra and interspecific behaviors in squid species (Hanlon 1996). Akre et al. (under review) studied the function of signaling in *L. brevis* and found that females make use of elaborate and redundant signaling during courtship, likely as a way to express receptivity to male sexual contact. It is also plausible that this redundancy is useful since individual components may not always be visible to males that approach females from multiple angles. Redundant signaling may help males track and stay close to receptive females during a highly mobile courtship. This species is sexually dimorphic and females are significantly larger than males. Akre et al. (under review) found no evidence suggesting that female signaling functions to lower risk of size-based sexual cannibalism, despite observations by Dr. Akre that sized based cannibalism did occur in captivity. Piperno (2015), studied male signal components in *L. brevis* and found that

while there are four signals that males make only in the presence of females, “the proportion of time spent signaling did not correlate with sexual contact” (p.23). She found that females signal less during male signaling, suggesting that females are aware of male signaling and that females are closer to males during male signaling than before or after. Further studies on the signaling behavior of the Atlantic brief squid are needed in order to better understand communication between these animals. These sexually specific signaling displays may be just one aspect of the mating behavior of this species. More research is needed about the sexual selection and mate preference in *L. brevis*.

Sexual selection was first proposed by Darwin in *The Descent of Man* (1871) to refer to the advantage some individuals have over others of the same sex for access to reproductive opportunities (Clutton-Brock, 2007). According to Andersson & Iwasa (1996), “competition over mates is the unifying aspect of all forms of sexual selection, including that occurring by mate choice, where individuals compete to be chosen by the other sex” (p.53). Generalizations about sexual selection include the idea that males compete for access to females and females choose which males they mate with. This idea stems from the fact that the investment in gametes and parental care (when applicable) is almost always higher in females than in males. Therefore males will mate as often as possible and females will be choosier about who they invest their reproductive resources on. This leads to biased operational sex ratios (OSRs), in which the number of mates available to one of the sexes, is higher than the number of mates available to the other. This bias in turn creates greater competition within one of the sexes and can lead to intense competition for mates, as well as ornate secondary sexual characteristics, usually in males. Less well studied, however, are the instances of elaborate female ornamentation



and intrasexual competition for mates among females (Clutton-Brock, 2007). Female Atlantic brief squid are significantly larger than males. This sexual dimorphism could be related to sexual selection in this species. Since signaling can be considered a form of ornamentation and female *L. brevis* have been observed to have more elaborate signaling than males (Akre et al., under review), the question remains as to whether females of this species are in competition with each other over mates. If so, what would make a female of this species more attractive to her potential mate? What features or behaviors of the Atlantic brief squid, both female and male, make them attractive to the opposite sex? Which sex does the choosing? Is there mutual mate selection? All of these questions require further research.

In many species of squid, females readily mate with multiple males present and do not appear to exert any sort of obvious preference (Hanlon, 1998; Shashar & Hanlon, 2013). Males take on a variety of reproductive strategies, which may change depending on the presence and size of other males in the area. Large males may interact in aggressive contests and small males may employ sneakier tactics to gain access to females (Hanlon, 1996). Sperm competition also plays a role in the reproductive strategies of many closely related squid species. Although a female may mate readily with more than one male, the sperm may be stored in different ways and may in itself have differential access to the unfertilized ova. Features of sperm competition may include, “a large testis that produce large quantities of sperm; sperm packaged in spermatophores; sperm stored by females; appropriate morphologies of the oviduct and spermatheca; polygamous mating systems; multiple styles of mating; delays between mating and egg laying; and intense mate guarding. In squids, many matings occur over

many months, and it is possible that sperm from the most recent matings might displace that from earlier matings,” (Hanlon, 1998, p. 93).

In a promiscuous species of squid, like *L. brevis*, it may be advantageous for males to be choosy when it comes to selecting a mate. Mating for male cephalopods is no guarantee of paternity since females readily mate with multiple males and can store spermatophores from many partners. Multiple paternity for clutches of eggs is very likely. Mating can be costly for both male and female cephalopods. Males risk sexual cannibalism in species, like the Atlantic brief squid, in which females are much larger than males. There are other costs to mating besides the well-studied phenomena of greater investment in gametes and parental care by females. Mating cuts back on foraging opportunities. Copulating pairs are often more vulnerable to predation, and in some species there may be a serious energetic cost to mating. The dumpling squid, *Euprymna tasmanica*, for example, mates for three hours, during which time the male actively restrains the female. The result of the activity on the part of the male and the lessened circulation on the part of the female, leads to a serious loss in energy post-copulation for both males and females (Franklin, Squires, & Stuart-Fox, 2012). Recovery can take up to thirty minutes in this species. Selecting the proper mate when the cost of mating is high should presumably be a priority for both males and females.

In at least one documented case of cephalopods, males appear to be choosy in their mate selection as a way to conserve resources. In the cuttlefish species, *Sepiadarium austrinum*, females regularly consume spermatophores from males. This can be costly to males. Since smaller females of this species consume more spermatophores than larger females, males appear to mate more with smaller females, however their investment of

spermatophores is not influenced by female size (Wegener, et al. 2013). Males of this species also “mated for longer and were more likely to transfer spermatophores to egg-carrying females” (p. 668). This likely increases the chance that their sperm will fertilize the female’s eggs rather than be rejected or eaten. This demonstrates that mating behavior of the males of this species is influenced by certain female characteristics.

Being the final male to mate with a female before she lays her eggs may greatly increase a male’s chance of paternity. This has been proven to be the case for many promiscuous taxa, and has been especially well studied in many insect species. Possible reasons for the advantage of being the final male to mate with a female include sperm displacement, sperm loss, and removal of sperm by rival males (Squires, et al. 2015). Because of this, mate guarding by males post-copulation is often seen in these promiscuous species. In the dumpling squid, *Euprymna tasmanica*, fertilization rates for males that mated last were significantly higher, especially for the initial eggs laid by females. In this species, it may be advantageous for males to mate with females that are close to laying their eggs.

Females of many species of squid may also exert post-copulatory choice (Hanlon & Messenger, 1996). Female squid may do this through various methods. They may choose to lay their eggs shortly following mating with a desirable male. They may choose to mate in different positions with different males, resulting in different sperm storage locations. Additionally, females may choose to consume spermatophores, rather than use them for fertilization. In all of these circumstances, females may exert control over which sperm will fertilize what proportion of their eggs. Because of this, it may be advantageous to females to mate with any available male. Not only does this give females

some control over the fertilization of her eggs, multiple paternity would increase the genetic diversity and therefore the potential adaptability of a female's offspring (Squires, et al. 2015). In a polyandrous species, it may be beneficial for males to be choosier in their mates than females and for females to exert choice post-copulation.

There are ways in which cephalopod species, despite their promiscuity, conform to classic notions of sexual selection. Rank among males has been observed in some species of squid, such as *Doryteuthis pealeii* (Shashar & Hanlon, 2013). This dominance between males is usually associated with size, with larger males being dominant over smaller ones. There are exceptions to this, however. Sato, Kasugai, & Munehara (2014), found that female Japanese pygmy squid appear to have a preference for smaller males and shorter copulations. They discovered that females may exert female choice in this species post-copulation by removing spermatangia from less desirable males. Females of this species were more likely to remove spermatangia from larger males and when copulation was longer. Perhaps females are choosing small males for their increased agility, and perhaps longer mating exposes the pair to greater risk of predation. It is also possible that larger males are giving extra spermatangia as a nuptial gift to the females in order to increase female fitness and reproductive success.

Prior access to and greater time spent in an area, as compared to a rival, is one factor that can determine dominance in certain species. In species that establish territories, "prior residents may be most knowledgeable of a territory's value and can subsequently exploit those resources with lower future investment than newcomers, so that they are more prepared to invest energy in territorial contests than new arrivals... Residents may also have a reduced vulnerability to predators,... a greater awareness of

established boundaries with neighbours,... and an increased investment in reproductive success” (Harwood, et al., 2002). Many animals from a wide variety of taxa including fish (Johnsson & Forser, 2002; Heuts & Nijman 1998; Nijman & Heuts 2000; Figler, Chaves, & Wazlavek, 1998; and Bronstein & Hirsch, 1985), birds (Beauchamp, 2000 and Dearborn & Wiley 1993), and echinoderms (Morishita et al., 2009), are known to establish dominance through prior-residency. There has been little to no research into whether prior residency plays a role in mate preference in cephalopods but it is unlikely that *L. brevis* forms any type of residency. This species forms schools and individuals do not establish territories. However, it is possible that while residency is not being formed, the amount of time an individual has been in an area could play a role in dominance and mating dynamics. For the purposes of this paper, extra time an individual spends in an area as compared to a same-sex rival will be referred as *prior presence* (term coined by Dr. Akre). Cephalopods are known for their intelligence and having prior presence in an area may help an individual squid have greater knowledge of that area, in a similar way that residents gain knowledge of their homes. This greater knowledge could be correlated with mating preference, as it appears to be in some species that establish residencies. Because *L. brevis* is a schooling species that lives in turbid waters, prior presence in an area is very difficult to study in the wild. The combination of unclear waters and the numbers of squid present at any given time would make it difficult to assess whether preference was given to individuals that had prior presence in a wild setting.

The current study makes use of an artificial setting in order to determine if there is any correlation between the amount of time an individual squid has to acclimate to an enclosure (prior presence) and the amount of time that individual spends in proximity to

an opposite sex individual, as well as the number of contacts made between opposite sex individuals. If a preference is found, this could be reflective of several factors. Females may prefer to mate with more established or acclimated males. Extra time spent in an area may reflect rank between males for access to females. Males may choose females that have been in an area longer, under the assumption that the female is getting closer to laying her eggs. Perhaps the longer a female stays in an area or the more acclimated she becomes to it, the closer she gets to laying a clutch of eggs. Females may communicate this to males either through visual, tactile, or possibly even olfactory cues (Hanlon, 1996). If the last male to mate with a female is more likely to fertilize a greater number of her eggs than previous males, the length of time a female has spent in a given area may be of great interest to males.

In order to begin to address these points, this study gave pairs of same-sex squid a different amount of time to acclimate to an enclosure and then measured two aspects of behavior: the number of contacts that each squid had with an opposite sex individual and the amount of time each spent in proximity to an opposite sex individual. These questions were examined in relation to both female and male pairs of squid. Given the findings of Akre, et al. (under review) that females have more elaborate ornamentation in signaling than males, it seems appropriate to ask if males show preference during mating. Are males of the species *L. brevis* indiscriminate in their choice of mate as classic notions of sexual selection would predict? Or do males exhibit preferences for certain females when selecting mates? Do males and females respond to the same characteristics or is there differentiation in the choices made by males and females? Does mutual mate selection play a role or is one sex choosier than the other?

This project used video footage captured by Dr. Akre to try to examine these questions in relation to *L. brevis*. Two treatment types were analyzed. In the first type of treatment, focal female treatments (FFTs), a focal female was introduced to an observation pool and allowed to acclimate for at least two minutes; subsequently a non-focal female was introduced into the tank and given at least two minutes to acclimate. Finally a male was introduced to the tank. After the addition of the third animal, video footage was recorded of the behavior of the squid for at least five minutes. In focal male treatments (FMTs), the same procedure was followed except the sexes were reversed. First a focal male was introduced, followed by a non-focal male, and lastly a female.

This projects attempts to explore whether having additional acclimation time in the observation tank changes the behavior of the squid. The hypothesis was that having exclusive access to the observation tank prior to the introduction of other animals will make the focal females more attractive to males and will make the focal males dominant over non-focal males for access to females. In order to test these hypotheses, video footage was examined in order to answer four different questions. 1) Do focal females receive a greater number of contacts from males than non-focal females? 2) Do focal males make a greater number of contacts to females than non-focal males? 3) Do focal squid (both female and male) spend a greater amount of time in proximity (within 1.5 body lengths) to opposite sex individuals than non-focal squid? 4) Are focal squid (both female and male) generally located in closer relative proximity to opposite sex individuals than non-focal squid? If a preference is shown by either females or males towards squid that have been given prior presence, this information could be valuable in

aquaculture, zoos, aquaria, or any other institution attempting to maintain or breed squid in captivity.

## Method

### Subjects

Subjects were 18 mature female and 20 mature male Atlantic brief squid, *Lolliguncula brevis*. Details of the capture and care of the squid are stated below (permission by Akre et al., under review).

We studied wild squid from the bays surrounding Port Aransas, TX during spring and summer (2007-2009). Squid were captured using seine nets or were donated by educational groups that used short duration trawling, and transferred immediately to cool, oxygenated seawater for transport to the University of Texas at Austin's Marine Science Institute. Laboratory holding tanks contained cylindrical tanks of 1.8m diameter filled to 40cm with filtered seawater. Temperatures varied with seasonal changes in flow-through seawater, but were slightly cooled due to building AC. We fed squid a natural diet of fish and shrimp three times daily.

All squid were observed exhibiting mating behavior in the holding tank prior to inclusion in the treatments and were therefore known to be of reproductive age.

### Apparatus

Videos were recorded using either a Sony Handycam Digital 8 or a Canon 2R 950. Videos were digitized using iMovie on a 13" 2012 MacBook Air. This same computer was used for analyzing relative squid distances during treatments.

### Procedure

Squid were observed in one of two treatment types. The focal female treatments (FFTs) contained one focal female (FF) that was introduced to the enclosure first, followed by a non-focal female (NFF), that was introduced at least 2 minutes later, and a



male squid (M) that was introduced at least 2 minutes after the NFF. Focal male treatments (FMTs) contained a focal male (FM) that was introduced to the enclosure first, followed by a non-focal male (NFM), that was introduced at least 2 minutes later, and a female squid (F) that was introduced at least 2 minutes after the NFM. Table 1 below lists the abbreviations used in this study. Details of the filming are given below, with permission of Akre et al. (under review).

Only individuals we observed mating in the holding tank were used in the tests, to assure correct sex identification, reproductive maturity, and receptivity. For each test the individual was placed in a testing tank (1.4m diameter, 40cm depth)...Focal individuals spent at least 2min alone in the tank prior to stimulus introduction. In condition SOS, we first introduced the same-sex squid, and at least 2min later introduced the opposite-sex squid. Curtains surrounding the tank blocked the squids' view of observers and cameras. Each trial was videotaped for 5min starting at the point of complete stimulus introduction. One video recorder was fixed overhead with a view of the entire tank. Another was hand held and followed only the focal individual. Stimulus presentation order was randomized, and each squid completed no more than one animal stimulus trial per day.

Table 1  
A list of abbreviations used in this study

<b>Abbreviation</b>	<b>Meaning</b>
<b>FFTs</b>	Focal Female Treatments
<b>FMTs</b>	Focal Male Treatments
<b>FF</b>	Focal Females (in FFTs only)
<b>NFF</b>	Non-Focal Females (in FFTs only)
<b>M</b>	Males (in FFTs only)
<b>FM</b>	Focal Males (in FMTs only)
<b>NFM</b>	Non-Focal Males (in FMTs only)
<b>F</b>	Females (in FMTs only)

### **Analysis**

Nine FFTs and ten FMTs were analyzed in total. The treatments were analyzed in two ways. First an ethogram of behaviors was recorded for each squid in all trials. The full ethogram included 17 distinct behaviors, divided into 4 main categories: swim,

proximity, contact, and other. With very little exception, the squid were always observed to be in motion. As Bartol, Patterson, & Mann (2001, p. 3670) point out, “as is the case with many squid species, *L. brevis* is negatively buoyant and consequently must generate lift to maintain position in the water column, an energetic expense that is not trivial at low speeds”. The full ethogram is presented in appendix 1 and complete data for all behavioral events and times for all trials is presented in appendices 2-13. The behaviors of interest for this study are shown in tables 2 and 3 below.

Table 2

The ethogram of the behaviors of interest under the category of proximity

<b>Behavior:</b>	<b>Description</b>
<b>Proximity</b>	
Opposite Sex	Individual is within 1.5 body lengths of a squid of the opposite sex
Same Sex	Individual is within 1.5 body lengths of a squid of the same sex
All Three	All three squid are within 1.5 body lengths of each other

Table 3

The ethogram of the behaviors of interest under the category of contact

<b>Behavior: Contact</b>	<b>Description</b>
To Opposite Sex	Individual initiates contact with a squid of the opposite sex
To Same Sex	Individual initiates contact with a squid of the same sex
From Opposite Sex	Individual is contacted by a squid of the opposite sex
From Same Sex	Individual is contacted by a squid of the same sex

All observed behaviors were recorded as events. Additionally, if a behavior lasted for longer than 3 seconds, the duration of the event was also recorded.

After videos were analyzed for behaviors, a second analysis was done to measure the relative distances of the squid from each other. Two of the FMT videos were unusable for this aspect of the analysis as the non-focal or opposite sex squid was off camera for too large of a proportion of the time to collect any real data. For this reason, only eight FMT videos were analyzed for relative closeness. Videos were viewed in full screen

mode and paused every 30 seconds. Using a standard ruler, a measurement of the distance between each of the individual squid was recorded in cm. If all three squid were not on screen at the given interval, a measurement was taken at the closest time to that interval in which all three individuals were seen on camera. Due to angle of the camera, issues of parallax made it impossible to obtain an absolute distance between any of the individual squid. Because of this, the initial measurements taken in cm were coded and converted into numbers 1-6 to reflect the relative distances of the squids. The distance between the three squid at any point in time can be represented by the sides of a triangle, with the distance between the closest pair represented by the shortest side of the triangle, the distance between the farthest pair represented by the longest side of the triangle, and the distance between the remaining pair represented by the third, middle length, side of the triangle. A description of the coded distances is shown in Table 4 below.

Table 4

The details of the coding system used to turn numerical distance between squid into relative distance. This was necessary due to issues of parallax caused by the angle of view from the recording device.

<b>Coded Number</b>	<b>Corresponding Distance</b>
1	The closest pair are less than 4cm apart
2	The closest pair are 4 or more cm apart
3	The middle pair are less than 4cm apart
4	The middle pair are 4 or more cm apart
5	The farthest pair are less than 4cm apart
6	The farthest pair are 4 or more cm apart

If the distance between 2 pairs was the same, an average of the two coded numbers was taken. For example, if the closest pair was 1cm apart and the next 2 pairs were both 4cm apart, the coded distances would be 1, 5, & 5 respectively. The two number “5”s represent  $(4+6)/2$ .

### **Statistics**

The hypothesis of this study was that focal individuals would have a greater number of contacts with opposite sex individuals than would non-focal individuals. Additionally, focal individuals would spend a greater amount of time in proximity to opposite sex individuals than would non-focal individuals. Because of this, dependent, one-tailed *t*-tests of all results were calculated using Vassar's online *t*-test calculator (<http://vassarstats.net/tu.html>). A standard *p* value of 0.05 was used to measure significance. There were a fixed and limited number of trials available for analysis in this project and it is possible that these small numbers may affect the results of any statistical analysis.

### **Camera Position**

It is important to note a few things about this study in relation to video analysis. The video footage captured by Dr. Akre was intended to study signaling behavior and not the effect of prior presence. For this reason, the primary video footage used for this project was from a camera positioned above the enclosure that left certain parts of the tank off camera. Additional hand held footage was used to supplement the fixed view footage but this camera followed the focal individual only. As such, the focal animal is the only individual whose behavior was guaranteed to be captured for the full five minutes of every trial. It is possible that this difference in time spent off camera had an effect on the results of this study. More information about time spent off camera is in appendices 14 & 15. Also, due to the position of the camera, it was impossible to

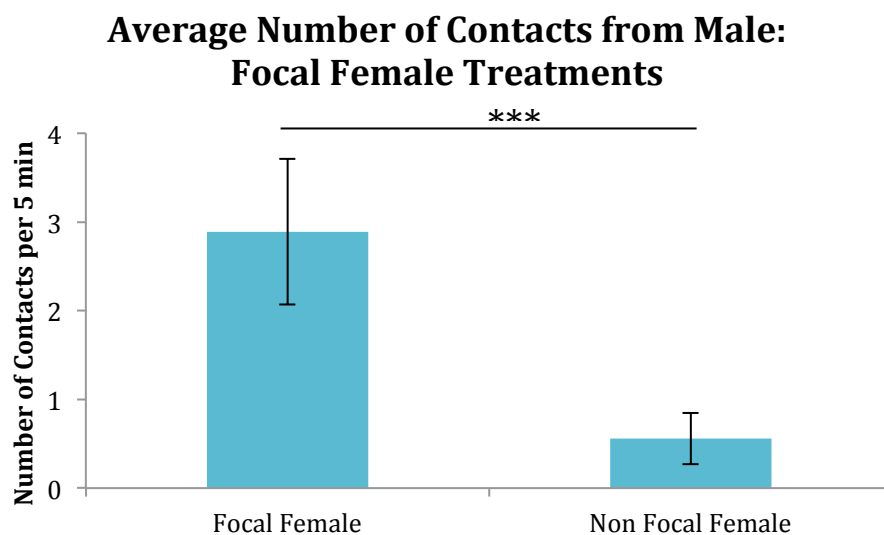
accurately ascertain relative size differences among same-sex squid. It is possible that this, in addition to prior presence, influenced the behavior of the animals in this study.

## Results

### Number of Contacts

In all of the treatments observed, the males always initiated contact between opposite sex squid. In only one treatment did a female make contact with another squid and it was a non-focal female that made contact with a focal female. Therefore, it appears that contact between animals is almost always male initiated in this species. In the treatments with a focal female, a non-focal female, and a male (focal female treatment, FFTs), there was a significantly greater number of contacts made by the male to the focal female than to the non-focal female,  $t(8) = 4.04$ ,  $p = 0.0019$ , one tailed (see Fig. 1).

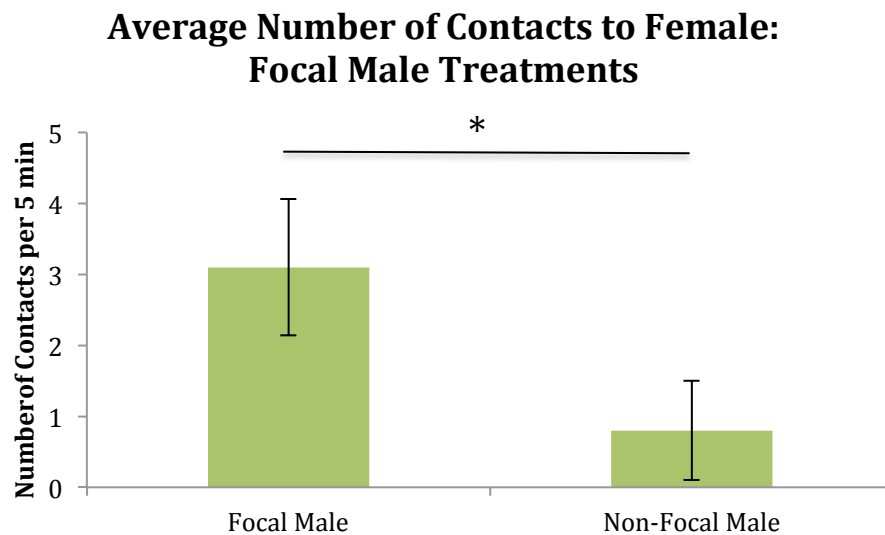
Figure 1  
The average number of contacts ( $\pm$  SEM) made by the male to the females in focal female treatments.



Note: \*\*\* denotes that significantly more contacts were made to the focal female,  $t(8) = 4.04$ ,  $p = 0.0019$ .

In the treatments with a focal male, a non-focal male, and a female (focal male treatments, FMTs), there were a significantly greater number of contacts made by the focal male to the female than by the non-focal male to the female,  $t(9) = 1.99$ ,  $p = 0.0389$ , one tailed (see Fig. 2).

Figure 2  
The average number of contacts ( $\pm SEM$ ) to the female by the males in focal male treatments.

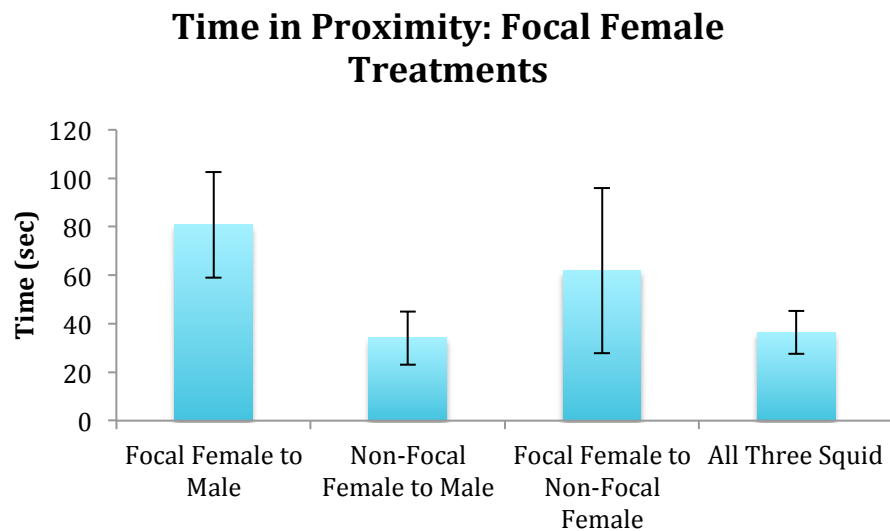


Note: \* denotes that focal males made significantly more contact with females than non-focal males,  $t(9) = 1.99$ ,  $p = 0.0389$ .

### Proximity

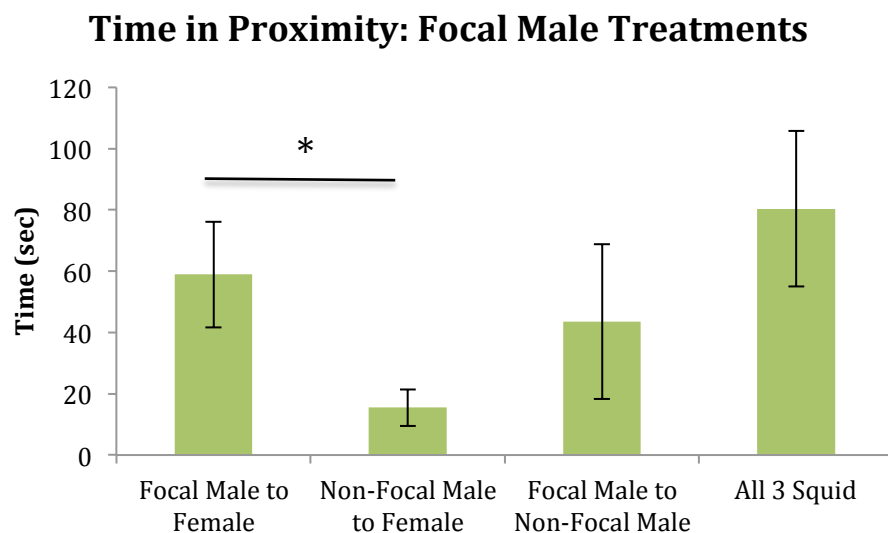
Proximity was measured in two different ways. The first was simply a measure of how much time each squid spent within one and a half body lengths of the other squid in the treatment. In the FFTs, while the focal females spent a greater amount of time in proximity to the males than the non-focal females spent in proximity to the males, it was just out of the range of significance,  $t(8) = 1.74$ ,  $p = 0.0600$ , one tailed (see Fig. 3).

Figure 3  
The time spent within 1.5 body lengths of other squid ( $\pm SEM$ ) in focal female treatments.



Note: The focal females did not spend significantly more time in proximity to the male than the non-focal females,  $t(8) = 1.74$ ,  $p = 0.0600$ .

Figure 4  
The time spent within 1.5 body lengths of other squid ( $\pm SEM$ ) in focal male treatments.



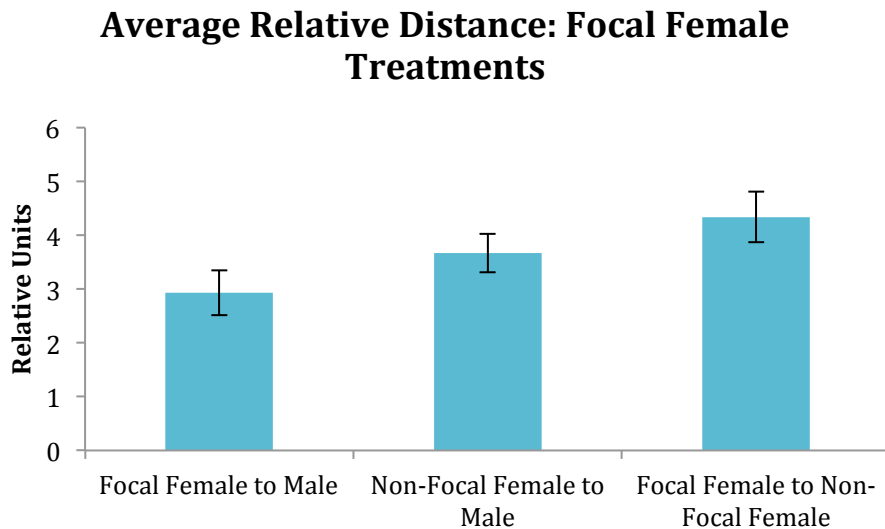
Note: \* denotes that the focal males spent significantly more time in proximity to the female than the non-focal males,  $t(9) = 2.1$ ,  $p = 0.0326$ .

In the FMTs, the focal males spent significantly more time in proximity to the females than the non-focal males spent in proximity to the females,  $t(9) = 2.1$ ,  $p = 0.0326$ , one tailed (see Fig. 4).

Proximity was also measured by finding the average distance between pairs of the three squid. This distance was then coded to represent relative, rather than absolute, distance between the animals. Details of this coding method are laid out in Table 4 above. For the FFTs, while the average relative distance between focal females and males was closer than between non-focal females and males, the difference was not significant,  $t(8) = -1.21$ ,  $p = 0.1304$ , one tailed (see Fig. 5).

Figure 5

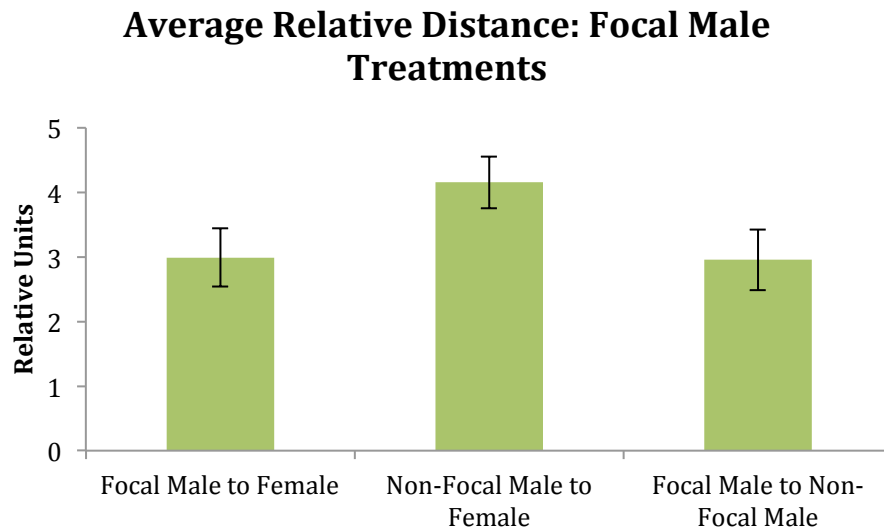
The average relative distance between individual squid in focal female treatments. For details on the relative units used, see Table 3. The differences were not significant.



In the FMTs, the average relative distance between the focal males and females was closer than to the average relative distance between the non-focal males and females; however these differences were not significant,  $t(7) = -1.53$ ,  $p = 0.0849$ , one tailed (See Fig. 6).



Figure 6  
The average relative distance between individual squid in focal male treatments. For details on the relative units used, see Table 4. Differences were not significant.



Complete data for all behavioral events and times for all trials can be found in appendices 2-13.

### Discussion

In the focal female trials, the males made significantly more contact with the focal females than with the non-focal females. Males also spent more time in proximity to focal females than to non-focal females; however the difference was just out of the range of significance. In the measurement of average relative distance, while the males had a closer relative distance to focal females than to non-focal females, the result was also not significant. In the focal male trials, the focal males made significantly more contacts with and spent significantly more time in proximity to females than did the non-focal males. However, while focal males were on average relatively closer to the females than were non-focal males, this difference was not significant. Being the focal individual and having prior presence in the enclosure made a difference for both female and male behavior.

### **Prior Presence and Prior Residency Effect**

The relationship between prior residency and dominance has been studied in many diverse animal species, including fish (Johnsson & Forser, 2002; Heuts & Nijman 1998; Nijman & Heuts 2000; Figler, Chaves, & Wazlavek, 1998; and Bronstein & Hirsch, 1985), birds (Beauchamp, 2000 and Dearborn & Wiley 1993) and echinoderms (Morishita et al., 2009). Prior residents in these species can exhibit dominance. In some species these effects may be mitigated by other intrinsic factors of the individual, such as body size. In the studies of these diverse species, individuals were given anywhere from 10 minutes to 45 days to establish residency.

Prior residence effects have not been well studied in cephalopods but there are a few reasons why prior residence is not an appropriate term for this experiment. In this study, the squid were only given 2 extra minutes of acclimation time. Furthermore, there was no shelter in the enclosure for this study and there were no real landmarks for the squid to learn. Lastly, *L. brevis* does not form territories in the wild. While the squid in this study did not form *residency*, it is possible that by having, what Dr. Akre termed, *prior presence*, some of the same factors that provide dominance to animals that establish residency are affecting the social interactions of the Atlantic brief squid. Given the findings of this study, it appears that prior presence for *L. brevis* affects behavior and maybe dominance, even if prior exposure to an area is just a couple of minutes. Cephalopods are known for their high levels of intelligence and may be more sensitive to these differences than other taxa.

Another possibility is that extra acclimation time simply allows the squid more time to adjust to changes in their environment, such as temperature or salinity. Perhaps in this study, the extra time allowed them to calm down after the stress of being moved. While this is a possibility, the fact that many squid, especially the males, moved directly toward opposite sex squid immediately after being introduced to the tank seems to suggest that no acclimation time was necessary for individuals to engage in mating behaviors. This may reflect the fact that in the wild, the environment where *L. brevis* lives can be very turbid. However, according to Dr. Akre, patches of clear “blue water” do wash in from the gulf and can suddenly create periods of high visibility. Perhaps this species is well adapted to taking advantage of mating opportunities that appear suddenly and last an unknown period of time. Simply needing time to acclimate and relax after being introduced to a new space cannot be ruled out, however.

#### **Male Mate Choice in Focal Female Treatments**

Focal females received a greater number of contacts from males than did non-focal females. While this study does not make a distinction between contacts made and successful mating or mating attempts, each contact does signal an interest by the male in the female for mating purposes. While the difference was just out of the range of significance, males also spent greater time in proximity to focal females than to non-focal females. The lack of significance is possibly the consequence of a very small sample size. However, the greater amount of time spent in proximity to focal females by males also suggests increased sexual interest. There are many possible reasons males made significantly more contacts with and spent more time in proximity to focal females than non-focal females.

Akre et al. (under review) found that female *L. brevis* have more elaborate signaling than males. For this reason, it is possible that male mate preference plays a role in the evolution of sexual selection for this species. Normally it is the less elaborately ornamented sex that is the choosier of the two and in terms of signaling, male *L. brevis* are less ornate than females. Perhaps males of this species are choosing females based on a measure of quality related to prior presence or greater time spent in the enclosure.

Female squid mate multiple times and store sperm from previous copulations. They also lay large numbers of eggs that are presumably fertilized by the sperm from more than one male. Females may exert post-copulatory choice by deciding which sperm receives the best access to eggs while laying, when to lay her eggs, and when to consume spermatophores rather than use it to fertilize eggs. If this is the case, it may be most beneficial for females to mate with any available male, and exert choice later. Even if females do not exert post-copulatory choice, it may still be advantageous for female *L. brevis* to not to be choosy and to mate with any available male. Since females lay a great number of eggs at a time and have semelparous reproduction, it is likely advantageous for females to increase the genetic diversity and adaptability of their offspring by using the sperm from multiple males to fertilize their eggs. For these reasons, males of this species may be choosier about their mates than females.

Since females may consume costly spermatophores, or may choose not to use the sperm of a particular male, or may use the sperm of a given male to fertilize only a small portion of her eggs, males may choose females based on their perceived chances of paternity. It is possible that the longer a female has spent in a given area may increase her attractiveness to males. This could be because females compete for access for the best

locations to lay their eggs and this is determined in some part by how long a female has been in an area. It is also possible that the longer a female has spent in an area the closer she is to laying her eggs. There is evidence that a male's chance of fertilizing the greatest proportion of a female's eggs is increased when he is the last male to mate with her before she lays. This can be the case for multiple reasons. Sperm may be displaced, females may not store sperm in the ideal placement to fertilize her eggs, or other rival males may attempt to remove it. In these circumstances, the increased time to acclimate to the enclosure may have signaled to the males that females were closer to laying their eggs and therefore the males were more likely to be the ones to fertilize a greater proportion of the clutch.

### **Dominance Between Males**

In focal male treatments, focal males spent significantly more time in proximity and made more contacts to females than non-focal males. As with the previous examination of focal female treatments, there are multiple possible reasons for the observed behavior in focal male treatments. It seems likely that males that spent a greater amount of time in the enclosure established some form of dominance. In many species of cephalopods, males will mate-guard females post-copulation as a way to ensure that rivals do not have a chance to mate with females subsequently (Hanlon & Messenger, 1996). There is also good reason to believe that once a male has mated with a female, he increases his chance of fertilizing a greater number of her eggs if no other males mate with her before she lays her eggs (Squires, et al., 2015). While the aim of this project was not to monitor mate-guarding behavior, there was no anecdotal evidence from this project that mate guarding is a behavior displayed by male *L. brevis* post-copulation. Greater

proximity to females and increased number of contacts may simply reflect a male's increased opportunity for mating attempts. If a focal male is closer to a female than a non-focal male is, presumably he is in a better position to 1) read and respond to her signals, and 2) initiate mating. This seems likely to be the case and is supported by the evidence that focal males made significantly more contacts with females than did non-focal males.

It is perhaps less surprising that the amount of time a males spends becoming acclimated to an area would have an effect on his potential to mate than the fact that it would for a female. This falls more closely in line with classic ideas of sexual selection, where males establish dominance and females choose the best mate. Prior presence and preferential access to females for dominant males is well documented.

When interpreting the results of this study, it is important to keep in mind that the experimental design of this project was not set up to test the specific questions this study set out to answer. The placement of the camera, the choice of focal animals, and the small sample size of this experiment mean that further research is needed before conclusive answers can be found. However, the results of this study are exciting and suggest that even a short amount of prior presence in an area or extra time to acclimate to it can have dramatic effects on the mating behavior of *L. brevis*. Both female and male *L. brevis* may use prior presence as a way to establish dominance during mating or as a way to evaluate the fitness or attractiveness of a mate. In the future, more studies are needed to investigate these behaviors further.

### **Application for Aquaculture, Zoos, Aquaria, & Other Breeding Programs**

Regardless of the cause, the fact that the amount of time a squid has to acclimate to an enclosure has an apparent effect on mate preference and mating behavior, should be of interest to anyone attempting to keep or breed these animals in captivity. Having an extra two minutes to acclimate to an area may not seem important enough to affect mating behavior and could therefore easily be overlooked or remain unknown. Zoos, aquaria, or any institution interested in maintaining this species in captivity would benefit from this information. Given how easy and inexpensive it would be to give animals prior presence to an enclosure before mating, it would be unfortunate for these institutions not to be aware of the potential outcomes of doing so. Furthermore, if having prior presence in an area truly does give both female and male Atlantic brief squid an advantage in mating, this information could easily be used by breeders as a way to artificially select for certain traits. If all that a breeder needs to do to increase the reproductive fitness of a particular squid is to give it prior presence in an enclosure, this gives the breeder easy control over mating. If there is a trait that someone would like to increase in a captive population, this could be an inexpensive, effective, and low impact way to do so.

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### Appendix 1

#### Appendix 1a

The ethogram of the behaviors of interest under the category of proximity

<b>Behavior:</b> <b>Proximity</b>	<b>Description</b>
Opposite Sex	Individual is within 1.5 body lengths of a squid of the opposite sex
Same Sex	Individual is within 1.5 body lengths of a squid of the same sex
All Three	All three squid are within 1.5 body lengths of each other

#### Appendix 1b

The ethogram of the behaviors of interest under the category of contact

<b>Behavior: Contact</b>	<b>Description</b>
To Opposite Sex	Individual initiates contact with a squid of the opposite sex
To Same Sex	Individual initiates contact with a squid of the same sex
From Opposite Sex	Individual is contacted by a squid of the opposite sex
From Same Sex	Individual is contacted by a squid of the same sex
Stacked and Still	One individual is located beneath a second individual and both are not swimming

#### Appendix 1c

The ethogram of swimming behaviors

<b>Behavior: Swim</b>	<b>Description</b>
Directed to OS	Fast movement towards an opposite sex individual
Directed to SS	Fast movement towards a same sex individual
Directed away OS	Fast movement away from an opposite sex individual
Directed away SS	Fast movement away from a same sex individual
Non-Directed	Slow swimming without an observable destination
Back and Forth	Individual swims forward the back in a repeated movement
Still	No movement observed

Note: Opposite Sex (OS), Same Sex (SS)

#### Appendix 1d

The ethogram of other behaviors

<b>Behavior: Other</b>	<b>Description</b>
Inked	Individual releases ink
Off Camera	Individual is not observable on camera

## Appendix 2

## Appendix 2

The behavioral events for focal females in focal female treatment

<b>Behavior</b>	<b>Average Number of Events</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	0.33	0.71	212.13	0.00	0.24
Directed to SS	0.11	0.33	300.00	0.00	0.11
Directed away OS	2.11	2.98	141.00	1.00	0.99
Directed away SS	0.00	0.00		0.00	0.00
Non-Directed	3.56	2.88	80.92	3.00	0.96
Back and Forth	2.22	1.20	54.08	2.00	0.40
Still	0.11	0.33	300.00	0.00	0.11
<b>Proximity</b>					
Opposite Sex	5.56	3.43	61.77	6.00	1.14
Same Sex	1.56	1.74	111.86	1.00	0.58
All Three	4.67	2.83	60.61	3.00	0.94
<b>Contact</b>					
To Opposite Sex	0.00	0.00		0.00	0.00
To Same Sex	0.00	0.00		0.00	0.00
From Opposite Sex	0.00	0.00		0.00	0.00
From Same Sex	2.89	2.47	85.57	2.00	0.82
Stacked and Still	0.00	0.00		0.00	0.00
<b>Other Behaviors</b>					
Off Camera	0.00	0.00		0.00	0.00
Inked	0.22	0.44	198.43	0.00	0.15

Note: Opposite Sex (OS), Same Sex (SS)

**Appendix 3**

## Appendix 3

The behavioral times in seconds for focal females in focal female treatments

<b>Behavior</b>	<b>Average Time (sec)</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	2.22	6.67	300.00	0.00	2.22
Directed to SS	0.78	2.33	300.00	0.00	0.78
Directed away OS	23.11	53.00	229.32	0.00	17.67
Directed away SS	0.00	0.00		0.00	0.00
Non-Directed	104.56	92.49	88.46	103.00	30.83
Back and Forth	168.67	113.89	67.52	213.00	37.96
Still	1.78	5.33	300.00	0.00	1.78
<b>Proximity</b>					
Opposite Sex	80.78	65.67	81.30	65.00	21.89
Same Sex	61.89	102.25	165.21	7.00	34.08
All Three	36.44	26.79	73.50	38.00	8.93
<b>Other Behaviors</b>					
Off Camera	0.00	0.00		0.00	0.00

Note: Opposite Sex (OS), Same Sex (SS)

**Appendix 4**

Appendix 4

The behavioral events for non-focal females in focal female treatments

<b>Behavior</b>	<b>Average Number of Events</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	0.89	1.36	153.48	0.00	0.45
Directed to SS	1.00	2.00	200.00	0.00	0.67
Directed away OS	0.89	1.36	153.48	0.00	0.45
Directed away SS	0.33	1.00	300.00	0.00	0.33
Non-Directed	4.22	3.53	83.55	3.00	1.18
Back and Forth	3.33	1.32	39.69	3.00	0.44
Still	0.00	0.00		0.00	0.00
<b>Proximity</b>					
Opposite Sex	3.33	2.55	76.49	4.00	0.85
Same Sex	1.56	1.74	111.86	1.00	0.58
All Three	4.67	2.83	60.61	3.00	0.94
<b>Contact</b>					
To Opposite Sex	0.00	0.00		0.00	0.00
To Same Sex	0.11	0.33	300.00	0.00	0.11
From Opposite Sex	0.00	0.00		0.00	0.00
From Same Sex	0.56	0.88	158.75	0.00	0.29
Stacked and Still	0.00	0.00		0.00	0.00
<b>Other Behaviors</b>					
Off Camera	3.63	2.97	82.02	4.00	0.99
Inked	0.00	0.00		0.00	0.00

Note: Opposite Sex (OS), Same Sex (SS)

## Appendix 5

## Appendix 5

The behavioral times in seconds for non-focal females in focal female treatments

<b>Behavior</b>	<b>Average Time (sec)</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	2.67	3.28	122.95	0.00	1.09
Directed to SS	6.00	14.10	234.96	0.00	4.70
Directed away OS	2.44	4.88	199.48	0.00	1.63
Directed away SS	0.00	0.00		0.00	0.00
Non-Directed	63.89	58.81	92.05	47.00	19.60
Back and Forth	186.89	109.33	58.50	196.00	36.44
Still	0.00	0.00		0.00	0.00
<b>Proximity</b>					
Opposite Sex	34.11	33.07	96.95	25.00	11.02
Same Sex	61.89	102.25	165.21	7.00	34.08
All Three	36.44	26.79	73.50	38.00	8.93
<b>Other Behaviors</b>					
Off Camera	35.88	41.76	116.40	34.50	13.92

Note: Opposite Sex (OS), Same Sex (SS)



## Appendix 6

## Appendix 6

The behavioral events for males in focal female treatments

<b>Behavior</b>	<b>Average Number of Events</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	5.11	2.67	800.00	6.00	0.89
Directed to SS	0.33	0.71	212.13	0.00	0.24
Directed away OS	3.00	1.66	55.28	2.00	0.55
Directed away SS	1.56	1.33	85.71	2.00	0.44
Non-Directed	0.00	0.00		0.00	0.00
Back and Forth	5.11	2.67	800.00	6.00	0.89
Still	0.33	0.71	212.13	0.00	0.24
<b>Proximity</b>					
Opposite Sex	8.89	5.25	59.11	8.00	1.75
All Three	4.67	2.83	60.61	3.00	0.94
<b>Contact</b>					
To Opposite Sex	3.44	3.28	95.31	2.00	1.09
From Opposite Sex	0.11	0.33	300.00	0.00	0.11
Stacked and Still	0.00	0.00		0.00	0.00
<b>Other Behaviors</b>					
Off Camera	2.50	2.73	109.02	2.00	0.91
Inked	0.00	0.00		0.00	0.00

Note: Opposite Sex (OS), Same Sex (SS)

## Appendix 7

## Appendix 7

The behavioral times in seconds for males in focal female treatments

<b>Behavior</b>	<b>Average Time (sec)</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	114.00	97.18	85.24	74.00	32.39
Directed to SS	0.00	0.00		0.00	0.00
Directed away OS	61.67	66.46	107.78	40.00	22.15
Directed away SS	109.50	131.41	120.01	39.50	43.80
Non-Directed	0.00	0.00		0.00	0.00
Back and Forth	114.00	97.18	85.24	74.00	32.39
Still	0.00	0.00		0.00	0.00
<b>Proximity</b>					
Opposite Sex	90.44	49.96	55.24	103.00	16.65
All Three	36.44	26.79	73.50	38.00	8.93
<b>Other Behaviors</b>					
Off Camera	26.50	41.85	157.92	4.00	13.95

Note: Opposite Sex (OS), Same Sex (SS)

## Appendix 8

## Appendix 8

The behavioral events for focal males in focal male treatments

<b>Behavior</b>	<b>Average Number of Events</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	3.10	2.23	72.05	3.50	0.71
Directed to SS	0.20	0.42	210.82	0.00	0.13
Directed away OS	0.30	0.67	224.98	0.00	0.21
Directed away SS	0.00	0.00		0.00	0.00
Non-Directed	2.20	1.93	87.83	1.50	0.61
Back and Forth	1.80	1.23	68.29	1.50	0.39
Still	0.10	0.32	316.23	0.00	0.10
<b>Proximity</b>					
Opposite Sex	3.90	2.81	71.97	5.00	0.89
Same Sex	1.20	0.92	76.58	1.00	0.29
All Three	4.10	2.85	69.42	4.50	0.90
<b>Contact</b>					
To Opposite Sex	3.10	3.03	97.90	3.00	0.96
To Same Sex	0.20	0.63	316.23	0.00	0.20
From Opposite Sex	0.00	0.00		0.00	0.00
From Same Sex	0.10	0.32	316.23	0.00	0.10
Stacked and Still	0.20	0.63	316.23	0.00	0.20
<b>Other Behaviors</b>					
Off Camera	0.10	0.32	316.23	0.00	0.10
Inked	0.00	0.00		0.00	0.00

Note: Opposite Sex (OS), Same Sex (SS)

**Appendix 9**

Appendix 9

The behavioral times in seconds for focal males in focal male treatments

<b>Behavior</b>	<b>Average Time (sec)</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	72.20	76.33	105.72	67.50	24.14
Directed to SS	0.70	2.21	316.23	0.00	0.07
Directed away OS	1.10	2.60	236.48	0.00	0.82
Directed away SS	0.00	0.00	0.00	0.00	0.00
Non-Directed	42.40	53.87	127.06	17.50	17.04
Back and Forth	169.50	116.32	68.63	192.50	36.78
Still	0.40	1.26	316.23	0.00	0.40
<b>Proximity</b>					
Opposite Sex	58.90	54.78	93.00	60.00	17.32
Same Sex	43.60	79.98	183.45	21.00	25.29
All Three	80.40	80.55	100.19	71.00	25.47
<b>Other Behaviors</b>					
Off Camera	0.60	1.90	316.23	0.00	0.60

Note: Opposite Sex (OS), Same Sex (SS)

## Appendix 10

## Appendix 10

The behavioral events for non-focal males in focal male treatments

<b>Behavior</b>	<b>Average Number of Events</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	2.70	3.47	128.36	1.50	1.10
Directed to SS	0.30	0.67	224.98	0.00	0.21
Directed away OS	0.10	0.32	316.23	0.00	0.10
Directed away SS	0.30	0.67	224.98	0.00	0.21
Non-Directed	1.80	1.48	81.98	1.50	0.47
Back and Forth	1.40	1.17	83.84	1.50	0.38
Still	0.20	0.42	210.82	0.00	0.13
<b>Proximity</b>					
Opposite Sex	1.60	2.01	125.69	1.00	0.64
Same Sex	1.20	0.92	76.58	1.00	0.29
All Three	4.10	2.85	69.42	4.50	0.90
<b>Contact</b>					
To Opposite Sex	0.80	2.20	275.13	0.00	0.70
To Same Sex	0.10	0.32	316.23	0.00	0.10
From Opposite Sex	0.00	0.00		0.00	0.00
From Same Sex	0.20	0.63	316.23	0.00	0.00
Stacked and Still	0.50	1.58	316.23	0.00	0.50
<b>Other Behaviors</b>					
Off Camera	2.00	2.11	105.41	1.50	0.67
Inked	0.00	0.00		0.00	0.00

Note: Opposite Sex (OS), Same Sex (SS)

## Appendix 11

## Appendix 11

The behavioral times in seconds for non-focal males in focal male treatments

<b>Behavior</b>	<b>Average Time (sec)</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	50.60	68.33	135.04	9.00	21.61
Directed to SS	1.10	3.48	316.23	0.00	1.10
Directed away OS	0.44	1.33	300.00	0.00	0.42
Directed away SS	0.56	1.67	300.00	0.00	0.53
Non-Directed	29.70	53.46	180.00	12.00	16.91
Back and Forth	108.90	115.40	105.97	85.00	36.49
Still	60.40	127.48	211.06	0.00	40.31
<b>Proximity</b>					
Opposite Sex	15.50	18.99	122.50	9.50	6.00
Same Sex	41.80	80.82	193.35	14.00	25.56
All Three	84.40	82.60	97.87	71.00	26.12
<b>Other Behaviors</b>					
Off Camera	41.90	53.26	127.10	8.00	16.84

Note: Opposite Sex (OS), Same Sex (SS)

**Appendix 12**

Appendix 12

The behavioral events for females in focal male treatments

<b>Behavior</b>	<b>Average Number of Events</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	0.20	0.42	210.82	0.00	0.13
Directed to SS	1.30	1.83	140.68	0.50	0.58
Directed away OS	3.10	2.38	76.71	2.50	0.75
Directed away SS	1.70	1.42	83.42	1.00	0.45
Non-Directed	0.00	0.00		0.00	0.00
Back and Forth	0.20	0.42	210.82	0.00	0.13
Still	1.30	1.83	140.68	0.50	0.58
<b>Proximity</b>					
Opposite Sex	5.50	2.27	41.33	5.50	0.72
All Three	4.10	2.85	69.42	4.50	0.90
<b>Contact</b>					
To Opposite Sex	0.00	0.00		0.00	0.00
From Opposite Sex	3.90	3.84	98.53	3.00	1.22
Stacked and Still	0.70	2.21	316.23	0.00	0.70
<b>Other Behaviors</b>					
Off Camera	2.90	1.97	67.90	3.00	0.62
Inked	0.00	0.00		0.00	0.00

Note: Opposite Sex (OS), Same Sex (SS)

## Appendix 13

## Appendix 13

The behavioral times in seconds for females in focal male treatments

<b>Behavior</b>	<b>Average Time (sec)</b>	<b>Standard Deviation</b>	<b>Coefficient of Variance</b>	<b>Median</b>	<b>SEM</b>
<b>Swim</b>					
Directed to OS	1.00	3.00	300.00	0.00	0.95
Directed to SS	7.80	12.57	161.13	0.00	3.97
Directed away OS	90.10	74.74	82.96	112.00	23.64
Directed away SS	102.60	109.20	106.43	49.00	34.53
Non-Directed	0.00	0.00		0.00	0.00
Back and Forth	1.00	3.00	300.00	0.00	0.95
Still	7.80	12.57	161.13	0.00	3.97
<b>Proximity</b>					
Opposite Sex	74.40	49.66	66.75	66.00	15.70
All Three	84.40	82.60	97.87	71.00	26.12
<b>Other Behaviors</b>					
Off Camera	87.00	96.38	110.78	59.50	30.48

Note: Opposite Sex (OS), Same Sex (SS)

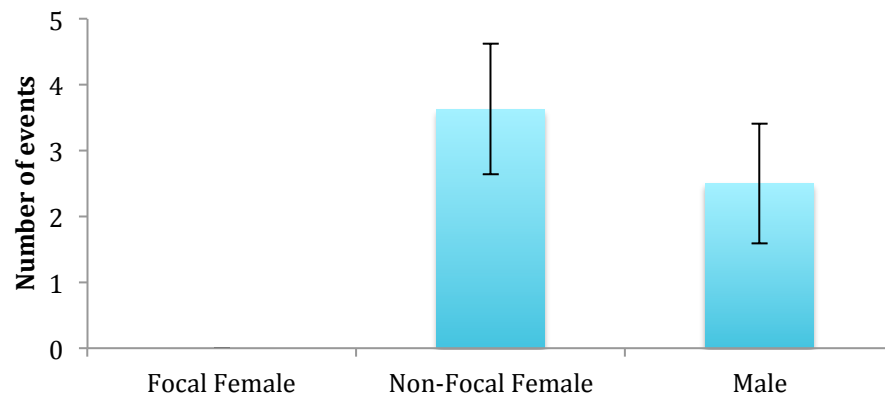


**Appendix 14**

Appendix 14a

The average number of times squid went off camera ( $\pm SEM$ ) during focal female treatments

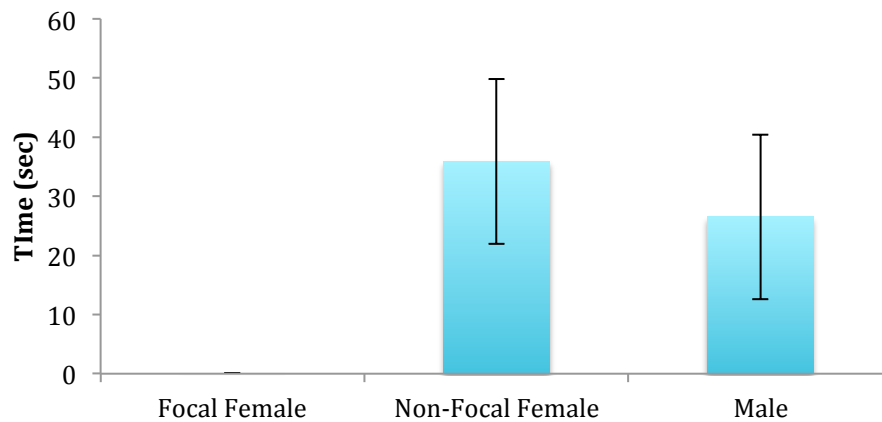
**Average Number of Off Camera Events:  
Focal Female Treatments**



Appendix 14b

The average time spent off camera ( $\pm SEM$ ) in seconds during focal female treatment

**Average Time Spent Off Camera: Focal  
Female Treatments**

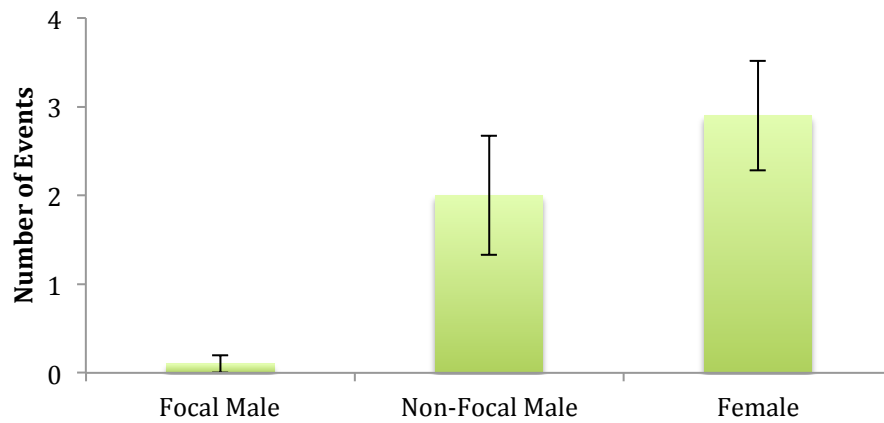


**Appendix 15**

Appendix 15a

The average number of times squid went off camera ( $\pm SEM$ ) during focal male treatments

**Average Number of Off Camera Events:  
Focal Male Treatments**



Appendix 15b

The average time spent off camera ( $\pm SEM$ ) in seconds during focal male treatments

**Average Time Spent Off Camera: Focal Male  
Treatments**

