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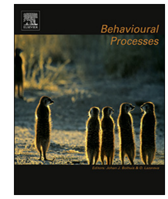

Temporal patterns and sex differences in dyadic interactions in a wild zebrafish population

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ABSTRACT

Male-female interactions in several group living organisms, including some fish species tend to be dynamic and play a key role in determining their mating and courtship behavior. Laboratory-bred zebrafish (*Danio rerio*) strains are one of the most widely used model systems in various fields of biology. While research on wild zebrafish behavior is gaining ground, our knowledge about their mating ecology and mating strategies is still limited. We investigated diel temporal patterns in inter-sex dyadic interactions among wild zebrafish and the occurrence of behavioral dimorphism in their interactive behaviors. We observed randomly paired male and female individuals at three distinct time intervals (early morning, morning and afternoon sessions) in the day and collected occurrence data for six discrete inter-individual interactive behaviors that were associated with mating, aggression, and display. We used generalized linear mixed models to examine the effect of time, sex of the individual and presence of oviposition substrate on these behavioral traits. We found a higher incidence of mating-associated behavior during the early parts of the day which declined by the afternoon. These mating-associated behaviors were also dependent on the presence of gravel substrate for egg-laying compared to other behaviors. This work is the first of its kind that details patterns in behavioral dimorphism between sexes in zebrafish. Our results throw light on the complex dynamics of male-female interactions in a group living externally fertilizing species and can have implications in designing experiments involving behavioral testing of zebrafish which is increasingly being done in toxicological studies and laboratory breeding.

1. Introduction

For group living organisms, interactions between sexes is dynamic (Adamo and Hoy, 1995; Gavish et al., 1983) and forms the basis for the evolution of mating behavior, through chemical (Gavish et al., 1983) as well as behavioral (Crews and Moore, 1986) signals for exchange of information. Inter-sexual interactive behaviors linked to reproduction are modulated by several ecological factors that themselves change across seasons and habitats. Seasonality in reproduction is determined by temporal variations in reproductive hormones and the subsequent physiological changes associated with mating (Reiter et al., 2009; Holland et al., 2001). This, in turn, could impact female receptivity, male-male competition, population density, operational sex ratios and even sexual selection forces within mating systems (Shuster and Wade, 2003). Several kinds of male-female interaction patterns have been observed in animals. In some species (such as in sheep and goats), males and females stay segregated except for a brief period of mating (Walkden-Brown et al., 1999) while among primates like bonobos that live in mixed groups, males and females not only interact for reproduction but also compete for food and resources, protect territories and forage (Furuichi, 2011). This study investigates the dynamics of interactions between males and females in a wild zebrafish (*Danio rerio*) population. As this species occurs naturally in mixed sex groups, we expect a combination of social and courtship/mating associated interactions among sexes. However, the temporal (diel) dynamics of these interactions have not been investigated in detail. Our study attempts to explore variations in behavioral repertoire across the day (i.e. early morning-morning-afternoon sessions) and potential sexual dimorphism in their occurrence frequencies.

Among fish with either internal and external fertilization, species exhibit diverse mating strategies that often show rhythmic (diurnal or seasonal) periodicity. In both temperate and tropical regions, seasonal periodicity can lead to differential allocation of reproductive effort among fish species (Winemiller and Rose, 1992). Periodicity is regulated by various external environmental factors that often show diel patterns. For example, the diel cycle of dawn, sunrise, sunset can affect fundamental biological activities among fish, including foraging, breeding and general activity (Helfman, 1986). Photoperiod has been

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reported as a primary regulator for many fish species such as rainbow trout (Duston and Bromage, 1986), Japanese medaka (Urasaki, 1976), Salmonidae and Cyprinidae (de Vlaming, 1972) and catfish (Sundararaj and Vasal, 1976). There are other environmental regulators like temperature which can work concomitantly with photoperiodic stimuli (de Vlaming, 1972; Lam, 1983). Among oviparous species, in addition to temporal dynamics, another important abiotic factor that plays a key role in successful reproduction is the quality of the oviposition substrate. The female's choice of an egg-laying site can influence the survivability and life-history of its offspring (Resetarits, 1996; Refsnider and Janzen, 2010). Through the choice of egg laying sites, the mother can not only increase the chances of the embryos' survival but may impart long term effects like natal philopatry and mate choice (Refsnider and Janzen, 2010).

Zebrafish is a small cyprinid that occurs in groups or shoals and are distributed across the Indian subcontinent (Engeszer et al., 2007; Spence et al., 2008). They are polygynandrous and their reproductive system is governed by photoperiod (Laale, 1977; Spence and Smith, 2005); they are known to mainly spawn at dawn in both laboratory and in the field conditions (Spence et al., 2008). Zebrafish courtship comprises of the male chasing the female multiple times, and the female leading the male towards the egg-laying substratum. They engage in courtship displays using their fins and moving in circles. The male will then initiate nudging with the belly of the female that stimulates the release of eggs and the male subsequently releases its sperms (Spence et al., 2008). Although they stay in large dynamic groups, it has been suggested that zebrafish are not group-spawning and mating occurs within a male-female dyad (Hutter et al., 2010). This makes it an interesting model for studying courtship behaviors and variations in the male-female dynamics. There have been a few earlier investigations towards a more detailed understanding of courtship behavior in zebrafish (Darrow and Harris, 2004; Sessa et al., 2008). Recently, Hutter et al. (2010) explored the interactions among shoals of zebrafish in laboratory tanks. However, specific temporal dynamics of intersex interactions and the important drivers of these behaviors are still not well understood. This study addresses a broader question of how individual pairs of males and females interact with regards to mating and courtship over a diurnal time scale. More specifically, our study aims to address the following aspects of male-female dyadic interactive behavior:

a) To explore the various interactive behaviors in a zebrafish dyad for a better understanding of the dyadic dynamics of a zebrafish pair.
b) To understand the temporal variation in the mating-related behaviors. Based on earlier studies (Spence et al., 2008) and our existing understanding on zebrafish intraspecific interactions, we hypothesized that mating related interactive responses within dyads would be variable across a temporal scale during the diel cycle, that is, early morning, morning and afternoon.
c) To explore sex-specific behavioral repertoire among zebrafish. Behavioral differences exist between males and females across several species of fishes (reviewed in Magurran and Garcia, 2000). Aggressive interactions have been shown to be mediated by age, social status (Ricci et al., 2013) and male aggression itself influence spawning and egg production in females (Spence and Smith, 2005). We therefore hypothesized that a dimorphism in male and female aggressive interactions would be observed among dyadic pairs among zebrafish and predicted that male zebrafish would show more aggressive behavior compared to females
d) To investigate the role of oviposition substrates on various social as well as sexual or mating related behavior. It has been shown that zebrafish females prefer gravel substratum compared to silt for egg-laying (Spence et al., 2008). Here, we studied the effect of oviposition substrate on dyadic interactions and assessed differences in specific interactive behaviors in the presence and absence of an oviposition substrate.

2. Methodology

We used a wild-caught zebrafish population collected from Howrah and Nadia districts (West Bengal, India). The individuals were maintained in large holding tanks (45.7 × 25.4 × 25.4 cm) in mixed sex groups. Holding room temperature was maintained between 23 °C–25 °C, with a light-dark cycle at 14 h L:10 h D to mimic natural LD cycle, necessary for normal reproductive cycles and behaviors. The fish were fed daily (between 10 am-12 noon) with commercially purchased food pellets and frozen dried blood worms alternating with brine shrimp (*Artemia* spp.). The tanks had standard corner filters for water circulation.

30 individuals of each sex were chosen for the experiment. The fish were isolated and kept in individual jars for four days. This was done to ensure individual identification of the fish and also to starve them of any other interactions with other individuals which might influence their interactions on the day of the experiment (Gerlach, 2006, Hutter et al., 2010). On the afternoon of day 4, male and female individuals were paired randomly and each pair was released in an open tank (30 × 20 × 20 cm) and left overnight. 15 pairs were kept in separate tanks with a petri-plate filled with small gravels as an egg-laying substrate in their tanks (Spence et al., 2007), while the other 15 pairs were devoid of this substrate. On day 5, observations commenced once the lights were switched on (at 900 h.). We video recorded (Sony DCR-PJ5, Sony DCR-SX22) their behavior at three different sessions—early morning (900 h.), morning (1130 h.) and afternoon (1330 h.). Their interactions were recorded for one hour for each session. The experiments were performed in the months of February to March 2017.

For our analysis, every one-hour observation was broken into 12 sections of 5 min each. We analyzed every alternate 5-minute section totaling to six sections or 30 min of observational data for each video recording. The 5-minute observations within sessions were combined to obtain data on 30 min of total observation for each pair. We recorded the count data for 6 different behaviors we had chosen from pilot experiments: Bites, Nudges, Chases, Leads, Circling and Quivers (Kalueff et al., 2013) (Table 1). We also used two different modes of classification of behavior. The first classification was based on the nature of the behavior, which categorized them into three classes: Aggressive (Bites and Chases), Mating-related (Nudges, Leads and Quivers) and

Table 1
Behavioral interactions (behaviors) included in this study and their description (modified from Kalueff et al., 2013).

INTERACTION	DEFINITION
Bite (B)	One individual bites the other on any part of the body, resulting in an evasive reaction (rapid fleeing) in the second individual.
Chase (C)	One individual chases the other, with a high relative speed. May or may not end in a Bite. The behavior is counted for the chaser.
Nudge (N)	One individual nudges the other on any part of the body, generally with the snout.
Lead (L)	One individual follows the other, at a certain distance, but at a low relative speed and generally the follower makes the same turns as the leader individual. The behavior is counted for the leader and not the follower.
Circling (Cr)	Involves both individuals making circles, with fins raised, sometimes also moving in upward spirals.
Quivering (Q)	Two individuals come close (within one body length), generally near the floor of the tank or the oviposition substrate, aligned parallelly or anti-parallelly, bodies quiver. Generally associated with repeated nudging by the male and also tight circling.

Circling. Circling was treated as a separate class since there is report of it being a part of zebrafish courtship as well aggressive repertoire (Kalueff et al., 2013). As we were unable to clearly distinguish the minute differences in circling during these two different cases during analyses of video, this behavior was considered separately. We also classified the behaviors into Contact behaviors (Bites, Nudges and Quivers) and Non-contact behaviors (Chases, Leads and Circling) based on the tactility of the behaviors. Bites, nudges, chases and leads were directed behaviors where one individual initiated the behavior towards the other. Circling and Quivers were joint behaviors in which both individuals participated and thus received equal count values.

3. Statistical analysis

All statistical analyses were performed in R studio (R Core Team, 2014, ver. 1.1.423). We obtained generalized linear mixed models using package 'lme4' (ver. 1.1–19, Bates et al., 2015), with fish pair (pair id) as the random factor and session, sex, and substrate as fixed factors. We constructed a whole model with all the factors (described in Supplementary File S1) and then subsequently reduced models with various combinations of the fixed factors. From these models the most parsimonious was chosen based on the lowest AIC values and model comparisons using 'Anova' (package 'car', ver.-3.0–3, Fox and Weisberg, 2019). Separate models were built for all individual behaviors as well as the behavioral classes described in the Methods section. The models that we selected were used for interpreting which specific behaviors were influenced by each factor (i.e. session, sex, presence of substratum).

For individual behaviors, the data fitted negative binomial distribution the best and we thus used the 'glmer.nb' class in 'lme4' package that uses Laplace approximation method. For the grouped behaviors, both mating-related behaviors and non-contact behaviors fit closest to a negative binomial distribution and hence we used the same GLMM method. We used the Poisson distribution 'family' function in glmm package for analysis of aggressive behaviors and contact behaviors.

Following model selection, post hoc tests (non-parametric paired Mann Whitney U tests) were then conducted to compare differences between sexes and presence/absence of substratum for a specific behavior. If session was found to be a significant predictor of a response factor (i.e. behavior), signed rank Wilcoxon tests (with Bonferroni corrections) were used to compare differences in behavior between session pairs.

4. Results

4.1. Predictors of interactive behaviors

The selected model for bites revealed that sex was the most important predictor (Table 2a) (Wald type II $\chi 2 = 29.002$, df = 1, $p << 0.01$). Post-hoc tests showed that males (mean = 79.14 ± 17.05) elicit significantly higher number of bites than females (mean = 21.18 ± 5.2) (Mann Whitney U test, W = 571.5, p = 0.003). In our final model for Nudges (Table 2b), sex was again found to be an important factor (Wald type II $\chi 2 = 44.62$, df = 1, $p << 0.01$) along with significant sex*substrate (Wald type II $\chi 2 = 8.55$, df = 1, p = 0.003) and sex*session (Wald type II $\chi 2 = 6.35$, df = 2, p = 0.04) interaction effects. Mann Whitney U test showed a significant difference between males (mean = 74.32 ± 17.08) and females (mean = 22.86 ± 4.55) (W = 545, p = 0.01). Males and females differed in terms of the total number of bites and nudges towards one another. Males exhibited higher number of both these behaviors towards females within dyads (Fig. 1). While there was no overall effect of session, there was a difference in nudges between males and females in early morning (W = 358, p = 0.04) as well as morning (W = 582.5, p = 0.002) sessions. Females also showed marginally significant difference in nudges between early morning and morning sessions (V = 172.5, p = 0.05).

Predictive models for chases did not reveal a significant effect of any specific predictor and hence we did not perform any further post-hoc analyses on this behavior. For Leads (Table 2c), session (Wald type II $\chi 2 = 6.29$, df = 2, p = 0.04), interaction effects between session*sex (Wald type II $\chi 2 = 9.91$, df = 2, p = 0.007) and sex*substrate (Wald type II $\chi 2 = 12.22$, df = 1, p = 0.0004) were found to be significant predictors in the selected model. Substrate was found to be a significant factor in our Wilcoxon analysis (W = 159, p = 0.005) for leads. Within the sexes, significant difference in leads was found only for females, between early morning and afternoon sessions (V = 194.5, p = 0.0009) and morning and afternoon sessions (V = 271, p = 0.0006), accounting for the significant effect of the session*sex interaction in the selected overall model. The females were also the more responsive sex to the presence of a substratum with a significant increase in their number of leads shown (W = 33, p = 0.003) compared to when a substratum was absent. Overall, males and females showed differing number of leads when a substrate was present (V = 32, p = 0.009).

Circling behavior seemed only to be dependent on the presence of substrate (Table 2d) (Wald type II $\chi 2 = 3.9$, df = 1 p = 0.05) with a significant difference in the number of circling with (mean = 18.9 ± 4.43) or without (mean = 7.33 ± 1.44) substrate (W = 1046.5, p = 0.009). Quivering behavior was also significantly affected by the presence of substrate (W = 495.5, p = 0.0005). Higher number of quivers were shown in the presence (mean = 78.54 +/-9.46) of substrate compared to its absence (mean = 35.8 ± 8.29) (Fig. 2).

4.2. Predictors of mating-related and aggressive behaviors

Session and sex were the important predictors for mating-related behavior (Table 2e). There was a significant reduction in mating-related behaviors from the morning to the afternoon session (V = 333.5, p = 0.003), for both males (V = 329, p = 0.004) and females (V = 300, p = 0.008) (Fig. 3).

Analysis of aggressive behavior (Table 2f) showed that there is a significant interaction effect of sex*substrate (Wald type II $\chi 2 = 240.57$, df = 1, $p << 0.01$). Wilcoxon test showed a significant difference only in females in presence and absence of substrate (W = 49.5, p = 0.03).

4.3. Predictors of contact and non-contact behaviors

The selected models revealed that sex was an important predictor for contact behaviors (Table 2g)(Wald type II $\chi 2 = 1536$, df = 1, $p << 0.01$).Males showed significantly higher number (mean = 182 ± 30.47) of contact-based behaviors (W = 595.5, p = 0.0008) compared to females (mean = 55.57 ± 9.5). There was also a significant session*sex interaction effect (Wald type II $\chi 2 = 106.66$, df = 2, $p << 0.01$). Males were found to differ significantly between morning and afternoon sessions (V = 236, p = 0.05). Furthermore, there was also a significant difference in contact behaviors exhibited between males and females in early morning (W = 401, p = 0.002) and morning (W = 598.5, p = 0.0007) sessions. The model also showed a significant sex *substrate interaction effect (Wald type II $\chi 2 = 65.24$, df = 1, $p << 0.01$). Males and females differed in contact-based behaviors, in the presence of a substrate (W = 157, p = 0.0002). GLMM model for non-contact behavior (Table 2h) also showed a significant sex*substrate interaction effect (Wald type II $\chi 2 = 12.79$, df = 1, p = 0.0003), as confirmed by subsequent Wilcoxon test which showed an effect on females based on substrate presence (W = 44.5, p = 0.02).

5. Discussion

Temporal patterns related to seasonal and diel fluctuations in

Table 2
Selected GLMM models showing independent factors, estimates, z-scores, degrees of freedom (df) and p values for the individual behaviors -a) Bite, b) Nudge, c) Lead, d) Circling and the grouped behaviors e) Mating-related, f) Aggressive, g) Contact and h) Non-contact behaviors.

a)
Bite ˜ sex + (1|pair.id)

AIC				
1132.3				
variable	**estimate**	**z-score**	**df**	**p**
intercept	1.82	5.65		< < 0.01
sex	1.35	5.39	1	< < 0.01

b)
Nudge˜ sex + session*sex + sex*substrate + (1|pair.id)

AIC				
1186.8				
variable	**estimate**	**z-score**	**df**	**p**
intercept	2.23	7.52		< < 0.01
sexmale	0.12	0.34	1	0.73
sessionE	0.03	0.1	2	0.92
sessionM	−0.38	−1.22	2	0.22
substrateY	−0.54	−1.55	1	0.12
sessionM:sexmale	1.09	2.45	2	0.014
sexmale:substrateY	1.17	2.93	1	0.003

c)
Lead˜session + sex + substrate + session*sex + sex*substrate + (1|pair.id)

AIC				
1250.7				
variable	**estimate**	**z-score**	**df**	**p**
intercept	1.83	6.72		< < 0.01
sessionE	1.11	3.57	2	0.004
sessionM	0.87	3.03	2	0.002
sexmale	1.24	3.58	1	0.003
substrateY	0.63	2.06	1	0.04
sessionE:sexmale	−1.33	−3.19	2	0.001
sessionM:sexmale	−0.77	−1.9	2	0.05
sexmale:substrateY	−1.28	−3.5	1	0.0005

d)
Circling ˜ substrate + (1|pair.id)

AIC				
1132.3				
variable	**estimate**	**z-score**	**df**	**p**
intercept	2.48	9.44		< < 0.01
substrate	−0.75	−1.99	1	0.05

e)
Mating-related ˜session + sex + (1|pair.id)

AIC				
1460.5				
variable	**estimate**	**z-score**	**df**	**p**
intercept	3.02	20.39		< < 0.01
sexmale	0.47	4.44	1	< < 0.01
sessionE	0.44	3.98	2	< < 0.01
sessionM	0.53	3.53	2	0.0004

f)
Aggressive˜session + sex + substrate + session*sex + sex*substrate + (1|pair.id)

AIC				
7823.1				
variable	**estimate**	**z-score**	**df**	**p**
intercept	3.04	13.46		< < 0.01
sessionE	0.13	3.46	2	0.005
sessionM	−0.14	−3.57	2	0.003
sexmale	0.58	12.56	1	< < 0.01
substrateY	0.93	2.85	1	0.004
sessionE:sexmale	−0.2	−3.53	2	0.0004
sessionM:sexmale	0.37	7.05	2	< < 0.01
sexmale:substrateY	−.71	−15.51	1	< < 0.01

g)
Contact˜session + sex + substrate + session*sex + sex*substrate + (1|pair.id)

(*continued on next page*)

Table 2 (*continued*)

g) Contact˜session + sex + substrate + session*sex + sex*substrate + (1|pair.id)				
AIC				
4992.6				
variable	**estimate**	**z-score**	**df**	**p**
intercept	2.77	13.52		< < 0.01
sessionE	−0.15	3.57	2	0.004
sessionM	−0.29	3.03	2	0.002
sexmale	0.55	3.58	1	0.003
substrateY	0.08	2.06	1	0.04
sessionE:sexmale	0.45	−3.19	2	0.001
sessionM:sexmale	0.72	−1.9	2	0.05
sexmale:substrateY	0.47	−3.5	1	0.0005
h) Non-contact ˜ sex + substrate + sex*substrate + (1|pair.id)				
AIC				
1601.1				
variable	**estimate**	**z-score**	**df**	**p**
intercept	3.9	23.86		< < 0.01
sexmale	0.23	1.6	1	0.1
substrateY	0.5	2.1	1	0.03
sexmale*substrateY	−0.7	−3.5	1	0.0003

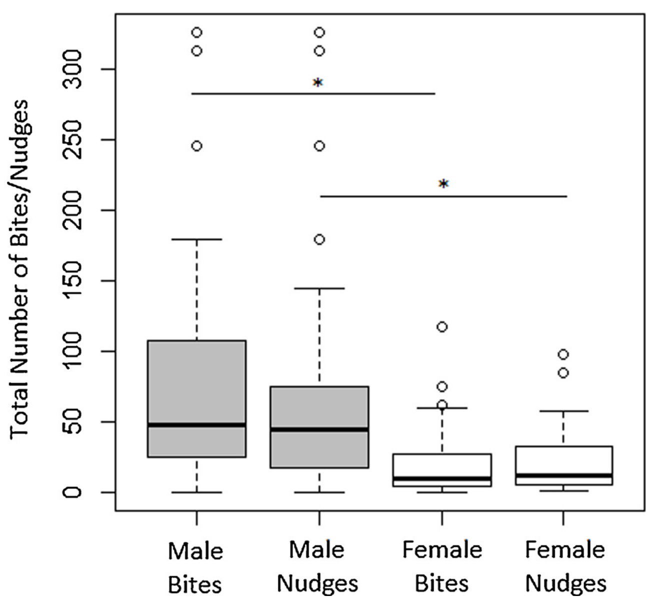

Fig. 1. Comparison of Bites and Nudges exhibited by males and females in dyadic pairs of zebrafish. Males show higher levels of bites ($p < 0.01$) and nudges ($p = 0.01$) than females both of which are contact behaviors. Asterisks indicate statistically significant differences ($p < 0.05$).

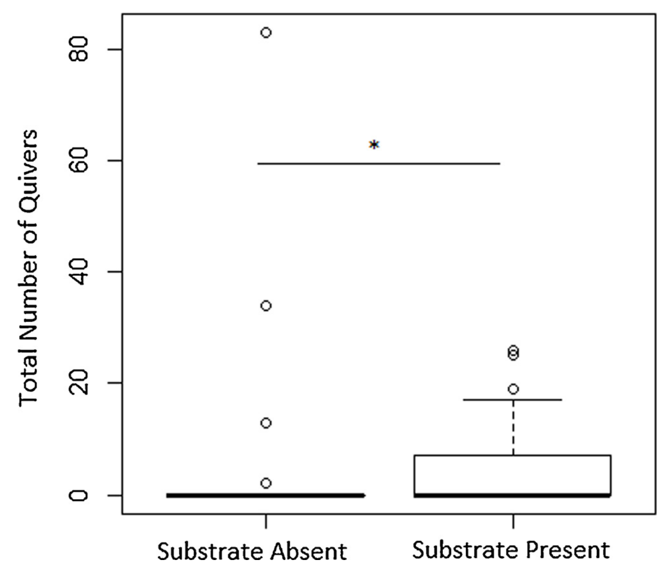

Fig. 2. Effect of substrate on quivering behavior among zebrafish dyadic pairs. In presence of substrate, quivering behavior is significantly increased ($p < 0.01$) compared to when substrate is absent. Statistically significant difference ($p < 0.05$) is indicated by an asterisk.

spawning and mating related activities have been reported in several fish species (de Vlaming, 1972). In zebrafish too, diel pattern in spawning and courtship behaviors have been observed both in the field and in laboratory conditions (Spence et al., 2008; Engeszer et al., 2007). Our study investigated the dynamics and factors regulating various interactive behaviors in a wild zebrafish dyad. Our results not only revealed the temporal dynamicity but also underlying sex differences in these behaviors. We found a clear temporal pattern to mating-related behaviors in zebrafish, supporting our predictions. We also found sexual dimorphism in the frequency of certain behaviors, especially for contact behaviors, which males exhibited more than females. Furthermore, our results established oviposition substrate as an important regulator of zebrafish behavior, especially for joint behaviors like circling and quivering.

5.1. Predictors of different types of interactive behaviors

While we did not find any behavior to be specific to either of the sexes, we did find that the frequency of many of the behaviors differed between the sexes. We found sex to be an important factor for bites as well as nudges. Males exhibited a significantly higher incidents of both these behaviors than females. Males showed higher levels of nudges especially in early morning and morning sessions, employing nudges act as tactile stimulation for the egg release from the females. The importance of males showing higher bites has been discussed in the last section.

We also found distinct interactions between the environmental factors (session and substrate presence) with sex, indicating that these factors affect behaviors differently for males and females. When substrate was present, females exhibited greater number of leads, especially in the morning. This could be indicative of her state of mating receptivity, as in the wild, courtship as well as mating are thought to

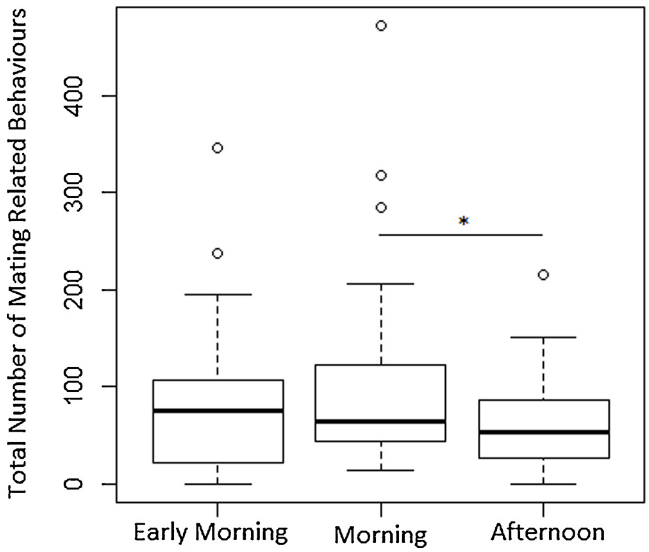

Fig. 3. Comparison of total number of mating-related behaviors across sessions among zebrafish dyads. The levels of mating-related behaviors are consistent across early morning (EM) and morning (M) sessions but significantly reduces ($p < 0.01$, indicated by an asterisk) in the afternoon (AF) session.

occur more in the early parts of the morning (Spence et al., 2008).

The lack of parental care in zebrafish may also make it more important for the females to spawn only when a proper egg-laying site is available. We found that presence of a substrate exacerbates the difference between males and females for certain specific behaviors. While females showed a higher number of leads, males showed higher number of nudges when substrate was present. Spatial aggregation patterns of the spawning sites can contribute to variation in reproductive success in a given species (Mück et al., 2013). In our experiment, as we used a single male-female pair with only one large oviposition substrate, we could not test for this variation, nonetheless it could be an important factor in natural systems.

We found that the number of quivers increased in the presence of oviposition substrate (Fig. 2). As it is a key behavior involved during pre-mating courtship, leading to mating, incidence of quivers can be considered a direct proxy to mating, (Darrow and Harris, 2004). Out of the 15 pairs that were observed without substrate, quivers were found in only 2 of them (13.3%), however, the number increased to 8 out of 13 pairs tested with substrate (61.5%). In species with no parental care, it is important for the female to find a suitable mating substrate (Deas and Hunter, 2013). The lack of a favored substrate can even lead to a block of oviposition in cabbage root fly *Delia radicum* (Kostal, 2013) and this may to explain the low frequency of quivers in our observational data in no-substrate condition. Incidences of circling behavior increased when substrate was present, which may be due to higher number of successful courtship attempts by the males as many of these circling behaviors would be associated with quivers.

5.2. Predictors of mating-related and aggressive behaviors

Photoperiod and temperature are also important factors controlling spawning in several fish families such as Salmonidae and Cyprinidae (de Vlaming, 1972) and also in catfish (Sundararaj and Vasal, 1976) to affect the reproductive physiology. In laboratory, as well as in the wild, zebrafish spawn at the onset of dawn (Darrow and Harris, 2004; Spence et al., 2008; Hutter et al., 2010) and it reduces as the day progresses. In our study, we found mating-related behaviors to be dependent on session i.e., time. We found comparable levels of the mating-related behaviors in both early morning as well as morning sessions but there was a significant reduction in the afternoon session which supports our hypothesis and the current literature (Fig. 3).

In a socially gregarious species like zebrafish, agonistic interactions can play an important role in territoriality, mate seeking as well as competition of food. As females can often be considered as a 'limiting resource', males may be expected to display strong aggressive behavior to gain access to females (Pyron, 2003). We had hypothesized that males would show more aggressive behavior than females. However, from our observations, effect of sex was not significant in predicting overall aggressive interactive behaviors. In general, however, we observed a high number of aggressive interactions within zebrafish dyads, as has also been reported in previous studies (Hutter et al., 2010). While both male-male and female-female dyads have been shown to display similar aggressive behaviors, dominant females direct lower aggression towards subordinate females, compared to males during dyadic interactions (Paull et al., 2010).

5.3. Predictors of contact and non-contact behaviors

An important finding in our study was the distinct higher levels of contact-based behaviors in males than in females (Fig. 1). Though our experimental design did not allow for testing of sexual coercion explicitly males are known to be coercive towards the females (Owen et al., 2012) which probably results in a high number of bites (as mentioned above) and as well as a significantly higher number of contact-based behavior among the males of this species. As with mating related behaviors, non-contact behaviors (that included chases, leads and circling), females were more sensitive to the presence (or absence) of substrate.

In conclusion, we found the zebrafish dyads to be dynamic in terms of temporal patterns, with specific behavioral repertoire for males and females. Our findings also provide insights into their behavioral phenotypes and can have potential applications in eco-toxicological research and medical fields that use zebrafish as model systems. For example, exposure to toxic chemicals have been shown to differentially affect behavioral mating responses and even their physiology among wild and laboratory-maintained zebrafish populations (Brown et al., 2012; Söffker et al., 2012) There have been very few studies which have investigated zebrafish interaction dynamics. Hutter et al. (2010) studied the interactions of zebrafish housed in a tank but to the best of our knowledge there has been no detailed study on dyadic interactions focusing on not only mating but also other interactive behaviors within the scope of the same design. As a group living species the design of our experiment is artificial in having a dyad, however, this allows us to gather data on various kinds of interactions between male and female zebrafish which can be further expanded on to a group or shoal paradigm. Also, even though we could not quantify coercive tactics this is an aspect that warrants further investigation. Incorporating observations on coercive behaviors in future investigations may provide a further understanding of social behaviors and mating strategies in wild zebrafish.

Ethical note

The study complied with the existing rules and guidelines outlined by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The study adhered to the Institutional Animal Ethics Committee's (IAEC) rules and guidelines of IISER Kolkata. No animals were euthanized or sacrificed during the study. All behavioral observations were conducted without any chemical or invasive treatment on the individuals. At the end of the experiments, no individuals were sacrificed and all were returned to stock tanks.

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