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ORIGINAL PAPER

Social network analysis resolves temporal dynamics of male dominance relationships

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Abstract Social organization is often studied through point estimates of individual association or interaction patterns, which does not account for temporal changes in the course of familiarization processes and the establishment of social dominance. Here, we present new insights on short-term temporal dynamics in social organization of mixed-sex groups that have the potential to affect sexual selection patterns. Using the live-bearing Atlantic molly (*Poecilia mexicana*), a species with pronounced male size polymorphism, we investigated social network dynamics of mixed sex experimental groups consisting of eight females and three different-sized males over a period of 5 days. Analyzing association-based social networks as well as direct measures of spatial proximity,

we found that large males tended to monopolize most females, while excluding small- and medium-bodied males from access to females. This effect, however, emerged only gradually over time, and different-sized males had equal access to females on day 1 as well as day 2, though to a lesser extent. In this highly aggressive species with strong social dominance stratifications, the observed temporal dynamics in male-female association patterns may balance the presumed reproductive skew among differentially competitive male phenotypes when social structures are unstable (i.e., when individual turnover rates are moderate to high). Ultimately, our results point toward context-dependent sexual selection arising from temporal shifts in social organization.

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Introduction

Males tend to differ in reproductive fitness (i.e., number of sired offspring, see Bateman 1948; Becher and Magurran 2004; Tatarenkov et al. 2008), and the resulting reproductive skew is largely attributable to female choice (intersexual selection) and male competition over mates (intrasexual selection, Bateman 1948 but see Gowaty et al. 2012 for a critical reevaluation of Bateman's studies). Intersexual and intrasexual selection can have similar effects on male trait evolution (e.g., both typically favor characters that increase fighting abilities, Berglund et al. 1996; Qvarnström and Forsgren 1998; Wong and Candolin 2005; Hunt et al. 2009). Nevertheless, males of many species exhibit pronounced variation in body size, even though large male body size is a good predictor not only of a high resource-holding potential (RHP, sensu Parker 1974) but also males' attractiveness

to females (e.g., Hunt et al. 2009). Explaining the existence of male size polymorphisms has been a frequent topic in behavioral ecology over the past several decades (field crickets Cade 1981; coho salmon Gross 1985; bluegill sunfish Dominey 1980; Pecos pupfish Kodric-Brown 1986), and live-bearing fishes (Poeciliidae) have been at the forefront of this research (*Xiphophorus variatus* Borowsky 1987; *Xiphophorus nigrensis* Zimmerer and Kallman 1989; Morris et al. 1992; Ryan et al. 1992; *Limia perugiae* Erbeling-Denk et al. 1994). Post-maturation body growth is strongly suppressed in male poeciliids (see Reynolds et al. 1993 for guppies, *Poecilia reticulata*), and variation in the onset of maturity is either determined genetically, via allelic variation at the so-called *P* locus (*Xiphophorus* spp. Kallman 1989; Lampert et al. 2010), or reflects a plastic response to environmental variation (*Poecilia latipinna* Trexler et al. 1990).

In theory, between-individual phenotypic variation can be maintained whenever different phenotypes have equal lifetime fitness at equilibrium when simultaneously considering differences in timing of the onset of reproduction, mating success, and longevity (for a review, see Wolf and Weissing 2010). The two most prominent evolutionary mechanisms proposed to stabilize (genetic or environmentally determined) between-individual phenotypic variation are the following: (a) frequency-dependent selection, where relative fitness advantages of different phenotypes depend on their frequency (Maynard-Smith 1982; Gross 1996; Sinervo and Lively 1996), and (b) context-dependent selection, whereby the relative fitness of either phenotype varies as a function of extrinsic factors that vary over time and/or space (Qvarnström 2001; Cornwallis and Uller 2010; Wolf and Weissing 2010).

In species such as poeciliids, small males adopt distinct behavioral phenotypes, often called alternative mating tactics, to achieve reproductive success (Parzefall 1969; Travis and Woodward 1989; Reynolds et al. 1993; Gross 1996; Taborsky and Brockmann 2010). For example, large males in swordtails and sailfin mollies regularly court females to solicit consensual mating (Parzefall 1969; Zimmerer and Kallman 1989; Niemeitz et al. 2002), while small males show sneak-like mating behavior instead (Travis and Woodward 1989; Bisazza 1993). Several studies suggest that the reproductive success of alternate behavioral and morphological phenotypes can be context-dependent (reviewed in Cornwallis and Uller 2010 as well as in Taborsky and Brockmann 2010). For instance, Bisazza et al. (2001) proposed that seasonal variation in densities and sex ratios in eastern mosquitofish (*Gambusia holbrooki*) populations creates alternating selective regimes that favor the small male phenotype when population densities are low and sex ratios female-biased (in spring) and, alternatively, the large phenotype with high RHP, when densities are high and sex ratios more male-biased (late summer). Small-bodied

males generally benefit from an earlier onset of sexual maturation, translating into a higher likelihood of actually reaching maturity and a longer reproductive life span (for coho salmon, *Oncorhynchus kisutch*, see Gross 1985). Upon reaching maturity, large males may be disproportionately targeted by predators, especially if they develop colorful sexual ornaments (Trexler et al. 1994; Pocklington and Dill 1995; Tobler et al. 2008).

Components of reproductive success of different poeciliid male phenotypes are typically estimated in laboratory experiments exploring female preferences (reviewed by Jennions and Petrie 1997), male reproductive effort (Travis and Woodward 1989; Reynolds et al. 1993; Magellan and Magurran 2007; Plath 2008), and male competition (reviewed in Hunt et al. 2009) or through the observation of focal individuals in the wild (Morris and Ryan 1992; Witte and Ryan 2002; Köhler et al. 2011). Similarly, genetic analyses can provide valuable insights into paternity patterns (Schartl et al. 1993; Becher and Magurran 2004; Tatarenkov et al. 2008; Girdt et al. 2012). However, recent advances in the field of animal social network analysis (Krause et al. 2007; Croft et al. 2008, 2011; Wilson et al. 2013) have been proposed for their potential application to the study of mating systems and alternative male mating tactics (McDonald et al. 2013). Nevertheless, social network analyses are often based on sampling methods that do not allow the exploration of temporal dynamics in social organization (but see Kelley et al. 2011; Darden et al. 2009).

Social groups of poeciliids exhibit some degree of stability in the wild (e.g., Croft et al. 2005). Still, turnover within and between social groups, as found in larger streams (Kelley et al. 1999) or after flood events (Plath et al. 2010), may reset familiarity among the members of social networks, thus breaking up social network structures. This instability of social organization ultimately has the potential to balance the aforementioned effects of social dominance on different males' reproductive success.

Our present study addresses the question of whether emerging familiarity among group members after a simulated disturbance event (i.e., combining unfamiliar individuals within a group) affects patterns of social organization and ultimately patterns of sexual selection. We used association-based network analysis (see Croft et al. 2008) to characterize social interactions between different male phenotypes, among females, and between males and females, in the live-bearing fish *Poecilia mexicana* during a familiarization period among group members. We composed small, mixed sex experimental groups of nonfamiliar individuals that covered the range of naturally occurring size polymorphism and scored measures of social proximity over a period of 5 consecutive days to establish individuals' position in their social network in relation to body size and sex. This enabled us to observe the temporal dynamics of social organization, including the

formation of dominance hierarchies, and their effect on males' opportunities to access females, a proxy for male reproductive success in poeciliids (see Walling et al. 2010 for the related *Xiphophorus hellerii*).

Methods

Study organisms and their maintenance

The Atlantic molly, *P. mexicana*, is widespread in freshwaters along the Central American Atlantic coast (Miller 2006). Atlantic mollies have a promiscuous mating system (Plath et al. 2003; Köhler et al. 2011), and males form dominance hierarchies by means of aggressive combat (Parzefall 1969; Bierbach et al. 2012, 2013). Based on qualitative laboratory observations, Parzefall (1969) proposed a linear dominance structure for this species, with large males monopolizing small groups of females and defending them against intruding smaller, subordinate males. Body size distributions of males from different Mexican populations show Gaussian distributions and pronounced variation around the population mean (Plath et al. 2003; Riesch et al. 2011). In contrast to some other mollies in which large males show elaborate courtship displays (Parzefall 1969; Niemeitz et al. 2002), even large-bodied Atlantic molly males do not court and defend distinct territories. They mostly rely on consensual mating, as females have a mating preference for large male body size (Plath et al. 2004; Bierbach et al. 2011a), while small males often exhibit “ambushing” behavior (i.e., they hide near groups of females and attempt forced copulations; Plath et al. 2003).

Fish used in our experiments were laboratory-reared descendants of individuals collected in coastal brackish ditches near the Mexican city of Tampico (Tamaulipas, Mexico) in 2003. They were reared in large (6,000-L) fish culture tanks at the aquaculture facilities of the Academic Division for Biological Sciences at Universidad Juárez Autónoma de Tabasco (DACBIOL-UJAT) in Villahermosa. Sex ratios in the rearing tanks were somewhat skewed to females, approximately 1:2 (males/females). Water temperature was maintained at a natural range of 27–29 °C under an ambient, approximately 12:12 h, light/dark cycle. Fish were fed at least twice daily with commercially available flake food (TetraMin® Tetra GmbH), frozen chironomid larvae, bosmids, *Artemia salina* shrimps, and frozen spinach.

Experimental design

For the construction of social networks, identification of individual group members is necessary. To this end, we briefly anesthetized all test individuals using clove oil and injected small spots of visible implant elastomer (VIE, Northwest Marine Technology, Inc.) under the dorsal epidermis following

the protocol described by Croft et al. (2003). Thus, each individual was given a unique identification tag enabling us to recognize individuals from above throughout the experiment. No mortality was associated with the tagging procedure, and all fish behaved calmly and showed no signs of distress after recovery from anesthesia. After the tagging procedure, fish were transferred in small groups (five to eight individuals from the same stock tank) into well-aerated 54-L stock tanks and were given 1 week to recover. Only 3 out of 77 tagged fish (4 %) lost the tag within this period of time.

We observed six independent experimental groups of *P. mexicana* comprising 11 individuals each. All individuals in an experimental group were taken from different stock tanks and were therefore not familiar. Our choice of group size was based on our need to be able to identify all group members at any given time (Kelley et al. 2011) and on the observation that small shoals of this size are common in the wild (i.e., ~5 to 20 individuals, Köhler et al. 2011). Natural wild *P. mexicana* populations are typically female-biased, with reported adult sex ratios (males/females) ranging from 0.07 to 0.5 (mean \pm SE = 0.32 \pm 0.06; Plath and Tobler 2010). Therefore, experimental groups were composed of three males and eight females (sex ratio=0.38). Table S1 lists the standard lengths (SLs) of all test fish. Previous studies also revealed pronounced body size variation in both sexes in natural populations, and so, we selected test individuals within each experimental group to roughly span the natural sex-specific size ranges (Plath et al. 2003; Riesch et al. 2011). We also made sure that body size variation did not differ between experimental groups (Levene's tests separate for each sex did not reveal significant differences in variance).

Behavioral observations were conducted between the 13th June and 24th August 2012 under a glass rooftop at the aquaculture facilities of the DACBIOL-UJAT in Villahermosa. We used a circular test arena (a blue wading pool, Simex Sport GmbH, Viersen, Germany) with a diameter of 160 cm. The water level was kept at 15 cm, and we installed an aquarium heater, a water filter (EHEIM professional™ 3 600, EHEIM GmbH & Co. KG), and a UV filter (Tetratrec UV 400, Tetra GmbH, Melle, Germany) to prevent algal blooms. The test arena was further aerated by an air pump. A plastic ruler was placed centrally on the bottom and served as a size standard. Above the test arena, we positioned a digital SLR camera (EOS 600D, Canon, Inc.) at approximately 2-m height. We used the Canon Utility™ (Canon, Inc.) to connect the camera with a personal computer. This system allowed us to automatically photograph the entire test arena periodically while creating minimal disturbance to the experimental fish group.

To start a trial, males and females were transferred into the test arena at 9:00 a.m. and were left undisturbed for 1 h. After this habituation period, we started photographing the test arena and automatically took one picture per minute over a period of 30 min. This interval was recently chosen in a study

by Kelley et al. (2011) to analyze social networks in the Trinidadian guppy (*P. reticulata*) with a similar experimental setup. Approximately 10 min before the 30-min observation period, the heater, filter, and air stone were gently removed from the test arena to ensure that all fish would be visible throughout the recording session. We repeated these recordings on 5 consecutive days, starting each day at 10:00 a.m. Bierbach et al. (2011b) found that *P. mexicana* males remembered the sexual activity of a rival after 1 week of familiarization, and as such, we assumed a 5-day period to be sufficient to observe patterns of emerging familiarity.

Fish were fed twice a day (at 8:00 a.m. and at 16:00 p.m.) with TetraMin® flake food. Water was exchanged by aged tap water once a 5-day session was completed and after test fish had been moved to a new stock tank; afterward, they were no longer involved in the current study.

Measuring associations among individuals

Studies using Trinidadian guppies often defined dyadic associations or interactions as occurring when two individuals were observed within a range of four body lengths (Croft et al. 2008). However, mollies show pronounced variation in body size (see Table S1 and above), rendering such an approach impracticable. We therefore defined individuals as interacting when they were within a radius of 15 cm measured from the middle of the head of a focal individual. To build networks for each of the six groups and for each day, we calculated a simple ratio association index (AI, see Whitehead 2008) by summing all events during which two individuals (either male-female, male-male, or female-female) were observed in association and divided this by the total number of possible interactions (30 recordings per day). The resulting values can range between 0 (dyad never observed) and 1 (individual dyad observed on all photos). Since we repeated our observations for each of the $N=6$ groups for 5 consecutive days, we constructed 30 social networks.

Based on the AI values of each individual, we calculated individuals' network strength as the sum of its AI values with all interaction partners (Newman 2004). Network strength reflects the general sociability of an individual (Lusseau et al. 2008). We further scored the distance of an individual to its nearest neighbor on each photo with FixFoto software (Joachim Koopmann Software, Inc.).

Statistical analysis

The major aim of our study was to investigate whether an individual's network position is defined by its sex and body size (within sexes) and how this is changing over time. In Trinidadian guppies, individual centrality (a measure of "network strength" in unweighed networks, see Croft et al. 2008)

appears to be linked to absolute body size, with some support for size-assortative association patterns (Croft et al. 2005). In Atlantic mollies, however, body size varies to a much larger extent (see above), rendering analyses based on absolute body size impractical. As such, we assigned group-specific size ranks to each individual such that the largest females in each group received a score of "1" and the smallest "8." We also used rank assignment to categorize males in each group, and sex-specific size ranks were used for the statistical analyses. Mean SLs (\pm SEM) of each size rank are depicted in Fig. 2a (see also Table S1).

Potential pitfalls in analyzing network data were pointed out by Croft et al. (2011), as relationships between individuals within a social network are highly interconnected. It was thus suggested to compare replicated networks each producing a single independent network-level metric, therein avoiding the analysis of individual-based data—a harsh limitation. To circumvent this limitation, we used individual network strength as a dependent variable in a generalized linear mixed model (GLMM, GENLIMIX procedure in SPSS 21, IBM, Inc.) that accounted for structural autocorrelations in the repeated measurements (i.e., network strength of size rank 1 at day 1 is more strongly connected to size rank 2 at day 1 than to size rank 1 at day 2 and so forth) using a first-order autoregressive covariance structure ("AR1" in SPSS, see Dormann et al. 2007 for a review). For the GLMM, we specified a normal error distribution and an identity link function. Throughout, Satterthwaite approximations were employed to determine degrees of freedom in the statistical models; so, df values in the final models might not represent actual sample sizes. As we were particularly interested in the network positions of different-sized individuals within a group rather than differences between groups, we entered "group ID" ($N=6$) as a random factor in our model for which "size rank (nested within sex)" (11 levels) and "day" (5 levels) were defined as repeated measures. Both repeated measure factors as well as their interaction term were initially included in the analytical model; however, the interaction term was not significant (see results). Nonetheless, the interaction term was kept in the final model as its removal decreased the model's statistical power (Δ Akaike information criterion (AIC)=23.1). Post hoc pairwise Fisher's least significant difference (LSD) tests were conducted based on estimated marginal means derived from the final model.

We proceeded to ask whether individuals' distance to the nearest neighbor differed between individuals in our experimental groups. We used mean distances to the respective nearest neighbors as the dependent variable in another GLMM with similar model structure but with a log link function. The interaction term "day \times size rank (nested within sex)" had no significant effect ($F_{40,206}=0.61$, $P=0.97$) and was excluded from the final model as removal increased the model's statistical power (Δ AIC=-1.5).

Since both “network strength” and “distance to nearest neighbor” derive from the same spatial distances, we could confirm a strong correlation of both measures through Spearman’s rank order tests (significant negative correlation in all groups and at all experimental days). Nevertheless, two separate analyses seem to be useful in our case as absolute distances have not yet been used for describing poeciliid social networks, and our analysis thus provides a link between a standard network metric (“network strength”) and a biologically relevant measure of interaction (“distance to nearest neighbor”).

In our third analysis, we asked whether the three different-sized males had differential access to females and whether males differ in AI determined from male-male interactions. We calculated mean AI values for each male size rank and each of the eight females and among males. These AI values (arcsine square root transformed to decouple the variance from the mean) were included as the dependent variable in a GLMM with normal error distribution and an identity link function. Again, group ID was set as random factor while “male size rank” and “day” were repeated measure factors (with a first-order autoregressive covariance structure). We included the repeated measure factors and size rank (nested within sex and group) as fixed factors and all possible interaction terms in our initial model. However, as male size rank is also part of size rank, only valid interactions were included in our model (i.e., no male size rank 1 × male size rank 1). Nonsignificant interactions were removed from the final model when removal increased the model fit (day × size rank (nested within sex and group) × male size rank $F_{68,568}=0.31$, $P=0.99$, $\Delta AIC=-135.2$; day × size rank (nested within sex and group) $F_{40,478}=0.27$, $P=0.99$, $\Delta AIC=-120.8$). Estimated marginal means were compared using post hoc pairwise LSD tests and back-transformed ($y=\sin(y')^2$) to the original scale for presentation.

Results

Network strength

Overall, mean network strength decreased gradually by ca. 50 % during the course of the experiment, indicating that individuals decreased their sociability, i.e., number and time spent with conspecifics (Fig. 1), and accordingly, the factor day was highly significant in the GLMM using network strength of different-sized individuals as the dependent variable (Table 1a). Also, the factor size rank (nested within sex) had a highly significant effect (Table 1a): The network strength (a measure of interaction with other males and females) of males of medium and small size rank was significantly lower than that of males of the largest size class. Network strength decreased—although to a much lesser

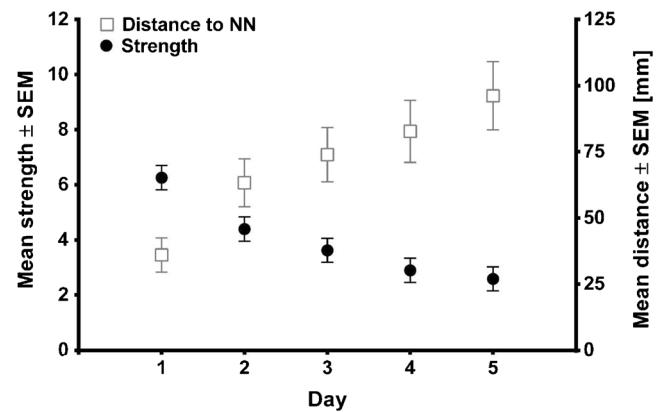


Fig. 1 Network strength and distance to the nearest neighbor (NN) during the course of the experiment (5 days). Depicted are estimated marginal means (\pm SEM) derived from two GLMMs (see main text). Post hoc LSD tests revealed that network strength was significantly higher on day 1 compared with all other days and on day 2 compared with days 4 and 5. Regarding mean distances to nearest neighbors, day 1 differed significantly from days 3 to 5 while day 2 differed from day 5 (LSD tests)

extent—with increasing size rank (i.e., smaller body size) also in females. Most of the larger-bodied females showed network strengths similar to the largest male; the smallest female in a group, however, had a network strength comparable with the smallest male (Fig. 2b).

Our model did not detect a significant effect of the interaction term day × size rank (nested within sex) (Table 1a), suggesting a high consistency of differences in network strength between different-sized individuals over the course of the experiment. The random factor “group” also had no significant effect (Table 1a).

Mean distance to the nearest neighbor

In congruence with the results from our analysis of network strength, individuals increased the distance to their nearest neighbor by almost 300 % during the course of the experiment (Fig. 1).

Our GLMM using mean distances to the nearest neighbor as the dependent variable found the factors day and size rank (nested within sex) to have significant effects (Table 1b). We found the pattern of size rank-dependent variation described for network strength (see above) to be largely mirrored by the variation seen in nearest neighbor distances, as low values for nearest neighbor distances translated into a high network strength and vice versa (Fig. 2c). The random factor group was not significant (Table 1b).

Association index and male size rank

The last GLMM used AIs of males as the dependent variable and tested how size ranks affect association patterns. We detected a significant effect of the interaction term male size rank × size rank (nested within sex and population) (Table 2),

Table 1 Results from GLMMs using “day” and “size rank (nested within sex)” as repeated measure factors with a first-order autoregressive (AR1) covariance structure (repeated subject group) and “group” as random

factor (see main text for details). (A) The dependent variable was the network strength per individual size rank. (B) The dependent variable was the distance to the nearest neighbors per individual size rank

(A) Network strength					
Fixed effects	<i>F</i>	<i>df</i> _{factor}	<i>df</i> _{error}	<i>P</i> value	
Day	15.37	4	29	<0.001	
Size rank	7.54	10	224	<0.001	
Day × size rank	0.33	40	199	0.99	
Random and residual effects	Variance (estimated)	SEM	Wald's <i>Z</i>	<i>P</i> value	
AR1, diagonal	1.95	0.33	5.96	<0.001	
AR1, rho	0.80	0.04	22.99	<0.001	
Group	0.40	0.38	1.38	0.29	
(B) Mean distance to nearest neighbor					
Fixed effects	<i>F</i>	<i>df</i> _{factor}	<i>df</i> _{error}	<i>P</i> value	
Day	16.12	4	172	<0.001	
Size rank	18.09	10	106	<0.001	
Random and residual effects	Variance (estimated)	SEM	Wald's <i>Z</i>	<i>P</i> value	
AR1, diagonal	1,332.67	116.49	11.44	<0.001	
AR1, rho	0.26	0.06	4.35	<0.001	
Group	0.09	0.06	1.50	0.14	

Significant *P* values are in boldface

suggesting that different-sized males showed contrasting interaction patterns (Fig. 3a). Post hoc LSD tests on estimated marginal means revealed that the largest males associated significantly more with large females (up to the size rank 6) than medium- and small-sized males. Interestingly, males from the smallest (but not the intermediate) size class interacted most with the smallest female (size rank 8); a tendency in this direction can also be seen for the second smallest female (size rank 7; Fig. 3a). By contrast, little variation was seen in association patterns among males (Fig. 3a).

The interaction term day × male size rank had a significant effect (Table 2), which reflects that mean AIs of different-sized males were indistinguishably high on day 1 but then decreased more strongly over the course of our experiment in males of medium and small size rank compared with large-bodied males (Fig. 3b; see also significant main effect of day in Table 2). After the second day, males from the largest size class had higher AIs than males of the two other size ranks (see also the significant main effect of the factor male size rank in Table 2), but the smallest-sized males had higher AI values than medium-sized males at the end of the experiment (Fig. 3b). The random factor group had no significant effect in our final model (Table 2).

Discussion

Our study demonstrates the utility of association-based social network analysis for the study of temporal dynamics in social

organization. Metrics derived from social network analysis provided remarkably detailed insights on the emergence of dominance relationships between different-sized males and their effect on males' access to females over time while confirming several presumed patterns (compare Parzefall 1969, 1979; Bierbach et al. 2012).

We found strong support for a social dominance hierarchy among males, with the largest male in a group invariably becoming dominant and monopolizing most females. Dominance hierarchies were not linear across different male size classes though, as has been proposed based on qualitative observations for our study species (Parzefall 1969) as well as other poeciliids (Morris et al. 1992; Schartl et al. 1993). Large *P. mexicana* males had considerably higher network strengths than medium and small-bodied males, which were directly mirrored by a closer spatial proximity to females. Over the course of our experiment, however, a pattern emerged wherein medium-sized males interacted even less with females than the smallest males. Bierbach et al. (2012) found that staged fights between *P. mexicana* males are most likely to escalate when body size differences between opponents are small (i.e., less than 12 % in SL). Similar patterns have been reported for swordtails (Beaugrand et al. 1991; Morris et al. 1992). We argue that medium-sized males were more easily detected by large-bodied males and, consequently, were subject to more aggressive attacks, leading to a peripheral position in the social networks. Also, *P. mexicana* females have a mating preference for large male body size (Bierbach et al. 2011a), rendering the medium-sized male in our experimental

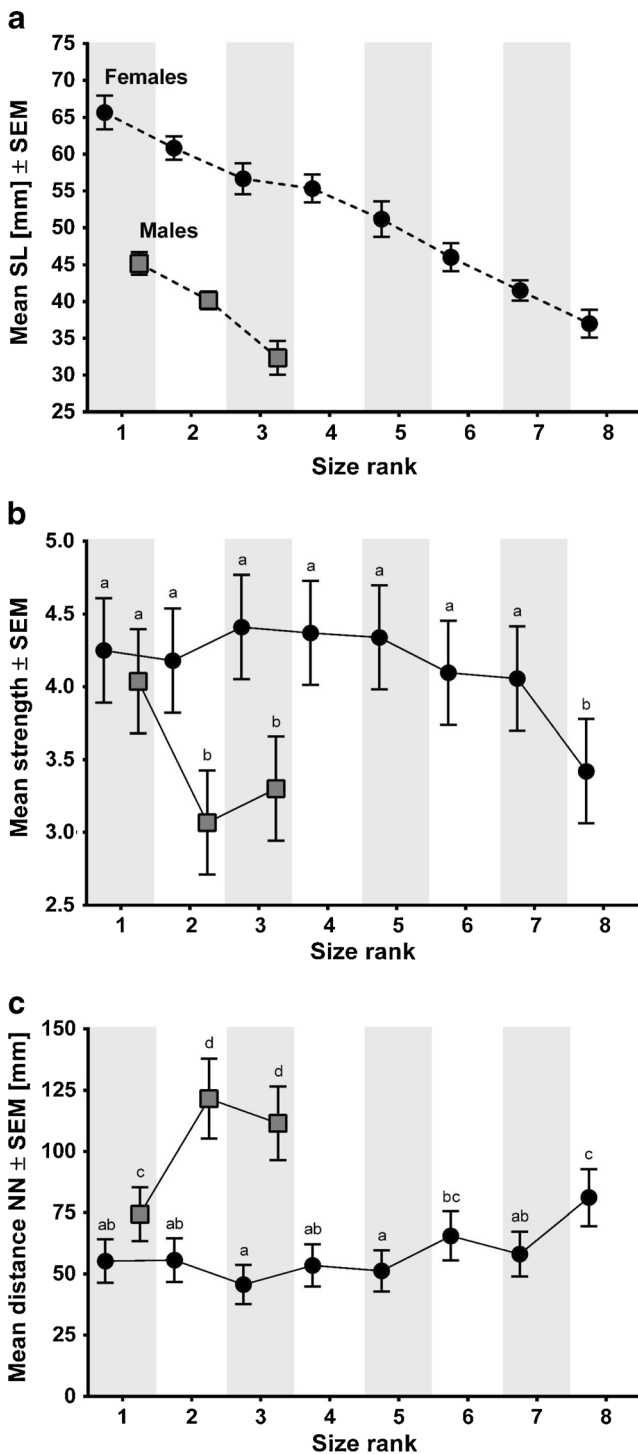


Fig. 2 **a** Mean body size (SL±SEM), **b** mean network strength, and **c** mean distance to nearest neighbor (NN) males and females sorted by size rank. Estimated marginal means (±SEM) derived from a GLMM (see main text) are depicted in **b** and **c**. Size ranks carrying the *same* letters did not differ in post hoc LSD tests

setup a larger threat to the dominant male compared with the smallest male in a group.

Our results imply a reproductive disadvantage for small- and especially medium-sized males, as they were largely

excluded from access to females. If we accept a correlation between interaction frequency (as well as spatial proximity) between males and females and male mating success, as shown for the related green swordtail (see Walling et al. 2010), this pattern ought to translate into directional selection in favor of large male body size (or potentially disruptive selection on male body size), which raises the question of why different size classes persist in natural populations (Plath et al. 2003; Riesch et al. 2011). We argue that temporal variation in social organization due to environmental disturbance could help solve this conundrum (see also Taborsky and Brockmann 2010): Our results suggest that there is an important time component in the establishment of social organization, with the most prominent changes occurring during the first to second day of the experiment. In general, network strength and association frequencies decreased over time—probably as a result of test fish habituating to their new environment (Brown 2001) and increasing familiarity among group members (Morrell et al. 2008), translating both into reduced shoal formation due to less risk averse behavior. However, also the aforementioned patterns of size-related differences in males' ability to access females arose gradually over time. Males of all size classes had similar access to females on day 1 and (with the exception of the medium-sized male) on day 2, while the largest male in a group appears to exclude subordinate males more successfully from day 3 onward. Dominance hierarchies and other social structures require some time to establish. For example, repeated aggressive interactions might be necessary to form stable hierarchies (Dugatkin and Dugatkin 2007), or group members might need to familiarize with one another (Griffiths and Magurran 1997; Darden et al. 2009). Interestingly, Kelley et al. (2011) did not find a comparable temporal effect on social network measures in groups of unfamiliar female guppies. This discrepancy is likely due to a somewhat different pretreatment of test fish. Although females in their study were caught at different sites (and thus presumed to be unfamiliar), those later assigned to the same groups had been transported to the laboratory within the same tanks. Test fish were then kept together for at least 2 days during elastomer tagging and acclimation before recording of social networks started. Given that noticeable changes in network strength and spatial proximity in our study occurred within the first 2 days after unfamiliar fish were cohobated, it seems likely that fish observed by Kelley et al. (2011) had already been familiar with each other. Hence, their findings and those of the present study might be indicative of the fast development of social organization in small groups of live-bearing fishes. Furthermore, fish used by Kelley et al. (2011) could have familiarized much faster as only females were used in that study, and it is known that the inclusion of males in social networks disrupts social structures and thus delays the development of familiarity (Darden et al. 2009).

Table 2 Results from GLMM using the strength of association (AI) obtained for each male size class as the dependent variable and “day” and “male size rank” as repeated measure factors (see main text) with a first-order autoregressive (AR1) covariance structure

Fixed effects	<i>F</i>	<i>df</i> _{factor}	<i>df</i> _{error}	<i>P</i> value
Day	91.21	4	397	<0.001
Size rank	0.66	10	170	0.76
Male size rank	49.82	2	526	<0.001
Day × male size rank	2.08	8	654	0.035
Size rank × male size rank	5.04	17	482	<0.001
Random and residual effects	Variance (estimated)	SEM	Wald’s <i>Z</i>	<i>P</i> value
AR1, diagonal	0.036	0.002	17.26	<0.001
AR1, rho	0.410	0.04	11.70	<0.001
Group	0.014	0.009	1.53	0.13

Significant *P* values are in boldface

Still, occasional or seasonal disturbance in aquatic ecosystems, like predator attacks or floods, as well as the death or emigration of individuals (and arrival of new individuals)

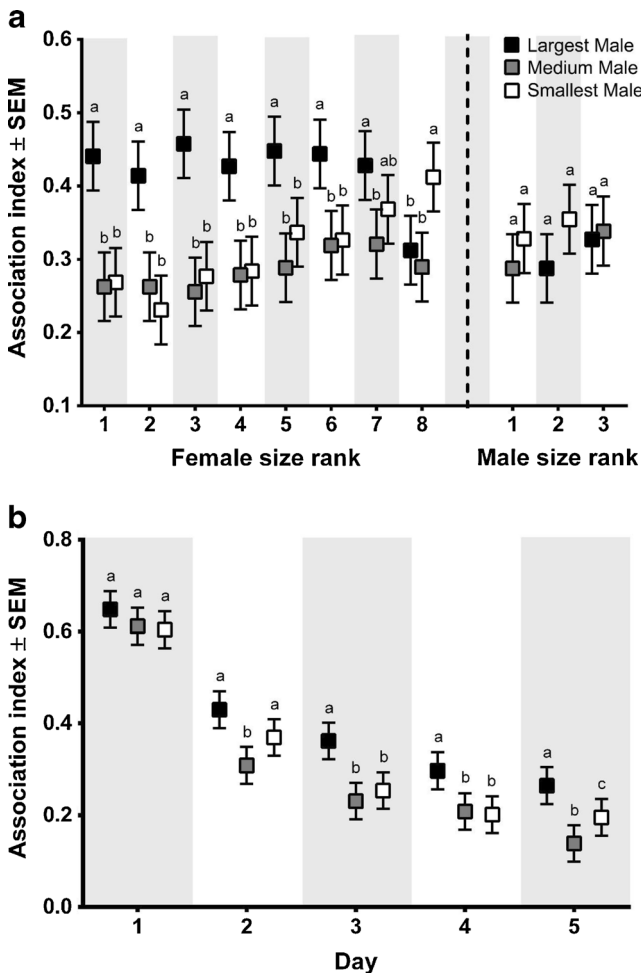


Fig. 3 a Association index (AI) between males and females of different size ranks (left) and among males (right). b Mean AI of males of different size ranks and female association partners during the course of the experiment. Identical letters indicate no significant difference in post hoc LSD tests. Depicted are estimated marginal means derived from a GLMM (±SEM, back-transformed from arcsine (square root)-transformed values used for the statistical analysis)

ought to reset the social organization of local fish societies at regular intervals (see discussion in Sih et al. 2009)—leading again to more equal mating opportunities of different male phenotypes. Given that small males have an earlier onset of maturity as well as a reduced risk of falling victim to predation, instability in social organization (due to environmental fluctuation) might provide them with sufficient mating opportunities to gain equal lifetime fitness compared with large, dominant males. It has been argued in another context that environmental fluctuation and/or disturbance maintains high species diversity in tropical rain forests and coral reefs (“intermediate disturbance hypothesis”; Connell 1978), because every disturbance will be followed by a phase of random success of differently competitive species. An example of how disturbance or seasonal changes affect different male phenotypes’ reproductive success comes from a study on maternally mouthbrooding cichlids (*Ctenochromis horei*) from Lake Tanganyika (Sefc et al. 2009). While dominant males monopolized females in the dry season, resulting in low rates of multiple paternity (ca. 14 % of the broods were multiply sired), the patterns changed completely during the rainy season as all broods had multiple sires (Sefc et al. 2009). Interestingly, turbidity increased strongly from the dry to rainy season, and the authors hypothesized that this causes dominant males to fail defending females from intruding subordinates.

A word of caution is required, as a previous study found dominant males of *L. perugiae* in stable social groups to sire almost all offspring when cohabited with only one subordinate male but fail to reproduce when kept with three subordinate males (Schartl et al. 1993). The authors hypothesized that dominant males spent most of their time attacking intruding subordinates in the latter situation. However, Schartl et al. (1993) used relatively small test aquaria (180-L, 100-cm length), which could explain why dominant males failed to monopolize females; still, the effects of varying sex ratios on male mating success in poeciliids certainly warrant further attention (see also Makowicz and Schlupp 2013). Furthermore, we are not aware of any study reporting on group turnover rates for our study species in the wild, although

annual flooding events are common and do have the potential to disrupt established social or spatial organizations entirely (e.g., Plath et al. 2010). However, we do not know yet how stable *P. mexicana* groups are over prolonged periods of time, which should be a subject of future research in this species.

We did not observe strong size-assortative interaction patterns among females; only the smallest females showed significantly fewer interactions with other females. This led to some degree of size-assortative interactions between sexes, as small-bodied males interacted most with small-bodied, peripheral females. Our results are not congruent with studies on wild guppy populations: Croft et al. (2003) found female shoals to be more homogenous in body size compositions than expected by chance. Another study found the body size of individual shoal members to be positively correlated with the body size of their interaction partners (Croft et al. 2005). Croft et al. (2003) proposed that shoal mate choice during shoal fission-fusion events and microhabitat choice based on body length may contribute to these patterns. In our study, shoal mate choice was obviously restricted, and no heterogeneous microhabitat structure was provided.

In summary, our study identifies social network analysis as a useful tool to detect temporal fluctuation in animal social organization. Specifically, we argue that the reported changes in male-female association patterns over the course of the experiment may balance mating opportunities of differently competitive male phenotypes (i.e., different-sized males) and thus represent context-dependent sexual selection.

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Ethical standards The experiments reported here comply with the current laws of the United States of Mexico and were run under the federal permits from Mexican agencies SAGARPA/CONAPESCA (DGOPA.09004.041111.3088) and SEMARNAT/Dirección General de Vida Silvestre (SGPA/DGVS/04315/1).

References

- Bateman AJ (1948) Intra-sexual selection in *Drosophila*. *Heredity* 2: 349–368
- Beaugrand J, Goulet C, Payette D (1991) Outcome of dyadic conflict in male green swordtail fish, *Xiphophorus helleri*: effects of body size and prior dominance. *Anim Behav* 41:417–424
- Becher SA, Magurran AE (2004) Multiple mating and reproductive skew in Trinidadian guppies. *Proc R Soc Lond B* 271:1009–1014
- Berglund A, Bisazza A, Pilastro A (1996) Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biol J Linn Soc* 58: 385–399
- Bierbach D, Klein M, Sassmannshausen V, Schlupp I, Riesch R, Parzefall J, Plath M (2012) Divergent evolution of male aggressive behaviour: another reproductive isolation mechanism in extremophile poeciliid fishes. *Intern J Evol Biol* 2012:148745
- Bierbach D, Makowicz AM, Schlupp I, Geupel H, Streit B, Plath M (2013) Casanovas are liars: behavioral syndromes, sperm competition risk, and the evolution of deceptive male mating behavior in live-bearing fishes [v3, indexed]. *F1000Research* 2:75
- Bierbach D, Schulte M, Herrmann N, Tobler M, Stadler S et al (2011a) Predator-induced changes of female mating preferences: innate and experiential effects. *BMC Evol Biol* 11:190
- Bierbach D, Girndt A, Hamfler S, Klein M, Müksch F et al (2011b) Male fish use prior knowledge about rivals to adjust their mate choice. *Biol Lett* 7:349–351
- Bisazza A, Vaccari G, Pilastro A (2001) Female mate choice in a mating system dominated by male sexual coercion. *Behav Ecol* 12:59–64
- Bisazza A (1993) Male competition, female mate choice and sexual size dimorphism in poeciliid fishes. *Mar Behav Physiol* 23:257–286
- Borowsky RL (1987) Genetic polymorphism in adult male size in *Xiphophorus variatus* (Atheriniformes: Poeciliidae). *Copeia* 1987: 782–787
- Brown C (2001) Familiarity with the test environment improves escape responses in the crimson spotted rainbowfish, *Melanotaenia duboulayi*. *Anim Cogn* 4:109–113
- Cade WH (1981) Alternative male strategies: genetic differences in crickets. *Science* 212:563–564
- Connell JH (1978) Diversity in tropical rain forests and coral reefs. *Science* 199:1302–1310
- Comwallis CK, Uller T (2010) Towards an evolutionary ecology of sexual traits. *Trends Ecol Evol* 25:145–152
- Croft DP, Arrowsmith BJ, Bielby J, Skinner K, White E, Couzin ID, Magurran AE, Ramnarine I, Krause J (2003) Mechanisms underlying shoal composition in the Trinidadian guppy, *Poecilia reticulata*. *Oikos* 100:429–438
- Croft DP, James R, Krause J (2008) Exploring animal social networks. Princeton University Press, Princeton
- Croft DP, James R, Ward AJW, Botham MS, Mawdsley D, Krause J (2005) Assortative interactions and social networks in fish. *Oecologia* 143:211–219
- Croft DP, Madden JR, Franks DW, James R (2011) Hypothesis testing in animal social networks. *Trends Ecol Evol* 26:502–507
- Darden SK, James R, Ramnarine IW, Croft DP (2009) Social implications of the battle of the sexes: sexual harassment disrupts female sociality and social recognition. *Proc R Soc Lond B* 276:2651–2656
- Dominey WJ (1980) Female mimicry in male bluegill sunfish—a genetic polymorphism? *Nature* 284:546–548
- Dugatkin LA, Dugatkin AD (2007) Extrinsic effects, estimating opponents' RHP, and the structure of dominance hierarchies. *Biol Lett* 3: 614–616
- Dormann FC, McPherson JM, Araújo MB, Bivand R, Bolliger J et al (2007) Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. *Ecography* 30:609–628
- Erbelding-Denk C, Schröder J, Schartl M, Nanda I, Schmid M, Epplen J (1994) Male polymorphism in *Limia perugiae* (Pisces: Poeciliidae). *Behav Genet* 24:95–101
- Girndt A, Riesch R, Schröder C, Schlupp I, Plath M (2012) Multiple paternity in different populations of the sailfin molly (*Poecilia latipinna*). *Anim Biol* 62:245–262
- Gowaty PA, Kim Y-K, Anderson WW (2012) No evidence of sexual selection in a repetition of Bateman's classic study of *Drosophila melanogaster*. *P Natl Acad Sci USA* 109:11740–11745
- Griffiths SW, Magurran AE (1997) Familiarity in schooling fish: how long does it take to acquire? *Anim Behav* 53:945–949
- Gross MR (1985) Disruptive selection for alternative life histories in salmon. *Nature* 313:47–48
- Gross MR (1996) Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol Evol* 11:92–98
- Hunt J, Breuker CJ, Sadowski JA, Moore AJ (2009) Male–male competition, female mate choice and their interaction: determining total sexual selection. *J Evol Biol* 22:13–26

- Jennions M, Petrie M (1997) Variation in mate choice and mating preferences: a review of causes and consequences. *Biol Rev* 72: 283–327
- Kallman KD (1989) Genetic control of size at maturity in *Xiphophorus*. In: Snelson GK (ed) *Ecology and evolution in livebearing fishes (Poeciliidae)*. Prentice-Hall, Englewood Cliffs, pp 163–184
- Kelley JL, Graves JA, Magurran AE (1999) Familiarity breeds contempt in guppies. *Nature* 401:661–662
- Kelley JL, Morrell LJ, Inskip C, Krause J, Croft DP (2011) Predation risk shapes social networks in fission-fusion populations. *PLoS ONE* 6: e24280
- Kodric-Brown A (1986) Satellites and sneakers: opportunistic male breeding tactics in pupfish (*Cyprinodon pecosensis*). *Behav Ecol Sociobiol* 19:425–432
- Köhler A, Hildenbrand P, Schleucher E, Riesch R, Arias-Rodriguez L, Streit B, Plath M (2011) Effects of male sexual harassment on female time budgets, feeding behavior, and metabolic rates in a tropical livebearing fish (*Poecilia mexicana*). *Behav Ecol Sociobiol* 65:1513–1523
- Krause J, Croft DP, James R (2007) Social network theory in the behavioural sciences: potential applications. *Behav Ecol Sociobiol* 62:15–27
- Lampert KP, Schmidt C, Fischer P, Volff J-N, Hoffmann C, Muck J, Lohse MJ, Ryan MJ, Schartl M (2010) Determination of onset of sexual maturation and mating behavior by melanocortin receptor 4 polymorphisms. *Curr Biol* 20:1729–1734
- Lusseau D, Whitehead H, Gero S (2008) Incorporating uncertainty into the study of animal social networks. *Anim Behav* 75:1809–1815
- Magellan K, Magurran AE (2007) Behavioural profiles: individual consistency in male mating behaviour under varying sex ratios. *Anim Behav* 74:1545–1550
- Makowicz AM, Schlupp I (2013) The direct costs of living in a sexually harassing environment. *Anim Behav* 85:569–577
- Maynard-Smith J (1982) *Evolution and the theory of games*. Cambridge University Press, Cambridge
- McDonald GC, James R, Krause J, Pizzari T (2013) Sexual networks: measuring sexual selection in structured, polyandrous populations. *Philos T Roy Soc B* 368:20120356
- Miller RR (2006) *Freshwater fishes of Mexico*. Chicago University Press, Chicago
- Morrell LJ, Croft DP, Dyer JRG, Chapman BB, Kelley JL, Laland KN, Krause J (2008) Association patterns and foraging behaviour in natural and artificial guppy shoals. *Anim Behav* 76:855–864
- Morris MR, Batra P, Ryan MJ (1992) Male-male competition and access to females in the swordtail *Xiphophorus nigrensis*. *Copeia* 1992: 980–986
- Morris MR, Ryan MJ (1992) Breeding cycles in natural populations of *Xiphophorus nigrensis*, *X. multilineatus*, and *X. pygmaeus*. *Copeia* 1992:1074–1077
- Newman MEJ (2004) Analysis of weighted networks. *Phys Rev E* 70: 056131
- Niemeitz A, Kreutzfeldt R, Schartl M, Parzefall J, Schlupp I (2002) Male mating behaviour of a molly, *Poecilia latipunctata*: a third host for the sperm-dependent Amazon molly, *Poecilia formosa*. *Acta Ethol* 5:45–49
- Parker GA (1974) Assessment strategy and the evolution of fighting behaviour. *J Theor Biol* 47:223–243
- Parzefall J (1969) Zur vergleichenden Ethologie verschiedener *Mollienesia*-Arten einschließlich einer Höhlenform von *Mollienesia sphenops*. *Behaviour* 33:1–38
- Parzefall J (1979) Zur Genetik und biologischen Bedeutung des Aggressionsverhaltens von *Poecilia sphenops* (Pisces, Poeciliidae). *Z Tierpsychol* 50:399–422
- Plath M (2008) Male mating behavior and costs of sexual harassment for females in cavernicolous and extremophile populations of Atlantic mollies (*Poecilia mexicana*). *Behaviour* 145:73–98
- Plath M, Hermann B, Schroder C, Riesch R, Tobler M, Garcia de Leon F, Schlupp I, Tiedemann R (2010) Locally adapted fish populations maintain small-scale genetic differentiation despite perturbation by a catastrophic flood event. *BMC Evol Biol* 10:256
- Plath M, Parzefall J, Korner K, Schlupp I (2004) Sexual selection in darkness? Female mating preferences in surface- and cave-dwelling Atlantic mollies, *Poecilia mexicana* (Poeciliidae, Teleostei). *Behav Ecol Sociobiol* 55:596–601
- Plath M, Parzefall J, Schlupp I (2003) The role of sexual harassment in cave and surface dwelling populations of the Atlantic molly, *Poecilia mexicana* (Poeciliidae, Teleostei). *Behav Ecol Sociobiol* 54:303–309
- Plath M, Tobler M (2010) Subterranean fishes of Mexico (*Poecilia mexicana*, Poeciliidae). In: Trajano E, Bichuette ME, Kapoor BG (eds) *The biology of subterranean fishes*. Science Publishers, Enfield, pp 283–332
- Pocklington R, Dill L (1995) Predation on females or males: who pays for bright male traits. *Anim Behav* 49:1122–1124
- Qvarnström A (2001) Context-dependent genetic benefits from mate choice. *Trends Ecol Evol* 16:5–7
- Qvarnström A, Forsgren E (1998) Should females prefer dominant males? *Trends Ecol Evol* 13:498–501
- Reynolds JD, Gross MD, Coombs MJ (1993) Environmental conditions and male morphology determine alternative mating behaviour in Trinidadian guppies. *Anim Behav* 45:145–152
- Riesch R, Plath M, Schlupp I (2011) Toxic hydrogen sulphide and dark caves: pronounced male life-history divergence among locally adapted *Poecilia mexicana* (Poeciliidae). *J Evol Biol* 24:596–606
- Ryan MJ, Pease CM, Morris MR (1992) A genetic polymorphism in the swordtail *Xiphophorus nigrensis*: testing the prediction of equal fitnesses. *Am Nat* 139:21–31
- Schartl M, Erbeling-Denk C, Hölter S, Nanda I, Schmid M, Schröder JH, Epplen JT (1993) Reproductive failure of dominant males in the poeciliid fish *Limia perugiae* determined by DNA fingerprinting. *P Natl Acad Sci USA* 90:7064–7068
- Sefc KM, Hermann CM, Koblmüller S (2009) Mating system variability in a mouthbrooding cichlid fish from a tropical lake. *Mol Ecol* 18: 3508–3517
- Sih A, Hanser S, McHugh K (2009) Social network theory: new insights and issues for behavioral ecologists. *Behav Ecol Sociobiol* 63:975–988
- Sinervo B, Lively CM (1996) The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* 380:240–243
- Taborsky M, Brockmann HJ (2010) Alternative reproductive tactics and life history phenotypes. In: Kappeler P (ed) *Animal behaviour: evolution and mechanisms*. Springer, Heidelberg, pp 537–586
- Tatarenkov A, Healey CIM, Grether GF, Avise JC (2008) Pronounced reproductive skew in a natural population of green swordtails, *Xiphophorus helleri*. *Mol Ecol* 17:4522–4534
- Tobler M, Franssen CM, Plath M (2008) Male-biased predation of a cave fish by a giant water bug. *Naturwissenschaften* 95:775–779
- Travis J, Woodward BD (1989) Social context and courtship flexibility in male sailfin mollies, *Poecilia latipinna* (Pisces: Poeciliidae). *Anim Behav* 38:1001–1011
- Trexler J, Tempe R, Travis J (1994) Size-selective predation of Sailfin mollies by two species of heron. *Oikos* 69:250–259
- Trexler JC, Travis J, Trexler M (1990) Phenotypic plasticity in the Sailfin molly, *Poecilia latipinna* (Pisces: Poeciliidae). II. Laboratory experiment. *Evolution* 44:157–167
- Walling C, Royle N, Lindström J, Metcalfe N (2010) Do female association preferences predict the likelihood of reproduction? *Behav Ecol Sociobiol* 64:541–548
- Whitehead H (2008) *Analyzing animal societies*. University of Chicago Press, Chicago
- Wilson ADM, Croft DP, Krause J (2013) Social networks in elasmobranchs and teleost fishes. *Fish Fish*. doi:10.1111/faf.12046

- Witte K, Ryan MJ (2002) Mate choice copying in the sailfin molly, *Poecilia latipinna*, in the wild. *Anim Behav* 63:943–949
- Wolf M, Weissing FJ (2010) An explanatory framework for adaptive personality differences. *Phil Transact R Soc B: Biol Sci* 365:1471–2970
- Wong BBM, Candolin U (2005) How is female mate choice affected by male competition? *Biol Rev* 80:559–571
- Zimmerer EJ, Kallman KD (1989) Genetic basis for alternative reproductive tactics in the pygmy swordtail, *Xiphophorus nigrensis*. *Evolution* 43:1298–1307