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Behavioural responses to season and adult sex ratio in the fantail darter *Etheostoma flabellare*

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Social behaviours such as competition for mates, which can indicate the strength and direction of sexual selection, may change either directly or indirectly in response to the operational sex ratio (OSR). Field studies indicate flexibility in social behaviours in response to a changing OSR, in particular as the OSR changes across the breeding season. Remaining unclear, however, is the degree to which these behavioural changes are a direct response to an unpredictably varying OSR, or are seasonal, developing in response to seasonal cues. In this experiment, we collected cohorts of male and female fantail darters at regular intervals over the course of two full breeding seasons and placed them in artificial streams in high, moderate and low adult sex ratios (ASRs). We observed streams for 5-day periods, during which we tallied aggressive and sexually solicitous behaviours. Both male and female intrasexual aggression responded to ASR manipulations as predicted, with males increasing and females tending to decrease intrasexual aggression in response to higher male numbers. Changes in intersexual aggression rates were not correlated with changes in intrasexual rates, indicating that these two classes of behaviour varied independently of one another. Male solicitations towards females increased at moderate ASRs, but decreased when the ratio of males to females exceeded the observed natural range. Contrary to predictions, female solicitations towards males increased with increasing male numbers. Male density and hence encounter rate was a confounding factor for interpreting male-directed (but not female-directed) behaviours in response to the ASR, and furthermore negatively affected overall reproductive success. We found no evidence that competitive or solicitous behaviour responds directly to seasonal cues in *Etheostoma flabellare*; rather, competition over mates and solicitation of mates appear to respond directly to changes in the ASR.

In sexually reproducing species, one sex is often forced, by limited availability of the opposite sex, to compete for access to mates (Darwin 1871). Because of unequal precopulatory investment, pregnancy and maternal care, females are normally considered to be the limiting sex (Bateman 1948; Trivers 1972; Clutton-Brock & Vincent 1991; Clutton-Brock & Parker 1992; Andersson 1994; Kvarnemo & Ahnesjö 1996). Where males provide a valuable resource such as paternal care, however, the investment balance may shift and males may become limiting (Trivers 1972; Berglund et al. 1989; Clutton-Brock & Vincent 1991; Clutton-Brock & Parker 1992; Emlen & Wrege 2004). The limiting sex is typically determined by the operational sex ratio (OSR): the ratio of reproductively receptive females to reproductively receptive males (Emlen & Oring 1977). When fixed over time, a skewed OSR predicts competition within the abundant sex for mates (Emlen & Oring 1977; but see Klug et al. 2010). Where the OSR is not fixed within a generation, however, the strength of sexual selection in a given sex also should not be fixed, and competition for mates within a sex may vary in intensity (Kvarnemo & Ahnesjö 1996; Johnstone 1997; Gowaty & Hubbell 2005; but see Klug et al. 2010).

In field and laboratory assays, variation in components of the OSR, including adult sex ratio (ASR), individual condition (including age), population density and resource availability, are correlated with changes in competitive and solicitous behaviours. For example, male mate-guarding behaviour increases in the water strider *Gerris remigis* and in the carrion beetle *Necrophila americana* when the ASR becomes male biased (Clark 1988; Knox & Scott 2005). In the pollen katydid *Kawanaphila nartee*, males reduce mate solicitation efforts in response to low food availability, a factor that reduces the number of available males (Gwynne & Simmons 1990). The competitiveness of both sexes of two-spotted sand gobies, *Gobiusculus flavescens*, shifts in correlation with observed seasonal changes in the OSR (Forsgren et al. 2004), and changes in female competitiveness also appear to be correlated with seasonal

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OSR changes in the shorebird *Phalaropus tricolor* (Colwell & Oring 1988) and in the blenny *Petroscirtes breviceps* (Shibata & Kohda 2006). A recent meta-analysis of both observational and manipulative studies indicated that, within a certain OSR range, competitive behaviour does appear to respond to the OSR as predicted (Weir et al. 2011).

An alternative hypothesis is that aggressive and courtship behaviours may not only respond directly to unpredictable changes in OSR, but may have evolved to develop seasonally in response to predictable seasonal changes in OSR. Seasonal changes in mating behaviours and strategies have been observed in other systems, ranging from seasonal changes in preferences for sexual versus asexual females in male sailfin mollies, *Poecilia latipinna* (Heubel & Schlupp 2008), to increased sexual cannibalism in early-season female praying mantis, *Mantis religiosa* (Prokop & Václav 2008), to a seasonally dependant reversal of sex roles in the butterfly *Bicyclus anynana* (Prudic et al. 2011). If the OSR develops predictably over the course of each nuptial season, then aggressive and courtship behaviours may be selected to develop directly with the season and, indirectly, with the OSR. A direct behavioural response to a rapidly changing social environment would seem ideal, but assumes constant access to reliable social cues and sufficiently rapid neurological processing and/or hormonal responses. A seasonal development of behaviours may be more advantageous if the social environment develops predictably within the nuptial season, if social cues are unreliable and/or if physiological response lag is substantial. The two hypotheses (social versus seasonal) are not mutually exclusive but rather represent two mechanisms by which an organism can respond to its environment in a way that increases relative fitness.

Males of fantail darter *Etheostoma flabellare* have been reported to precede females to the nuptial grounds (Lake 1936) and may become scarcer relative to females as the season progresses (see below). We sought here to assess whether aggressive and solicitous behaviours in the fantail darter develop predictably over the course of the season regardless of the ASR, a component of the OSR, and whether these behaviours respond directly to manipulations of the ASR. These benthic stream fish mate promiscuously in stream riffles from March to May in Maryland (U.S.A.), with males providing substantial paternal care until the eggs hatch (Lake 1936; Winn 1958a; Page 1983). Over the course of the nuptial season, females of this polygynandrous species invert to lay one or more clutches in nest cavities, which are maintained and defended by males (Lake 1936; Winn 1958b; Moretz & Rogers 2004). Males are, on average, larger than females, with more vivid black and orange nuptial coloration and a greater tendency to develop fleshy 'egg mimics' on their anterior dorsal fin during the nuptial season (Page 1983).

We performed an initial field survey to confirm a seasonally developing ASR. We then manipulated male number in artificial streams at three seasonal points and observed the effect on per capita rates of intrasexual aggression, intersexual solicitation and intersexual aggression. Under strong selection to compete for mates, a sex is predicted to show increased rates of intrasexual aggression and/or intersexual solicitation. The social hypothesis predicts that both sexes will change their levels of intrasexual aggression and intersexual courtship directly in response to our ASR manipulations; the seasonal hypothesis predicts that the behaviours of both sexes will be correlated with the normal seasonal ASR development, regardless of the ASR of their treatment group. Because we manipulated only male number, we were able to make some predictions that were exclusive to the effect of ASR and independent of encounter rate.

METHODS

Field Sex Ratio Counts

To verify earlier reports of a seasonally developing ASR within the nuptial grounds (Lake 1936), we seined three discrete 20 m stretches within a 500 m length of Meadow Branch, a tributary of Big Pipe Creek near Westminster, MD, U.S.A., to exhaustion in March, April and May 2009. Seined areas were mostly composed of riffles, the preferred nesting grounds of *E. flabellare* (Lake 1936; Page 1983). Collected fish were dissected, and the sexes were counted to provide estimates of the ASR in the nuptial grounds of this stream over the course of the nuptial season. To ensure that collection points fell within and also spanned the nuptial season, we counted egg-containing nests within a fixed 20 m stretch of riffle on each collection date.

Subject Collections

In March, April and May 2010 and 2011, we collected fish by kick seining a 100 m stretch of Meadow Branch, Carroll Co., MD (39°36′36″N, 77°6′36″W), and in March, April and May 2011, we also kick-seined fish from a 100 m stretch of Cabbage Run, Frederick Co., MD (39°29′24″N, 77°19′12″W). To avoid effects of habitat disturbance, monthly samples within a nuptial season occurred at nonoverlapping points within the same stream length. Fish were transported to the campus greenhouse at the University of Maryland Baltimore County (39°15′36″N, 76°42′36″W) within 5 h of collection and were sorted by sex and placed in 56.78-litre aquaria for overnight acclimation.

Behavioural Observations

Acclimated fish were elastomer-tagged at two of six consistent landmarks to identify both individual and sex, and were loaded into six artificial streams ($1.25 \times 0.6 \times 0.6$ m). Riffles and pools were created using pond pumps and pea gravel, and each stream was outfitted with seven nests constructed of 10.16 cm square slate tiles propped up approximately 2.0–2.5 cm along one edge to create a single opening that faced a viewing window through which we observed the fish. The number of males never exceeded the number of nests. Flow rates and water pH approximated that observed in the subjects' native stream, and water and air temperatures were kept as close to the native temperatures as possible. To reduce accumulation of secreted hormones and chemical cues, water changes were conducted throughout the day so that at least half of the volume of each stream was replaced with fresh water over the course of each 24 h period. Because the streams were in a greenhouse within close proximity of the field sites, the native photoperiod was preserved. Fish were fed frozen chironomid larvae twice daily to satiation. Stream mortalities were rare (<2%) and were immediately replaced using reserve stocks of the same collection cohort. Cohorts were euthanized and preserved for dissection at the end of each 5-day trial period, and eggs were counted within each nest, including any remaining from the pretrial acclimation period.

Within each seasonal point, streams were assigned either 'low male' adult sex ratios (0.33–0.57 males per female, $N = 25$), representing the late-season field ASR, 'moderate male' ASRs (0.80–0.83 males per female, $N = 18$), representing the early-season field ASR, or 'high male' ASRs (1.25–3.50 males per female, $N = 5$), representing a supernormally high ASR. Sexes were confirmed by dissections following the trial periods. Because there was one water source for every two streams, a semi-randomized block design was used in which each water source fed one high