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Dynamic resource allocation between pre- and postcopulatory episodes of sexual selection determines competitive fertilization success

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In polyandrous mating systems, male reproductive success depends on both mate-acquisition traits (precopulatory) and sperm competitive abilities (postcopulatory). Empirical data on the interaction between these traits are inconsistent; revealing positive, negative or no relationships. It is generally expected that the investment in pre- and postcopulatory traits is mediated by environmental conditions. To test how dietary resource availability affects sexual ornamentation, sperm quality and their interrelationship in three-spined sticklebacks (*Gasterosteus aculeatus*), full-sibling groups were raised under three conditions differing in food quantity and/or quality (i.e. carotenoid content): (i) high-quantity/high-quality, (ii) high-quantity/low-quality or (iii) low-quantity/low-quality. After 1 year of feeding, food-restricted males developed a more intense breeding coloration and faster sperm compared with their well-fed brothers, indicating that they allocated relatively more in pre- and postcopulatory traits. Moreover, they outcompeted their well-fed, carotenoid-supplemented brothers in sperm competition trials with equal numbers of competing sperm, suggesting that food-restricted males maximize their present reproductive success. This may result in reduced future reproductive opportunities as food-restricted males suffered from a higher mortality, had an overall reduced body size, and sperm number available for fertilization. In accordance with theory, a trade-off between the investment in pre- and postcopulatory traits was observed in food-restricted males, whereas well-fed males were able to allocate to both traits resulting in a significantly positive relationship.

1. Background

Sexual selection is known to force the evolution of male fitness-enhancing life-history traits [1]. In polyandrous mating systems, this can be achieved through both mate-acquisition traits (precopulatory) and sperm competitive abilities (postcopulatory). In precopulatory sexual selection, male mating success depends on the expression of extravagant phenotypic traits such as sexual ornamentation, or weapons [1]. In addition, when females copulate or spawn with more than one male sexual selection continues after mating, with sperm of two or more males competing against each other for the fertilization of eggs [2]. It is expected that sperm competitiveness is related to both sperm quantity and sperm quality traits (see [3] and citations therein). Consequently, a male's competitiveness during both pre- and postcopulatory sexual selection has a considerable effect on his overall fitness [4]. Thus, disentangling the contribution of pre- as well as postcopulatory mechanisms is important [5–7]. However, empirical data on the intraspecific level are inconsistent, showing either a positive relationship (e.g. [8]), a trade-off (e.g. [9]) or no association (e.g. [10]) between both sexual selection episodes.

The evolution of life-history strategies within a population is known to be affected by environmental conditions (e.g. [11]). The availability of resources strongly influences an individual's ability to modulate investment in sexually

selected traits. As the production and maintenance of traits mediated by pre- and postcopulatory sexual selection is energetically demanding [12,13], an increased investment in traits subject to both episodes of sexual selection can be expected under conditions of high resource availability. By contrast, under limitation an increased allocation in one function often results in a decreased resource availability for alternative functions [14], leading to one of the major predictions of sperm competition theory that a male's investment should be traded-off between pre- and postcopulatory traits [15].

Experimental manipulation of diet is a suitable approach to explore the impact of variation in resource availability on the expression of pre- and postcopulatory traits and their interrelationship. For example, a low-quantity diet is known to negatively affect reproductive output (e.g. [16]), whereas high food quality positively influences male reproductive performance (e.g. [17]), for instance by enhancing sperm competitiveness [18]. The latter is probably owing to increased antioxidant levels in the ingested diet, which are involved in the protection of sperm plasma membrane and DNA against oxidative stress (e.g. [19]). In addition to several examples on the adverse consequences of low food quantity and/or quality on postcopulatory sexual traits across a wide range of taxa [20,21], precopulatory sexual traits can also be negatively affected. In particular, the expression of conspicuous sexually selected male colour traits depends on food quantity and/or quality (e.g. [22]). Male sexual coloration is often based on carotenoid pigments that have to be acquired through diet and are an important source of antioxidants and immunostimulants [23]. Thus, the limited availability of carotenoids can lead to trade-offs between fitness-relevant life-history traits, such as ornamental pigmentation, sperm function as well as antioxidant defence and immunocompetence, thereby maintaining the honesty of carotenoid-based signals (e.g. [24], but see [25]). However, the majority of studies dealing with the impact of diet on trait expression in males have focused on either pre- or postcopulatory sexual selection. Empirical research has only recently started to take diet effects on the interrelationship between both stages into account (e.g. [26–29]).

In the three-spined stickleback (*Gasterosteus aculeatus*), the carotenoid-based orange-red breeding coloration of males is one of the key determinants of female mate choice decisions (e.g. [30]). During the breeding cycle (April–August), territorial stickleback males accumulate the eggs of several females in their nest. The risk of sperm competition is high under natural conditions as stickleback males often try to steal fertilizations in nests of neighbouring males (e.g. [31]). A former study revealed that under non-competitive conditions the expression of male breeding coloration is positively correlated with the proportion of fertilized eggs and males fed a high carotenoid diet have an increased functional fertility [32]. Moreover, sperm velocity in sticklebacks was recently found to be positively related to male attractiveness [33]. These findings are in accordance with the so-called phenotype-linked fertility hypothesis (PLFH), which predicts that males with conspicuous sexual ornaments signal their superior fertilization efficiency to females, which will gain direct fecundity benefits by preferring highly ornamented males [34]. Empirical evidence for the PLFH has also been provided by some further studies revealing positive correlations between pre- and postcopulatory traits (e.g. [8]). However, a recent meta-analysis found little support for the PLFH [35] and in line with the aforementioned sperm competition theory, a trade-off between the investment in pre- and postcopulatory traits is expected especially under resource limitation [15].

In this study, we used laboratory-bred stickleback males to examine how resource availability in terms of experimentally manipulated diet quality (i.e. carotenoid content) and quantity affects life-history traits and the relationship between the expression of both pre- and postcopulatory sexually selected traits. Individuals were raised under standardized conditions and underwent three different feeding treatments: (i) high-quantity/high-quality [+ +], (ii) high-quantity/low-quality [+ −] or (iii) low-quantity/low-quality [− −]. Across all treatments full-sibling groups were used, thereby controlling for genetic background. After 1 year of feeding, the effects on growth and mortality as well as on pre- (i.e. breeding coloration) and postcopulatory sexual traits (i.e. testis mass, sperm number, sperm swimming ability) were quantified. Our prediction was that males from the two high-quantity treatments would be able to highly invest in the expression of both pre- and postcopulatory traits, which should become even more pronounced in males that received the carotenoid-supplemented diet. By contrast, food-limited males were expected to show a negative relationship between the expression of pre- and postcopulatory traits indicating an allocation trade-off. In addition, to obtain a direct measure of fertilization success we compared sperm competitiveness of brothers from different treatments using the *in vitro* fertilization technique and subsequent paternity analysis.

2. Material and methods

(a) Experimental subjects

Three-spined sticklebacks from a large anadromous population were caught during their spring migration in April 2010 on the island of Texel, the Netherlands. To create different family groups, a randomly chosen male was allowed to spawn with a randomly chosen female (25 May–19 June 2010). In total, 25 clutches were produced and parents were only used once. To exclude paternal effects clutches were removed from the males' nests 2 h after fertilization and split into three equally portioned full-sibling sub-groups, which were separately placed in 1 l containers measuring $10 \times 17 \times 11$ cm (length × width × height). After hatching, juveniles were fed daily using the same diet (*Artemia* nauplii). With increasing age and body size, groups were transferred into larger holding tanks (age: 3 weeks, $30 \times 20 \times 20$ cm; 10 weeks, $50 \times 30 \times 30$ cm). To control for density effects, group size was reduced to 30 individuals per tank at an age of eight weeks. At this time point, each of the three family sub-groups was randomly assigned to one out of three diet treatments, which differed in food quantity and/or food quality. Overall, two types of food were provided; first, 'normal' red mosquito larvae (*Chironomus* spp.), which contain moderate levels of lutein [36] and second, carotenoid-enriched red mosquito larvae, which additionally contain high levels of astaxanthin (AHA Frostfutter, Duisburg, Germany). Astaxanthin is an important component of sticklebacks' natural diet and represents one of the main carotenoids incorporated in the integument of stickleback males [37,38]. Carotenoid-enriched larvae were commercially produced by depositing them in water that contained 2 g of 10% pure astaxanthin per litre for 1 h before they were frozen. Individuals of one group were fed with carotenoid-enriched larvae every day (high-quantity/high-quality diet, [+ +]). The second group received a daily portion of 'normal' larvae (high-quantity/low-quality diet, [+ −]), whereas the third group was provided with

'normal' larvae every second day (low-quantity/low-quality diet [− −]).

Holding tanks were placed in an air-conditioned room (17 ± 1°C) under standardized light-regimes (summer until 19 September 2010: day length 16 L : 8 D; autumn until 14 November 2010: 12 L : 12 D; winter until 28 March 2011: 8 L : 16 D; spring until 9 May 2011: 12 L : 12 D; summer until 14 August 2011: 16 L : 8 D). On 15 November 2010, body measures (body size and mass, to the nearest millimetre and milligram, respectively) of six randomly chosen sub-adult individuals per tank were taken to determine average developmental growth rate. In addition, for each group mortality was determined for a time period of about nine months, i.e. between the beginning of the feeding treatment and the start of the reproductive phase (8 May 2011).

(b) Experimental procedure

As sexual maturation in sticklebacks is stimulated by long photoperiods [39], data acquisition took place during summer conditions (2 June–8 July 2011). Males that showed signs of nuptial coloration were individually isolated in separate tanks (30 × 20 × 20 cm), each equipped with a sand-filled Petri dish (Ø 9 cm) and 2 g Java moss (*Vesicularia dubyana*) for nest building. Shortly before isolation male's body size and mass were measured. Food-restricted males were isolated only on days on which they received food. After isolation, all males were still fed according to their previous feeding treatment. In standardized *in vitro* fertilization experiments, three full-sibling brothers were allowed to compete pairwise against each other for egg fertilization ([+ +] versus [− −], [+ +] versus [+ −] and [+ −] versus [− −]) (see below for details). *In vitro* fertilization experiments took place on average 4–7 days after nest completion and only on days on which all males were fed. In addition, all brothers used in one experiment had to have finished nest building within the same 2 day time interval.

Prior to an *in vitro* fertilization experiment, a receptive female (wild-caught in April 2011 from the same population) served to stimulate the male. Directly after stimulation, male body measurements were taken again. The expression of breeding coloration was then quantified using a reflectance spectrophotometer (Avantes AvaSpec-2048, Eerbeek, The Netherlands) connected to a deuterium–halogen light source (Avantes DH-S) [40]. To quantify how female conspecifics might perceive the breeding coloration of males, the measured spectra were incorporated in a physiological model on stickleback colour vision. A detailed description of the reflectance measurements and the used parameters for the formulation of the model is given in a previous study [40] and in the electronic supplementary material. Directly after reflectance measurements, a male was quickly killed by decapitation in order to dissect and weigh the testes to calculate the relative testis mass (gonadosomatic index, GSI) after de Vlaming *et al.* [41]. To quantify sperm swimming abilities, the left testis was pestled in 500 µl artificial 'ovarian fluid' (3.0 g NaCl, 0.1 g KCl, 0.07 g $CaCl_2$ in 1 l distilled water after [42]; see [31] and the electronic supplementary material for further details). In addition, the right testis was put in 500 µl of a non-activating medium (for mixture see [43]) for subsequent *in vitro* fertilization.

(c) *In vitro* fertilization experiment

For all three brothers that participated in one *in vitro* set of fertilization experiments data acquisition (reflectance measurements, measurements of sperm swimming abilities) was time-shifted by 15 min. To exclude sequence effects, this was done in random order. The same receptive female was used for each male's stimulation.

For sperm number quantification the right testis of each male (previously stored in 500 µl non-activating medium) was pestled (see [44] for details). Sperm number was quantified prior to fertilization to ensure that an equal number of each male's sperm was used during the fertilization process as males showed considerable variation in sperm number (range: 2.75–23.5 × 10^7). Thereafter, different volumes of each male's sperm suspension (containing 10 million spermatozoa) were filled up with non-activating medium to obtain a constant total volume of 500 µl for fertilization. This resulted in three Eppendorff-tubes containing 20 million spermatozoa in total, each comprising an equally portioned sperm mixture of two brothers for each sub-trial ([+ +] versus [− −], [+ +] versus [+ −] or [+ −] versus [− −]). By keeping sperm number constant, it was possible to control for potential confounding effects between sperm quantity and quality (e.g. [45]).

Immediately thereafter, the same female, which had been used for the stimulation of the males was gently stripped, which is a common method and does not harm the fish (e.g. [46–48]). All eggs were counted and in each case 40 eggs (39.46 ± 0.97, mean ± standard deviation, 2486 in total) were placed in each of three small glass Petri dishes that already contained 1 ml of tap water, resulting in a sperm to egg ratio of 250 000 : 1 per male. Using a pipette, one sperm mixture was carefully released over one egg portion. One hour later, the fertilization process was stopped using sparkling water, which does not harm the eggs [46]. Eggs of each sub-trial were separately stored in an aerated container (1 l). After 24 h, fertilization rate was checked using a binocular microscope (Leica S8AP0, Wetzlar, Germany). In total, 2318 eggs were fertilized and stored in 99.8% ethanol at −18°C for paternity analyses. The whole procedure was repeated 21 times using different families, resulting in 63 sub-trials. Females that participated in an *in vitro* fertilization experiment ($n = 21$) were marked by cutting the tip of a spine that was preserved in 99.8% ethanol at −18°C for subsequent paternity analysis. Directly thereafter, they were returned to their holding tank to avoid repeated use and thus pseudoreplication. Furthermore, spine and tissue samples of the 63 putative fathers were additionally preserved in 99.8% ethanol at −18°C for paternity analysis.

(d) Paternity analysis

To check for differences in fertilization success, nine highly polymorphic microsatellite markers were available (see the electronic supplementary material, table S2). DNA-samples of all parents ($n_{\text{putative fathers}} = 63$, $n_{\text{mother}} = 21$) and eggs were extracted via Chelex (Bio-Rad; after [49]). In a subsequent PCR two, three or four microsatellite loci were multiplexed. PCR-products were run on a CEQ 8800 Genetic Analysis System (Beckman Coulter, Krefeld, Germany; see the electronic supplementary material, tables S2 and S3 for details). Only eggs that could be clearly (100%) assigned to one father were included in the statistical analyses (see the electronic supplementary material, table S2 for details).

(e) Statistical analyses

Statistical analyses were performed using SPSS 15.0 and R 3.0.2 statistical packages. Data were tested for normality (Kolmogorov–Smirnov tests with Lilliefors correction) and homogeneity of variances (Levene tests). Parametric statistics were used when these criteria were fulfilled, otherwise data were transformed (inverse transformation of body mass of adult individuals) or non-parametric statistics were applied.

In detail, linear mixed effects models were fitted using the 'lme' function of the 'nlme' package. Models were constructed using a measured variable as dependent variable (see table 1 for an overview, except percentage motile sperm and mortality, see below) and feeding treatment and each fertilization sub-trial (pairwise comparison), respectively, as explanatory variable. To investigate the effect of sperm velocity on fertilization success, mean values for both variables were calculated for the respective focal male relative to both competitors [((focal male − competitor 1) + (focal male − competitor 2))/2]. In addition, a potential interaction between sperm velocity and feeding treatment on fertilization

Table 1. Results of the comparisons between the three feeding treatments including subsequent pairwise comparison of the different sub-trials. (High-quantity/high-quality males [+ +] were fed with carotenoid (astaxanthin)-enriched larvae every day, high-quantity/low-quality males [+ −] were provided with a daily portion of 'normal' larvae, and low-quantity/low-quality males [− −] received 'normal' larvae every second day. Significant *p*-values are printed in bold.)

				pairwise comparison of the different sub-trials					
				[+ +] versus [− −]		[+ +] versus [+ −]		[+ −] versus [− −]	
dependent variable		χ^2	*p*-value	χ^2	*p*-value	χ^2	*p*-value	χ^2	*p*-value
sub-adult (approx. 5 months)	body size (cm)	135.06	**<0.001**	87.01	**<0.001**	0.70	0.404	96.57	**<0.001**
	body mass (g)	108.73	**<0.001**	72.99	**<0.001**	0.61	0.437	85.16	**<0.001**
adult (approx. 1-year old)	body size (cm)	53.55	**<0.001**	41.06	**<0.001**	7.70	**0.006**	26.81	**<0.001**
	body mass (g)	41.45	**<0.001**	31.77	**<0.001**	2.94	0.087	28.29	**<0.001**
development	mortality (%)	80.17	**<0.001**	68.84	**<0.001**	3.01	0.083	44.26	**<0.001**
breeding coloration	*theta* (hue longitude)	24.98	**<0.001**	20.92	**<0.001**	15.28	**<0.001**	2.26	0.133
	achieved chroma (r_A)	12.60	**0.002**	14.32	**<0.001**	2.43	0.119	3.95	**0.047**
testis traits	relative testis mass (GSI)	2.61	0.271	0.08	0.781	1.59	0.208	3.14	0.076
	absolute testis mass (g)	28.92	**<0.001**	21.19	**<0.001**	0.04	0.850	25.16	**<0.001**
sperm traits	sperm number	23.19	**<0.001**	13.69	**<0.001**	1.33	0.249	29.28	**<0.001**
	motile sperm (%)	5.08	0.079	3.55	0.060	0.37	0.544	3.82	0.051
	sperm velocity	14.40	**<0.001**	11.01	**<0.001**	0.83	0.363	6.97	**0.008**
	sperm linearity	2.71	0.258	2.51	0.113	0.08	0.784	1.19	0.276

success was tested. To check for differences between the daily-fed (irrespective of food composition) and food-restricted males concerning their investment in pre- and postcopulatory sexually selected traits (achieved chroma (r_A, i.e. colour intensity) and sperm velocity) a further linear model was conducted. Here, sperm velocity was used as dependent variable. Achieved chroma (r_A), food restriction (yes/no) and the interaction between these two variables were included as explanatory variables. Generalized mixed effects models using the 'glmer' function with a binomial error distribution and logit link function in the 'lme4' package were fitted to analyse the proportional data percentage of motile sperm and mortality. In all models, family identity was integrated as a random factor and never removed to control for genotype influences (see the electronic supplementary material, table S4 for a list of all fitted models). Non-significant factors were removed from the model and tests of significance were based on likelihood-ratio tests following a χ^2 distribution. Test probabilities are two-tailed throughout and the level of significance was set at 0.05.

3. Results

(a) Feeding effects

The results revealed that male phenotype was considerably affected by the three feeding treatments (see table 1 for an overview and the electronic supplementary material, table S5 for means ± standard deviations). In detail, the pairwise comparisons showed that under food limitation [− −] sub-adult fish were on average significantly smaller and lighter than their full-siblings (all $p < 0.001$; table 1), whereas daily-fed sub-adults ([+ +] and [+ −]) did not differ significantly in body measures irrespective of food quality (both $p \geq 0.404$; table 1). At the adult stage, males that received the restricted diet were still significantly smaller and lighter than their daily-fed brothers (all $p < 0.001$; table 1). However, after about 1 year of feeding males that belonged to the high-quantity/high-quality [+ +] group were significantly larger ($p = 0.006$; table 1) but not heavier than their high-quantity/low-quality [+ −] fed brothers ($p = 0.087$; table 1). Data analyses of adult males were done with averaged body variables as they were determined twice during the experimental procedure. In addition, significantly fewer individuals from the low-quantity/low-quality [− −] diet group reached the reproductive season (both $p < 0.001$; table 1), whereas the daily-fed males did not differ significantly concerning their mortality until adulthood ($p = 0.083$; table 1). However, mortality was determined over a period of about nine months and it is worth mentioning that the absolute number of individuals which died during this time was comparatively low (mean ± standard deviation: 2.48 ± 1.60 [+ +], 3.33 ± 2.87 [+ −] and 7.62 ± 2.84 [− −]). In addition, during the experimental procedure only one male died after isolation and before nest completion (high-quantity/low-quality [+ −]) and was thus replaced.

Males that received the astaxanthin-enriched larvae [+ +] had a significantly more red-shifted breeding coloration (lower *theta*) in comparison with their brothers (both $p < 0.001$; table 1; figure 1). The two groups that were fed with 'normal' larvae ([+ −] and [− −]) did not differ significantly in their rather orange-red breeding coloration ($p = 0.133$; table 1; figure 1). However, males from the low-quantity/low-quality group [− −] developed a more intense breeding

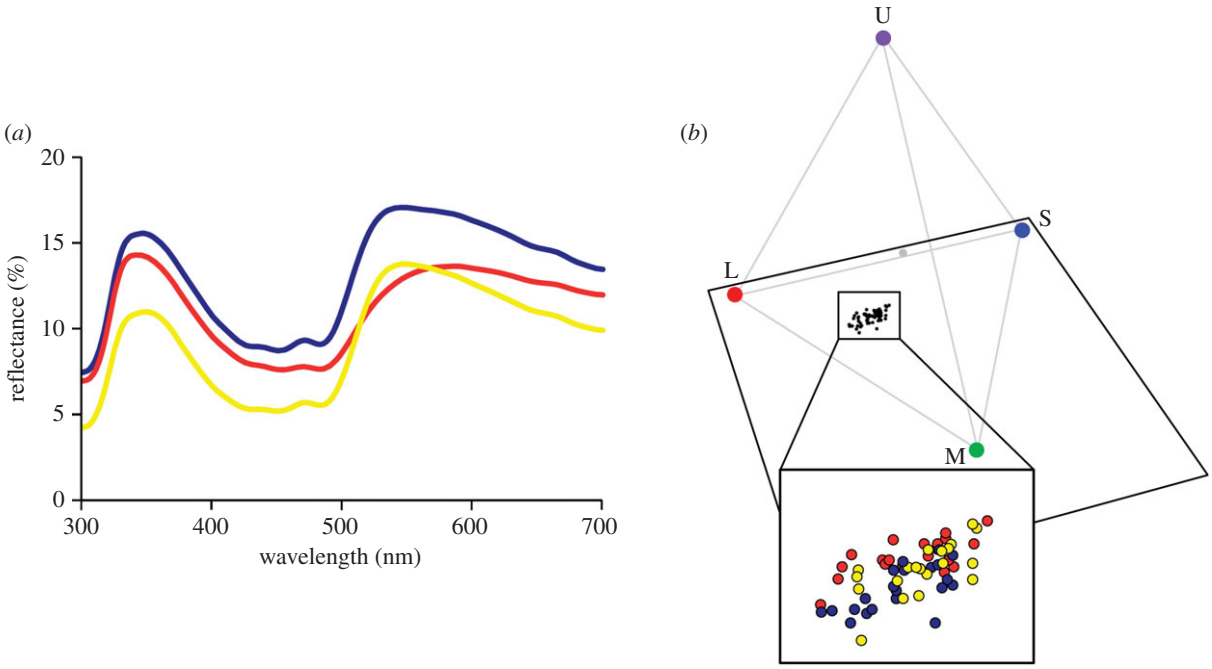

Figure 1. Reflectance spectra of males from the different diet treatments and the distribution of analysed spectra in tetrahedral colour space. (*a*) Mean reflectance between 300 and 700 nm of the cheek region of reproductively active males fed a high-quantity/high-quality ([+ +], grey line), a high-quantity/low-quality ([+ −], light grey line) and a low-quantity/low-quality diet ([− −], dark grey line). (*b*) Location of the orange-red cheek coloration of males from the three diet treatments in three-dimensional tetrahedral colour space. Based on the spherical coordinates *theta*, *phi* and *r* (see the electronic supplementary material for details) the colour of each male is plotted as a point within this tetrahedron ([+ +] grey dots, [+ −] light grey dots, [− −] dark grey dots). Vertices of the tetrahedron represent exclusive excitation of one of the four cones (U, S, M, L). The achromatic point (equal stimulation of all cones) is shown at the centre of the colour space (small grey dot). (Online version in colour; [+ +] red, [+ −] yellow, [− −] blue.)

coloration (achieved chroma r_A) that differed significantly from the breeding coloration of their brothers (high-quantity/high-quality [+ +], $p < 0.001$; high-quantity/low-quality [+ −], $p = 0.047$; table 1; figure 1). Achieved chroma (r_A) was not significantly different between the two daily-fed groups ($p = 0.119$; table 1; figure 1).

Relative testis mass (GSI) was not significantly different between the brothers of the three feeding treatments (all $p \geq 0.076$; table 1) but daily-fed males ([+ +] and [+ −]) exhibited both a significantly higher absolute testis mass and consequently more sperm when compared with their food-restricted brothers [− −] (all $p < 0.001$; table 1). However, males from the high-quantity/high-quality [+ +] and the high-quantity/low-quality [+ −] diet group did not differ significantly with respect to absolute testis mass and sperm number (both $p \geq 0.249$; table 1).

Low-quantity/low-quality [− −] fed males had significantly faster sperm than their brothers (high-quantity/high-quality [+ +], $p < 0.001$; high-quantity/low-quality [+ −], $p = 0.008$; table 1). Sperm velocity of daily-fed males ([+ +] and [+ −]) did not differ significantly from each other ($p = 0.363$; table 1). Sperm linearity and the percentage of motile sperm were not significantly different between the three treatment groups (all $p \geq 0.051$; table 1).

(b) Paternity analysis

Food-restricted males [− −] had a higher fertilization success when competing against high-quantity/high-quality fed males [+ +] ('lme', $n = 42$, $\chi^2 = 5.092$, $p = 0.024$), whereas in the two other sub-trials ([+ +] versus [+ −] and [+ −] versus [− −]) fertilization success did not differ significantly between males from the different treatments ('lme', $n = 42$, both $\chi^2 \leq 1.626$, both $p \geq 0.202$). Moreover, mean fertilization success was significantly positively associated with mean sperm velocity ('lme', $n = 63$, $\chi^2 = 10.076$, $p = 0.002$; figure 2). This relationship was not significantly affected by the different feeding treatments ('lme', $n = 63$, $\chi^2 = 0.855$, $p = 0.652$).

(c) Relationship between pre- and postcopulatory traits

There was a significant interaction between males from the three feeding treatments concerning the investment in pre- and postcopulatory sexually selected traits ('lme', $n = 63$, $\chi^2 = 12.690$, $p = 0.002$; figure 3). Under food limitation [− −], a significantly negative association between achieved chroma (r_A) and sperm velocity was observed (Pearson correlation: $n = 21$, $r_P = -0.454$, $p = 0.039$; figure 3). By contrast, males from the daily-fed groups ([+ +] and [+ −]) developed a significantly positive relationship between achieved chroma (r_A) and sperm velocity (Pearson correlation: $n = 42$, $r_P = 0.326$, $p = 0.035$; figure 3).

4. Discussion

Our study provides experimental evidence that life-history traits such as body growth, mortality and reproductive performance in full-sibling stickleback males were considerably affected through environmental conditions. Food-restricted males [− −] showed a phenotypically plastic response in terms of a substantial reduction in body size and mass during sub-adult and adult stages compared with their daily-fed brothers. A reduction in growth and body size is one of the most profound effects associated with lower levels of energy intake during ontogeny (e.g. [50]). Food limitation

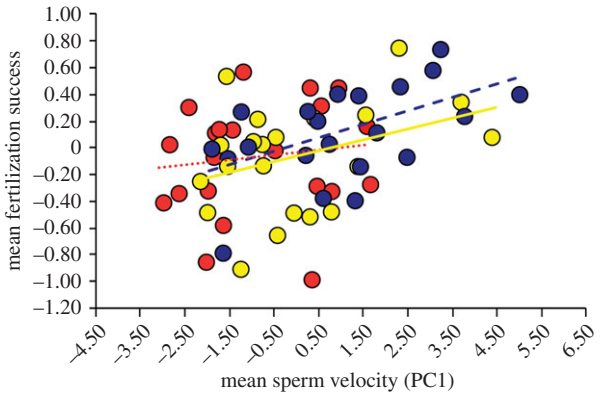

Figure 2. Significant positive relationship between mean fertilization success and mean sperm velocity (PC1, calculated between sub-trials; 'lme', $n = 63$, $\chi^2 = 10.076$, $p = 0.002$). This association was not affected by feeding treatment (high-quantity/high-quality [+ +] grey (dotted line), high-quantity/low-quality [+ −] light grey (solid line), low-quantity/low-quality diet [− −] dark grey (broken line)). (Online version in colour; [+ +] red, [+ −] yellow, [− −] blue.)

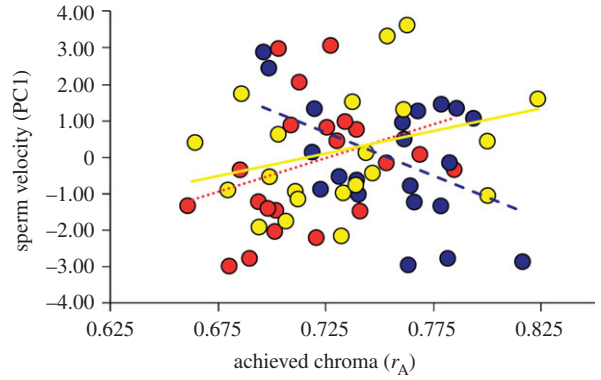

Figure 3. Food limitation ([− −] dark grey (broken line)) resulted in a negative relationship between pre- (achieved chroma (r_A)) and postcopulatory (sperm velocity, PC1 calculated for low-quantity/low-quality males separately) sexual selected traits, whereas a daily portion of either high-quality food ([+ +] grey (dotted line)) or 'normal' food ([+ −] light grey (solid line)) resulted in a positive association between sperm velocity (PC1, calculated for the daily-fed males [+ +] and [+ −]) and the intensity of male's breeding coloration (achieved chroma (r_A)). This resulted in a significant interaction term ('lme', $n = 63$, $\chi^2 = 12.690$, $p = 0.002$) across the three feeding treatments. (Online version in colour; [+ +] red, [+ −] yellow, [− −] blue.)

had further consequences as fewer individuals reached the reproductive phase suggesting that the chosen treatment represents a considerable stress factor during early development. Males fed high-quality food in high quantities [+ +] showed an enhanced body size compared with the high-quantity/low-quality [+ −] diet group at the adult stage but this was not apparent in sub-adult fish. This reveals that supplementation of carotenoids promoted growth only at later developmental stages possibly owing to an increased antioxidant demand at the initiation of reproductive activity [51]. In sticklebacks, larger body size is associated with fitness advantages as larger males have better chances in dominance fights [52] when establishing a territory under natural conditions. Moreover, females prefer to mate with larger males possibly owing to their higher paternal and territorial quality [53].

Although being smaller, males from the low-quantity/low-quality [− −] group had a more intense breeding coloration compared with males from the two other diet groups, which is in line with further studies on sexual signalling effort in sticklebacks (see [54] and citations therein). This suggests that, in accordance with theoretical predictions [55], males with a lower overall food intake showed an enhanced relative investment of carotenoid pigments in sexual ornamentation at least for the first breeding cycle that was studied here. An increased concentration of carotenoid pigments in the cheek region leads to a more saturated male breeding coloration [56], which is generally preferred by female conspecifics during mate choice [30]. Our findings thus reveal that food-restricted males may have improved their chances of recent reproduction through an increased attractiveness to females, which in turn might be associated with a reduction in future signalling effort in contrast with larger well-fed males. For instance, a study by Candolin [57] showed that larger males who completed several breeding cycles were able to increase their red coloration over the season, whereas small males that completed only few cycles did not. Pike *et al.* [58] demonstrated that stickleback males reared on a carotenoid-limited diet maintain their breeding coloration at the expense of total body carotenoids resulting in negative consequences for future reproductive investment and longevity. Males fed the carotenoid-enriched diet in the present study had a more red-shifted coloration, despite showing a less intense breeding coloration. Apparently, this resulted from a higher proportion of astaxanthin deposited in the integument of these males [56]. By contrast, male coloration in the low-quality dietary treatments (irrespective of food quantity ([+ −] and [− −]) was predominantly based on lutein that was ingested from the untreated chironomid larvae and differs from astaxanthin in its spectral absorbance characteristics, giving these males a rather orange 'hue' in human terms [38]. Although visual modelling suggests that sticklebacks are sensitive to variation in not only the concentration but also composition of carotenoids in a male's breeding signal [56], the role of carotenoid ratios in mate choice is less clear (but see [59]) and deserves further behavioural investigations.

In addition to the effects on precopulatory traits, the availability of dietary resources had an impact also on postcopulatory traits. Food-limited males [− −] had faster sperm compared with their daily-fed brothers ([+ +] and [+ −]) revealing that this component of sperm quality is sensitive to variation in nutritional conditions. Contrary to our findings, recent studies found positive effects of food quality on different measures of ejaculate quality (e.g. [18,20]). Despite the lower sperm swimming velocities, daily-fed males ([+ +] and [+ −]) in our study had a higher absolute testis mass and greater sperm numbers compared with their food-limited brothers. This may be explained by the fact that males in these groups were significantly larger than those from the low-quantity/low-quality [− −] group. A larger body size in combination with an increased ejaculate size should be beneficial for future reproductive opportunities [57]. On the other hand, the increased sperm velocity and elaborated sexual ornamentation in the low-quantity diet males implies that under food limitation available resources are allocated from somatic functions to increased expression of both pre- and postcopulatory traits, presumably at the expense of future reproductive performance (see also

[60]). One might assume that selective survival or group density effects owing to a higher mortality during development in the [− −] treatment may account for our findings that the food-restricted males showed an increased investment in pre- as well as postcopulatory sexually selected traits. However, the absolute difference in survival was about four individuals of unknown sex from a total of 30 fish per tank (see also the electronic supplementary material, table S5). We reran our statistical analyses without the four least intensely coloured males from the [+ +] as well as from the [+ −] diet group. Results were qualitatively the same (see the electronic supplementary material, table S6 for statistics), indicating that significant selection in the [− −] group relative to the males in the other diet groups ([+ +] and [+ −]) can be ruled out.

During pairwise sperm competition trials, food-limited males [− −] outcompeted their brothers from the high-quantity/high-quality group [+ +] by achieving a significantly greater paternity share when males contributed equal sperm numbers. Moreover, for all diet groups mean paternity success was best explained by mean sperm velocity, revealing that sperm velocity is an important determinant of sperm competitiveness in sticklebacks, as it has been described for other species (e.g. [61]). In this study, sperm number was kept constant during *in vitro* fertilization assays. Nevertheless, a potential interaction between sperm number (in fact ejaculate size, e.g. [62]) and velocity, as suggested by a recent stickleback study [45], affecting fertilization success under natural conditions cannot be ruled out and requires further investigations. Three-spined stickleback males are sperm-limited over the course of one breeding season as spermatogenesis only occurs during the short photoperiods [39]. Thus, apart from sperm velocity, sperm quantity additionally represents an important factor of a male's reproductive success (e.g. [62]). Therefore, it can be speculated that under natural conditions males from the high-quantity/high-quality [+ +] as well as the high-quantity/low-quality [+ −] diet groups might have an overall greater lifetime reproductive success owing to their higher number of stored sperm.

Consistent with our predictions and sperm competition theory [15], a negative relationship between the intensity of male breeding coloration and sperm velocity was found in food-restricted males [− −], suggesting that these individuals had to allocate their resources between pre- and postcopulatory sexually selected traits. By contrast, their daily-fed brothers ([+ +] and [+ −]) showed a positive relationship between the intensity of their orange-red breeding coloration and sperm velocity irrespective of food quality (see also [33]), indicating the ability to allocate to both pre- and postcopulatory traits under conditions of high resource availability [14]. The intensity of carotenoid-based breeding signals should provide females with honest information about male foraging ability [63] and the amount of carotenoids available for immunostimulation [24] or antioxidant functions [64]. Our results indicate that this may only account for well-fed males while under food limitation some dishonesty may occur [60]. This could lead to evolutionary constraints on the direction of pre- and postcopulatory sexually selected traits, which may have considerable consequences even on population level. Oxidative stress was identified as an important factor mediating the condition-dependent expression of both precopulatory (e.g. [65]) and postcopulatory traits (e.g. [18]). Although variation in male oxidative status is also likely to provide a proximate explanation for our findings (see also [28]), further research effort, including measures of antioxidant capacity and oxidative damage, is required before more definitive conclusions can be drawn.

5. Conclusion

To summarize, resource availability leads to a considerably plastic response by strongly affecting life-history traits when stickleback full-sibling brothers were raised under environmental conditions varying in food quantity and/or quality. Dietary manipulation simultaneously affected body growth, mortality and the expression of pre- and postcopulatory sexually selected traits as well as male competitive fertilization success through modulation of sperm swimming performance. Interestingly, under dietary limitation males showed an overall increased investment in both pre- and postcopulatory sexual traits, presumably to maximize their chances of present reproduction at the expense of future reproductive opportunities. Recent studies found that dietary restriction in male guppies does not expose an allocation trade-off between pre- and postcopulatory sexual traits [26,27]. While a direct comparison between stickleback and guppy mating systems is difficult owing to the fact that the guppy is an internally fertilizing species exhibiting cryptic female choice (e.g. [66]), our findings illustrate that, consistent with various theoretical predictions [15,34], reproductive investment between pre- and postcopulatory traits is traded-off under resource limitation, whereas a positive relationship between pre- and postcopulatory traits is present when resources are not limited.

Ethics. The study conforms to the legal requirements of Germany.

Data accessibility. Data have been submitted as the electronic supplementary material.

Authors' contributions. M.M. and T.C.M.B. designed the study. M.M. bred, fed and raised the used study animals, collected the data and analysed them. I.P.R. performed the visual model calculations. M.M. wrote the manuscript supported by I.P.R. and T.C.M.B. All authors gave final approval for publication.

Competing interests. The authors declare that they have no competing interests.

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Supplementary material

Reflectance measurements and physiological model on stickleback colour vision [see also 1 for further details]

Reflectance measurements were performed by holding a bifurcated 200-µm fiber-optic probe perpendicular to the male's skin. The probe end was inserted into a darkened pipette tip to exclude ambient light and to collect scans at a fixed distance (0.3 cm). Reflectance was quantified relative to a 98 % Spectralon white standard. Measurements were taken from the orange-red cheek region directly below the eye. For each male about 28 scans (mean ± standard deviation (SD): 28.40 ± 11.61) were recorded in quick succession without changing probe contact and then averaged. Spectral data were collected using Avantes Avasoft 7.5 and imported into Microsoft Excel. Each measurement took only about 30 seconds so that short-term colour changes could be excluded (IPR, personal observation).

The calculation of the physiological model on stickleback color vision was done as follows; for each of the four stickleback cone receptors (UV, S, M, L) absolute stimulation was calculated between 300 and 700 nm by multiplying spectral reflectance per individual male by stickleback retinal cone sensitivities and an ambient irradiance spectrum for D65 standard daylight. Absolute cone stimulation values were normalized to sum to one, resulting in relative cone stimulation values, which were converted to Cartesian coordinates (x, y, z), following Endler & Mielke [2], which were then converted to spherical coordinates for each color point. Angle *theta* (hue longitude) represents variation around the horizontal plane (L, M, S cone) in tetrahedral color space. Lower values of *theta* indicate red-shifted hues while higher values indicate orange-shifted hues. Thus, *theta* was used as measure of carotenoid composition of male breeding coloration [see 3]. Following Stoddard & Prum [4] the achieved chroma r_A was calculated as a measure of spectral purity, which sets the chroma r (distance to the achromatic centre) of a point in color space in relation to the potential maximum chroma r_{max} for its hue ($r_A = r/r_{max}$) using the

pavo package in R (version 3.0.2) [5]. The magnitude of r_A was used as an estimate of the concentration of carotenoids allocated to a male's breeding signal with higher values describing a more saturated coloration. Angle *phi* (hue latitude), which represents variation around the vertical plane of the colour space (stimulation of the UV cone) was not considered in the present study as we were only interested in analyzing variation in the composition (*theta*) and concentration (r_A) of carotenoids in male breeding coloration [see 3].

Quantification of sperm swimming abilities (i.e. percentage of motile sperm, sperm velocity and sperm linearity)

Prior to the quantification of sperm swimming abilities the pestled left testis was set at a constant temperature (16 °C; Thriller, PEQLAB_V0410E, Erlangen, Germany). Exactly, two minutes later a Leja counting chamber (depth 12 µm, Nieuw-Vennep, The Netherlands) was loaded with 3 µl of the mixed sperm suspension to achieve the following variables: (1) percentage of motile sperm, (2) velocity curvilinear (VCL [µm s^{-1}]), (3) velocity average path (VAP [µm s^{-1}]), (4) velocity straight line (VSL [µm s^{-1}]), (5) straightness (STR=VSL/VAP × 100 [%]), (6) linearity (LIN=VSL/VCL × 100 [%]) and (7) wobble (side to side movement of the sperm head; WOB=VAP/VCL × 100 [%]). For each male subsequent data analyses were based on sperm speed measurements of about hundred single sperm (mean ± SD: 126.26 ± 72.11). Threshold values for excluding immotile sperm were specified as 10 µm s^{-1} for VCL, 5 µm s^{-1} for VAP and 2 µm s^{-1} for VSL [see also 6, 7]. To receive a single significant factor (Kaiser criterion > 1) for sperm velocity (VCL, VAP and VCL) and sperm linearity (LIN, STR and WOB) principal component analyses were conducted (see below, table S1).

table S1. Principal component analyses of measured sperm variables.

So far it is unknown which sperm swimming variables are the most relevant during sperm competition in sticklebacks, thus principal component analyses were performed to obtain a single significant factor (Kaiser criterion > 1) of sperm velocity (VCL, VAP and VCL) and sperm linearity (LIN, STR and WOB) [see also 6, 7].

	principal component	Eigenvalue	proportion of variance
high-quantity/high-quality [+ +]	PC1sperm velocity	1.654	91.15 %
	PC1sperm linearity	1.556	80.68 %
high-quantity/low-quality [+ −]	PC1sperm velocity	1.643	89.99 %
	PC1sperm linearity	1.484	73.38 %
low-quantity/low-quality [− −]	PC1sperm velocity	1.646	90.31 %
	PC1sperm linearity	1.577	82.94 %
high-quantity/high-quality and low-quantity/low-quality	PC1sperm velocity	1.663	92.21 %
([+ +] and [− −])	PC1sperm linearity	1.523	77.34 %
high-quantity/high-quality and high-quantity/low-quality	PC1sperm velocity	1.648	90.50 %
([+ +] and [+ −])	PC1sperm linearity	1.554	80.49 %
high-quantity/low-quality and low-quantity/low-quality	PC1sperm velocity	1.655	91.29 %
([+ −] and [− −])	PC1sperm linearity	1.510	75.97 %
all three feeding treatments	PC1sperm velocity	1.659	91.74 %
	PC1sperm linearity	1.526	77.58 %

Principal component analyses were done separately (for each treatment, between the sub-trials and across all three feeding treatments) as the PC1-values were included in several different analyses as well (see main manuscript). Higher values for PC1 sperm velocity and PC1 sperm linearity indicated faster and sperm swimming with a more linear path, respectively. This was true for each diet treatment separately (Pearson correlations: $N = 21$, all $r_P \geq 0.616$, all $p \leq 0.003$), between the sub-trials (Pearson correlations: $N = 42$, all $r_P \geq 0.689$, all $p < 0.001$) and across all treatments (Pearson correlations: $N = 63$, both $r_P \geq 0.718$, both $p < 0.001$). In two cases (high-quantity/high-quality vs. high-quantity/low-quality, [+ +] vs. [+−] and high -quantity/low-quality [+−]) VCL was logarithmically transformed to achieve normal distribution according to Kolmogorov-Smirnov tests with Lilliefors correction.

table S2. List of all nine microsatellite markers [8, 9]. that were used for paternity analyses.

Locus	GenBank accession no.	combined	dye label	tail	tail-sequence 5'- 3‘
GAC1116PBBE	AJ010353	mix X	D3 (green)	T7	TAATACGACTCACTATAG
GAC7033PBBE	AJ010360	mix X	D4 (blue)	M13	TGTAAAACGACGGCCAGT
GAC3133PBBE	AJ010356	mix Y	D4 (blue)	Sp6	GATTTAGGTGACACTAT
GAC4174PBBE	AJ010358	mix Y	D3 (green)	T7	TAATACGACTCACTATAG
GAC7010PBBE	AJ311863	mix Y	D2 (black)	M13	TGTAAAACGACGGCCAGT
GAC1097PBBE	AJ010352	mix Z	D2 (black)	M13	TGTAAAACGACGGCCAGT
GAC1125PBBE	AJ010354	mix Z	D2 (black)	M13	TGTAAAACGACGGCCAGT
GAC4170PBBE	AJ010357	mix Z	D4 (blue)	Sp6	GATTTAGGTGACACTAT
GAC5196PBBE	AJ010359	mix Z	D3 (green)	T7	TAATACGACTCACTATAG

Same letter mix (X, Y or Z) indicated that they were combined during PCR reaction [using the tailed primer method; see 10] and genetic analyses (Beckman Coulter). All putative fathers were full-sib brothers but they originated from a large anadromous and hence genetically heterogeneous population [8]. Nevertheless, for each sub-trial it was separately decided which mix (X, Y or Z, see also table S3) was used for egg-genotyping because in many cases some microsatellite markers were not meaningful (i.e. 100 % informative). On average, each egg was successfully genotyped with 3.49 ± 1.44 (mean ± standard deviation (SD)) microsatellites markers of which at least one (1.63 ± 0.70; mean ± SD) was 100 % informative.

For each sub-trial [+ +] vs. [+ −], [+ +] vs. [+ −] or [+ −] vs. [− −], 30 randomly chosen eggs were extracted and genotyped, apart from five exceptions; in four sub-trials less than 30 eggs had been fertilized and in one case there was a miscount leading to the analysis of 31 eggs. Thus, the total sample size constitutes 1826 eggs, of which 1718 eggs could be successfully assigned to one father (mean ± SD: 27.27 ± 4.87 eggs per sub-trial). In total, 108 eggs had to be discarded due to contamination (49 eggs; more than 2 alleles), no available informative microsatellite marker (54 eggs) or no successful fertilization (5 eggs; no ‘father-alleles’).

table S3. Details of used PCR conditions.

Primer mix X		1 x µl
GAC1116PBBE	reverse (10 pmol/µl)	0.400
	labeled (10 pmol/µl)	0.400
	forward (2.5 pmol/µl)	0.200
GAC7033PBBE	reverse (10 pmol/µl)	0.100
	labeled (10 pmol/µl)	0.100
	forward (2.5 pmol/µl)	0.050
	Qiagen Multiplex PCR	5.000
	distilled water	2.000
	(extracted) DNA	2.000

PCR program mix X	
preheating	94°C 15 min.
30 cycles	
denaturing	94°C 60 sec.
annealing	56°C 45 sec.
elongating	72°C 60 sec.
8 cycles	
denaturing	94°C 60 sec.
annealing	53°C 45 sec.
elongating	72°C 60 sec.
final extension cycle	72°C 30 min.

Primer mix Y		1 x µl
GAC3133PBBE	reverse (10 pmol/µl)	0.100
	labeled (10 pmol/µl)	0.100
	forward (2.5 pmol/µl)	0.050
GAC4174PBBE	reverse (10 pmol/µl)	0.200
	labeled (10 pmol/µl)	0.200
	forward (2.5 pmol/µl)	0.100
GAC7010PBBE	reverse (10 pmol/µl)	0.160
	labeled (10 pmol/µl)	0.160
	forward (2.5 pmol/µl)	0.080
	Qiagen Multiplex PCR	5.000
	distilled water	1.850
	(extracted) DNA	2.000

PCR program mix Y	
preheating	94°C 15 min.
30 cycles	
denaturing	94°C 60 sec.
annealing	56°C 45 sec.
elongating	72°C 60 sec.
8 cycles	
denaturing	94°C 60 sec.
annealing	53°C 45 sec.
elongating	72°C 60 sec.
final extension cycle	72°C 30 min.

Primer mix Z		1 x µl
GAC1097PBBE	reverse (10 pmol/µl)	0.250
	labeled (10 pmol/µl)	0.250
	forward (2.5 pmol/µl)	0.125
GAC1125PBBE	reverse (10 pmol/µl)	0.060
	labeled (10 pmol/µl)	0.060
	forward (2.5 pmol/µl)	0.030
GAC4170PBBE	reverse (10 pmol/µl)	0.150
	labeled (10 pmol/µl)	0.150
	forward (2.5 pmol/µl)	0.075
GAC5196PBBE	reverse (10 pmol/µl)	0.400
	labeled (10 pmol/µl)	0.400
	forward (2.5 pmol/µl)	0.200
	Qiagen Multiplex PCR	5.000
	distilled water	0.850
	(extracted) DNA	2.000

PCR program mix Z	
preheating	94°C 15 min.
30 cycles	
denaturing	94°C 60 sec.
annealing	58°C 45 sec.
elongating	72°C 60 sec.
8 cycles	
denaturing	94°C 60 sec.
annealing	53°C 45 sec.
elongating	72°C 60 sec.
final extension cycle	72°C 30 min.

Each PCR product (10 µl) was diluted with 90 µl distilled water and 2 µl of this dilution were mixed with 0.1 µl of GenomeLabTM DNA Size Standard 400 and 30 µl of GenomeLabTM Sample Loading Solution (Beckman Coulter). Genotypes of all parents were scored on a CEQ 8800 Genetic Analysis System (Beckman Coulter) and analyzed via GenomeLabTM GeXP (version10.2).

92 **table S4.** Overview of all fitted models (see main manuscript for further details).

dependent variable	explanatory variable	random factor
body size [cm] (sub-adults and adults)	feeding treatment and sub-trial	family
body mass [g] (sub-adults and adults)	feeding treatment and sub-trial	family
mortality [%]	feeding treatment and sub-trial	family
theta (hue longitude)	feeding treatment and sub-trial	family
achieved chroma (r_A)	feeding treatment and sub-trial	family
relative testis mass (GSI)	feeding treatment and sub-trial	family
absolute testis mass [g]	feeding treatment and sub-trial	family
sperm number	feeding treatment and sub-trial	family
motile sperm [%]	feeding treatment and sub-trial	family
sperm velocity	feeding treatment and sub-trial	family
sperm linearity	feeding treatment and sub-trial	family
paternity [%]	sub-trial	family
mean fertilization success	feeding treatment x mean sperm velocity	family
sperm velocity	food restriction (yes/no) x achieved chroma (r_A)	family

feeding treatment: [+ +] vs. [+ −] vs. [− −]
sub-trial: [+ +] vs. [− −], [+ +] vs. [+ −] or [+ −] vs. [− −]
food restriction (yes/no): [− −] vs. [+ +] and [+ −]
calculation of mean fertilization success and mean sperm velocity: [((focal male – competitor 1) + (focal male – competitor 2))/2]
x means interaction term

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94 In all models family identity was included as random factor and never removed to control for
95 genotype influences.

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table S5. Descriptive data (mean ± standard deviation (SD)) of all measured variables for high-quantity/high-quality [+ +], high-quantity/low-quality [+−] and low -quantity/low-quality [− −] fed males.

		[+ +]		[+ −]		[− −]	
		mean	SD	mean	SD	mean	SD
sub-adult	body size [cm]	3.21	0.21	3.17	0.18	2.43	0.19
(~ 5 months)	body mass [g]	0.393	0.070	0.382	0.056	0.193	0.047
adult	body size [cm]	4.76	0.22	4.55	0.25	3.99	0.37
(~ 1 year old)	body mass [g]	1.355	0.220	1.241	0.212	0.835	0.279
development	mortality [%] *****	8.25	5.34	11.11	9.56	25.40	9.46
breeding	*theta* (hue longitude)	0.293	0.109	0.423	0.106	0.474	0.121
	achieved chroma (r_A)	0.716	0.030	0.731	0.042	0.754	0.032
testes traits	relative testis mass (GSI)	0.674	0.168	0.742	0.187	0.659	0.214
	absolute testis mass [g]	0.0090	0.0026	0.0091	0.0024	0.0053	0.0020
sperm traits	sperm number	$108.5 * 10^6$	$53.6 * 10^6$	$126.5 * 10^6$	$49.0 * 10^6$	$59.4 * 10^6$	$20.3 * 10^6$
	motile sperm [%]	37.62	8.62	37.94	9.06	36.44	7.32
	sperm velocity	-0.639	1.464	-0.325	1.367	0.964	1.733
	sperm linearity	-0.239	1.555	-0.126	1.500	0.365	1.526

***** This equals an absolute number of on average 2.48 ± 1.60 [+ +], 3.33 ±2.87 [+ −] and 7.62 ± 2.84 [− −] dead individuals over a period of about 9 months.

table S6. Results of the statistical analyses of pre- and postcopulatory traits after removing the 4 least intensely coloured males out of each of the two diet groups [+ +] and [+−] ($N_{[+\,+]} = 17$; $N_{[+\,-]} = 17$; $N_{[-\,-]} = 21$).

dependent variable				pairwise comparison of the different sub-trials					
				[+ +] vs. [− −]		[+ +] vs. [+ −]		[+ −] vs. [− −]	
		χ^2	p-value	χ^2	p-value	χ^2	p-value	χ^2	p-value
breeding coloration	*theta* (hue longitude)	20.34	**<0.001**	27.92	**<0.001**	11.71	**<0.001**	1.38	0.240
	achieved chroma (r_A)	8.08	**0.018**	8.85	**0.003**	3.13	0.077	0.95	0.330
testes traits	relative testis mass (GSI)	3.12	0.211	<0.01	0.940	2.25	0.134	3.15	0.076
	absolute testis mass [g]	25.56	**<0.001**	17.98	**<0.001**	0.30	0.581	22.72	**<0.001**
sperm traits	sperm number	20.99	**<0.001**	11.68	**<0.001**	1.65	0.199	24.88	**<0.001**
	motile sperm [%]	5.48	0.064	2.00	0.158	1.80	0.179	4.31	**0.038**
	sperm velocity	8.97	**0.011**	6.46	**0.011**	0.12	0.729	5.50	**0.019**
	sperm linearity	1.05	0.592	0.74	0.390	0.04	0.834	0.34	0.560

Analyses were performed following the same procedure as described for the main results. After removal of the 4 least intensely colored males results were qualitatively the same (see also Table 1 in the main manuscript) except the pairwise comparison of [+−] vs [− −] with respect to achieved chroma (r_A) (p = 0.047 changed to p = 0.330) and percentage of motile sperm (p = 0.051 changed to p = 0.038). This rather conservative testing clearly shows that significant selection due to higher mortality during development in the [− −] group relative to the males in the other diet groups ([+ +] and [+−]) cannot account for our main findings.

Accordingly, males from the daily-fed groups ([+ +] and [+ −]) developed a significant positive relationship between achieved chroma (r_A) and sperm velocity also for the reduced data set (Spearman correlation: N = 34, $r_S = 0.348$, p = 0.044). The significant interaction between males from the three feeding treatments with regard to their investment in pre- and postcopulatory traits also remained significant after data reduction ("lme", N = 55, $\chi^2 = 9.112$, p = 0.011; see also figure 3 in the main manuscript).

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