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Effects of nutrient limitation on sperm and seminal fluid: a systematic review and meta-analysis

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ABSTRACT

Theory predicts that costly sexual traits should be reduced when individuals are in poor condition (i.e. traits should exhibit condition-dependent expression). It is therefore widely expected that male ejaculate traits, such as sperm and seminal fluid, will exhibit reduced quantity and quality when dietary nutrients are limited. However, reported patterns of ejaculate condition dependence are highly variable, and there has been no comprehensive synthesis of underlying sources of such variation in condition-dependent responses. In particular, it remains unclear whether all ejaculate traits are equally sensitive to nutrient intake, and whether such traits are particularly sensitive to certain dietary nutrients, respond more strongly to nutrients during specific life stages, or respond more strongly in some taxonomic groups. We systematically reviewed these potential sources of variation through a meta-analysis across 50 species of arthropods and vertebrates (from 71 papers and 348 effect sizes). We found that overall, ejaculate traits are moderately reduced when dietary nutrients are limited, but we also detected substantial variation in responses. Seminal fluid quantity was strongly and consistently condition dependent, while sperm quantity was moderately condition dependent. By contrast, aspects of sperm quality (particularly sperm viability and morphology) were less consistently reduced under nutrient limitation. Ejaculate traits tended to respond in a condition-dependent manner to a wide range of dietary manipulations, especially to caloric and protein restriction. Finally, while all major taxa for which sufficient data exist (i.e. arthropods, mammals, fish) showed condition dependence of ejaculate traits, we detected some taxonomic differences in the life stage that is most sensitive to nutrient limitation, and in the degree of condition dependence of specific ejaculate traits. Together, these biologically relevant factors accounted for nearly 20% of the total variance in ejaculate responses to nutrient limitation. Interestingly, body size showed considerably stronger condition-dependent responses compared to ejaculate traits, suggesting that ejaculate trait expression may be strongly canalised to protect important reproductive functions, or that the cost of producing an ejaculate is relatively low. Taken together, our findings show that condition-dependence of ejaculate traits is taxonomically widespread, but there are also many interesting, biologically relevant sources of variation that require further investigation. In particular, further research is needed to understand the differences in selective pressures that result in differential patterns of ejaculate condition dependence across taxa and ejaculate traits.

Key words: ejaculate, post-copulatory, diet, protein, nutrient limitation, condition dependence, life history, body-size, sperm, semen.

CONTENTS

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I. INTRODUCTION

(1) Male ejaculate traits are expected to exhibit condition-dependent expression

Persistent directional sexual selection can lead to trait exaggeration, such that morphological appendages, organs, physiological functions or behaviours whose expression is positively correlated with fitness evolve enlarged or elaborated expression (Darwin, 1859, 1871). However, expressing such exaggerated traits is thought to impose a variety of costs, including the costs of increased resource allocation and trade-offs with other organismal functions (Kotiaho, 2000). Consequently, theory predicts that trait exaggeration will also be associated with the evolution of heightened condition dependence, whereby high-condition individuals (i.e. those able to acquire more metabolic resources) are better able to invest in such costly traits (Andersson, 1982; Nur & Hasson, 1984; Rowe & Houle, 1996; Cotton, Fowler, & Pomiankowski, 2004). Condition-dependence theory has been prominent in sexual selection theory for several decades, and heightened condition dependence has been demonstrated empirically in male secondary sexual traits such as ornaments and weapons (e.g. Moller & Delope, 1994; Emlen, 1997; Kotiaho, 2000). However, selection can also favour the exaggeration of sperm and semen traits (e.g. Cameron, Day, & Rowe, 2007; Crudgington *et al.*, 2009; Rowe *et al.*, 2015; Lüpold *et al.*, 2016; Godwin *et al.*, 2017). Indeed, Lüpold *et al.* (2016) demonstrated that sperm traits can, in some cases, show even greater trait exaggeration than secondary sexual traits. The expression of sperm and semen traits can impose non-trivial metabolic costs (e.g. Dewsbury, 1982; Olsson, Madsen, & Shine, 1997; Marcotte, Delisle, & McNeil, 2007; Perry & Tse, 2013; Lüpold *et al.*, 2016; Godwin *et al.*, 2017), resulting in the expectation that males in low condition should be less able to invest in sperm and semen traits compared to males in high condition. In other words, ejaculate traits may be expected to exhibit heightened condition dependence for the same reasons as other secondary sexual traits, such as ornaments and weaponry.

Parker (1970) recognised that sperm competition can generate sexual selection on sperm production, and proposed the 'raffle principle' whereby male fertilisation success is expected to depend on the number of sperm transferred relative to competitors (Parker, 1990). Many subsequent theoretical and empirical studies have investigated selection on sperm number (i.e. the number of sperm in storage, or within an ejaculate) (Parker *et al.*, 1997; Gage & Morrow, 2003; Parker & Ball, 2005; Boschetto, Gasparini, & Pilastro, 2011). Testes size, often used as a proxy for sperm production, has been shown to be correlated with levels of sperm competition (reviewed in Gage, 1994; Simmons & Fitzpatrick, 2012), and several studies have also shown that testes size (i.e. sperm production) and the number of sperm transferred can be correlated with paternity share (e.g.

Engqvist *et al.*, 2007; Vellnow *et al.*, 2018); demonstrating that selection can indeed favour increased sperm number. However, in the last decade, there has been a shift of focus to selection on sperm quality (e.g. Gomendio *et al.*, 2006; Gasparini *et al.*, 2010; Tourmente, Gomendio, & Roldan, 2011; Mehlis, Rick, & Bakker, 2015), spurred on by Snook (2005). For example, increased sperm viability can be correlated with risk of sperm competition (e.g. Gomendio *et al.*, 2006), and increased sperm velocity can be associated with increased paternity (Boschetto *et al.*, 2011; Beausoleil *et al.*, 2012). Also, in some cases, increased sperm length has been correlated with sperm competition intensity (e.g. Gage, 1994; Radwan, 1996; LaMunyon & Samuel, 1999), but this effect is less consistent across taxa (reviewed in Simmons & Fitzpatrick, 2012) and is likely due to differences in selection environments among taxonomic groups (see Immler *et al.*, 2011; Liao *et al.*, 2018). For example, selection for sperm length is clearly favoured in *Drosophila* species but not in passerine birds and such differences are likely driven by differences in sperm competition mechanisms (i.e. raffle sperm competition in passerines but sperm displacement in *Drosophila*) (Immler *et al.*, 2011).

Non-sperm ejaculate components – i.e. protein and peptides in the seminal fluid – can also be highly important for male fitness and may be expected to evolve heightened condition dependence. Such components can increase sperm survival, as well as confer advantages in sperm competition by altering female physiology (Chapman, 2001; Ramm, Parker, & Stockley, 2005; Avila *et al.*, 2011; South & Lewis, 2011; Crean, Adler, & Bonduriansky, 2016). Additionally, components in the ejaculate can alter offspring development and quality (reviewed in Bromfield, 2014). Therefore, the non-sperm components of the ejaculate are also likely to be under strong selection (Macartney, Crean, & Bonduriansky, 2018*a*). A large body of literature has focused on males that confer large, nutrient-rich spermatophores and nuptial gifts (e.g. Gwynne, 1993; Jia, Jiang, & Sakaluk, 2000; Perry & Rowe, 2010; Duplouy *et al.*, 2017). However, empirical evidence also suggests that selection can favour increased ejaculate expenditure in species where males do not transfer nutrient-laden ejaculates. For example, a study on *Drosophila pseudoobscura* demonstrated that increased risk of sperm competition selected for males with larger accessory glands (Crudgington *et al.*, 2009), and Linklater *et al.* (2007) demonstrated that *Drosophila melanogaster* males in male-biased populations invest more accessory gland products per mating compared to sperm, suggesting that intense sperm competition can also select for increased investment in the non-sperm ejaculate components (see also Cameron *et al.*, 2007).

(2) Special challenges in research on ejaculate condition dependence

There is now much theoretical and empirical support for the hypothesis that sperm competition can select for increased investment in sperm and/or semen (e.g. Parker, 1990; Wedell, Gage, & Parker, 2002; Gage & Morrow, 2003; Williams, Day, & Cameron, 2005; South & Lewis, 2011), and that such investment can be strongly condition dependent (e.g. Tazzyman *et al.*, 2009). However, the nature and degree of sperm and semen condition dependence presents several complications and challenges for research. Ejaculate traits are typically cryptic: they encompass sperm quantity, morphology, motility and viability (i.e. quality), as well as seminal fluid quantity and composition. Variation in such traits can be subtle, and the energetic and resource-allocation costs of investment in such traits are difficult to quantify. Ejaculate investment is also highly multi-faceted. For example, sperm production comprises multiple different traits (i.e. number, morphology, motility and viability) that affect sperm quality and can be targets of selection (Lüpold & Pitnick, 2018), and seminal fluid contains hundreds of different proteins and peptides with varying (and largely unknown) functions (Avila *et al.*, 2011; Perry, Sirot, & Wigby, 2013), making it difficult to identify the direct targets of sexual selection and predict which particular ejaculate traits should exhibit heightened condition dependence (Poiani, 2006). The nature and degree of condition dependence of ejaculate traits therefore remain less well understood than the condition dependence of pre-copulatory secondary sexual traits such as ornaments and weapons.

A key source of variation in condition is nutrient availability, which determines the quantity of metabolic resources available for investment in all fitness-enhancing traits (Andersson, 1982; Rowe & Houle, 1996; Morehouse, 2014). However, like life-history traits such as ornaments and weaponry, ejaculate traits could exhibit complex patterns of plasticity. For example, ejaculate investment could be strongly dependent on specific nutrients, and particularly sensitive to nutrient limitation at specific ontogenetic life stages. Ejaculate investment strategies are also likely to vary among (and sometimes within) species. Such factors could contribute to variable results of empirical studies. While many studies have indeed demonstrated condition-dependent responses of male sperm and semen traits through experiments manipulating nutrient availability (e.g. Droney, 1998; Rahman, Kelley, & Evans, 2013; Vega-Trejo, Jennions, & Head, 2016; Dávila & Aron, 2017), some studies have found that males may increase their investment in ejaculate traits when nutrients are reduced (e.g. Simmons, Tomkins, & Hunt, 1999; Perry & Rowe, 2010; Mehlis *et al.*, 2015), while others have found no effect of diet on such traits (e.g. Sitzmann *et al.*, 2010; Sullivan, Brown, & Clotfelter, 2014). Identifying and accounting for key sources of biological variation in ejaculate investment could help to make sense of such variable results.

Different sperm and seminal fluid traits could be subject to varying selection pressures within and among species, resulting in different levels of costliness and thus differences in condition dependence. For example, there is generally thought to be a trade-off between sperm quality and quantity, and taxonomic differences in the selection environment can alter this trade-off (Immler *et al.*, 2011; Liao *et al.*, 2018). Within species, Bunning *et al.* (2015) demonstrated that sperm number and sperm viability increased linearly with an increase in protein and carbohydrates in the

cockroach *Nauphoeta cinerea* and investment peaked at the same protein: carbohydrate ratio, but sperm number responded more strongly to nutrient availability than did sperm viability. Additionally, males in high *versus* low condition may employ differing reproductive strategies, and therefore exhibit differential responses to nutrient limitation. For example, Perry & Rowe (2010) found that male ladybirds (*Adalia bipunctata*) alter their investment in sperm and non-sperm components depending on their condition, with high-condition males (i.e. males reared on a nutrient-rich diet) investing more in non-sperm components of the ejaculate, and low-condition males (i.e. males reared on a nutrient-poor diet) investing more in absolute sperm number. This suggests that different ejaculate traits are differentially sensitive to nutrient abundance, and that males may employ different investment strategies based on their condition and mating system. Hence, the strength and direction of a trait's response to diet can vary depending on the species and on the particular ejaculate trait measured.

(3) Sources of variation in ejaculate condition dependence

Most studies examining the condition dependence of male ejaculate traits have manipulated dietary intake of nutrients. Many such studies have manipulated total calories without altering nutrient ratios (e.g. Vermeulen, Engels, & Sauer, 2008; Kahrl & Cox, 2015; Mehlis *et al.*, 2015; Kaldun & Otti, 2016; Vega-Trejo *et al.*, 2016). However, particular macro- or micronutrients may be particularly important for ejaculate trait expression. For example, protein is essential for oogenesis (Chippindale & Leroi, 1993; Adler *et al.*, 2013), but there is also evidence to suggest that protein can be important for male sperm and semen traits (e.g. Droney, 1998; Melo *et al.*, 2014; Dávila & Aron, 2017). Other studies have suggested that micronutrients such as carotenoids, amino acids and vitamins can affect ejaculate trait expression (Lederhouse *et al.*, 1990; Locatello *et al.*, 2006; Lambrot *et al.*, 2013; Yossa *et al.*, 2015; Tomášek *et al.*, 2017). But we do not know how different dietary components contribute to the variation in ejaculate responses.

The ontogenetic stage when nutrients are limited (i.e. during juvenile development *versus* during the adult stage) could also be very important. The adult diet could affect ejaculate trait expression because spermatogenesis typically occurs throughout adulthood, and the amount of metabolic resources available to adult males has been shown to influence investment in ejaculate traits in some species (e.g. Droney, 1998; Perry & Rowe, 2010; Evans, Rahman, & Gasparini, 2015; Kahrl & Cox, 2015; Bailey, Legan, & Demas, 2017). This may be particularly true in animals that have indeterminate growth, such as fish, molluscs and some reptiles. Such species require energy throughout life to maintain growth and reproduction, and energy gained through diet may be reallocated to growth instead of reproduction when nutrients are scarce (Heino & Kaitala, 1999). But the developmental environment can alter metabolic pathways in adults (e.g. Gheorghe *et al.*, 2010), and differences in developmental nutrients could change how resources are mobilised and allocated to sperm and semen traits. Investment in adult reproductive traits may be programmed during development (e.g. *via* changes in epigenetic factors such as DNA methylation, chromatin structure and non-coding RNAs), and this may depend on nutrient availability during development (Macartney *et al.*, 2018*a*). Also, effects of nutrient limitation during development may be more prevalent in some taxa. For example, holometabolous insects rely on developmental nutrient acquisition for investment in adult reproductive traits (Boggs, 1981), including ejaculate traits (e.g. Dávila & Aron, 2017; Macartney *et al.*, 2018*b*). However, there has been no systematic assessment of how male ejaculate traits respond to nutrient limitation at different ontogenetic stages, or how these effects differ among taxa.

(4) Systematically reviewing ejaculate condition dependence

Although many experimental studies have investigated the effects of nutrition on expression of a range of ejaculate traits in a diverse range of species, there has, as yet, been no systematic synthesis of these data. We conducted a systematic review with comparative meta-analyses by combining published data across a variety of species where male condition was manipulated by experimentally limiting dietary nutrient availability relative to a 'standard' or '*ad libitum*' baseline. We then used meta-regression and sub-analyses to determine: (*i*) which male ejaculate traits respond most strongly to nutrient limitation; (*ii*) which nutrients have the strongest effects on ejaculate trait expression; (*iii*) whether the degree of nutrient limitation alters the strength of condition dependence; (*iv*) what ontogenetic life stage (i.e. juveniles or adults) is most sensitive to nutrient limitation; and (*v*) how these effects vary across taxa.

Studies of condition dependence of morphological traits such as signals and weapons typically compare the responses of these traits with the response of body size (Cotton *et al.*, 2004). While we do not have *a priori* predictions of how most ejaculate traits should scale with body size, some ejaculate traits (such as testes size and therefore sperm quantity) are usually correlated with body size (e.g. Locatello *et al.*, 2008; O'Dea, Jennions, & Head, 2014; Macartney *et al.*, 2018*b*; but see Mautz, Møller, & Jennions, 2013). We therefore conducted parallel analyses of treatment effects on ejaculate traits and on body size, allowing us to compare the degree of condition dependence in ejaculate traits with the degree of condition dependence in overall growth.

II. METHODS

(1) Data collection and effect-size extraction

ISI Web of Science and *Scopus* were used to search for studies between January 1900 and June 2017 that manipulated

diet with an *ad libitum* or control diet and a diet-limitation manipulation and measured at least one ejaculate trait. Topics (i.e. title, key words and abstracts) were searched using the search string (condition OR diet* OR nutrient* OR food OR resource*) AND (sperm* OR semen OR ejaculate OR test?s). This resulted in 1086 papers from *ISI Web of Science* and 1493 papers from *Scopus*. 2117 papers remained after removing duplicates (this number is inflated as some replicates were missed due to differences in title formatting between databases). Titles and abstracts were then screened using Abstrackr (Wallace *et al.*, 2012) which uses machine learning to help order papers from most to least relevant. Of the 2117 papers screened, 138 papers met our initial selection criteria where diet was experimentally manipulated and at least one ejaculate trait was measured. All studies included in this meta-analysis were studies on animals reared in the laboratory. We excluded all studies on humans as it is not possible to manipulate human nutrient intake or to control for other environmental variables to the same extent as in laboratory studies. We also excluded all experiments on domestic and agricultural animals due to the likely high artificial selection for reproductive output that may result in inflated condition dependence that is not representative of ejaculate traits in natural populations. We also excluded diets where increased consumption likely results in decreased condition rather than an increase in condition such as males fed 'Western diets' (i.e. high trans- and saturated-fats, and refined sugar), toxins and carcinogens. We identified another 26 papers through backward and forward searching (i.e. systematically checking the bibliography of the 138 studies that met our initial search criteria and checking for relevant papers that had cited those 138 studies). This resulted in 164 papers for further screening. Briefly, we excluded all ejaculate traits that could be the result of female differential allocation or cryptic female choice rather than male investment in the trait, such as paternity share and offspring quality, as well as studies that reported gonado-somatic indices or traits corrected for body size as these may confound effects of diet on body size with effects on ejaculate traits. We also excluded studies that had ambiguous diet manipulations where we were unable to tell which diet would be an *ad libitum*/control or a limited diet, and studies with missing data (for three out of six studies with missing data, we were unable to obtain the data after contacting the authors). This resulted in 71 studies with 348 ejaculate trait effect sizes across 50 species [see Fig. 1 for the preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram, Fig. 2. for the distribution of taxa across the studies, and online Supporting information, Fig. S1, for a phylogenetic tree of the included species].

(2) Effect-size calculation

Standardised mean differences (SMDs) were used to compare the 'high' and 'low' diets and were calculated as Cohen's *d* coefficients:

$$SMD = \frac{mean_c - mean_l}{S_{pooled}}, \quad (1)$$

$$S_{pooled} = \sqrt{\frac{(n_c - 1)\, s_c^2 + (n_l - 1)\, s_l^2}{n_c + n_l - 2}}. \quad (2)$$

Where $mean_c$ are the mean measures of a trait for individuals from the *ad libitum*/control diet treatment, $mean_l$ are the mean measures of a trait for individuals from the nutrient-limited diet treatment, and S_{pooled} are the pooled standard deviations whereby more weight is given to groups of individuals with a larger sample size (n), and s_c^2 and s_l^2 are the standard deviations of the trait in individuals from the control and limited diet treatments, respectively. An effect size (SMD) was calculated for each trait across all papers that remained in the meta-analysis. We used conventional benchmarks for what is considered a 'high' (0.8/−0.8), 'moderate' (0.5/−0.5) or 'low' (0.2/−0.2) effect of diet on traits (Cohen, 1988).

Sampling variance for each SMD was calculated as:

$$s_d^2 = \frac{n_c + n_l}{n_c n_l} + \frac{SMD^2}{2\,(n_c + n_l)}. \quad (3)$$

Descriptive statistics such as means and standard deviations/errors were used to calculate SDM where possible (see Noble *et al.*, 2017). If descriptive statistics were not available, inferential statistics such as F and χ^2 values were used if the assumption of non-independence was met (Noble *et al.*, 2017). If experiments included a split-brood design and therefore resulted in non-independence due to genetic relatedness of individuals within a treatment, we used family number instead of individual number as n when calculating S_{pooled} in order to calculate a more conservative estimate of the effect of diet on traits.

(3) Moderator variables for meta-regression

Moderator (predictor/explanatory) variables were included in meta-regression analyses to test for correlations between moderator variables and ejaculate trait expression when nutrients are limited. The moderator variables included in the meta-regression analyses were: the degree of diet limitation (%), the type of ejaculate trait (e.g. sperm and seminal fluid quantity, and measures of sperm quality), the ontogenetic stage at which diet was manipulated (i.e. juveniles *versus* adults), and the type of diet manipulation (e.g. reduction of specific nutrients, or overall food reduction). The degree of diet reduction was calculated as a per cent of food limitation relative to the fully fed/control diet for the studies that provided quantitative details in their methods (mean ± S.D. % reduction = 56.89 ± 31.76%, range = 0.3–100%). This was then z-transformed to improve interpretability (*sensu* Schielzeth, 2010). Ejaculate traits were divided into six categories: total sperm length (note that we did not include effects on multiple parts of the sperm such as flagella, midpiece and head length as these are expected to be highly correlated and the number of studies that measured these individual parts was too low for analysis), sperm movement (including sperm velocity, flagella beat

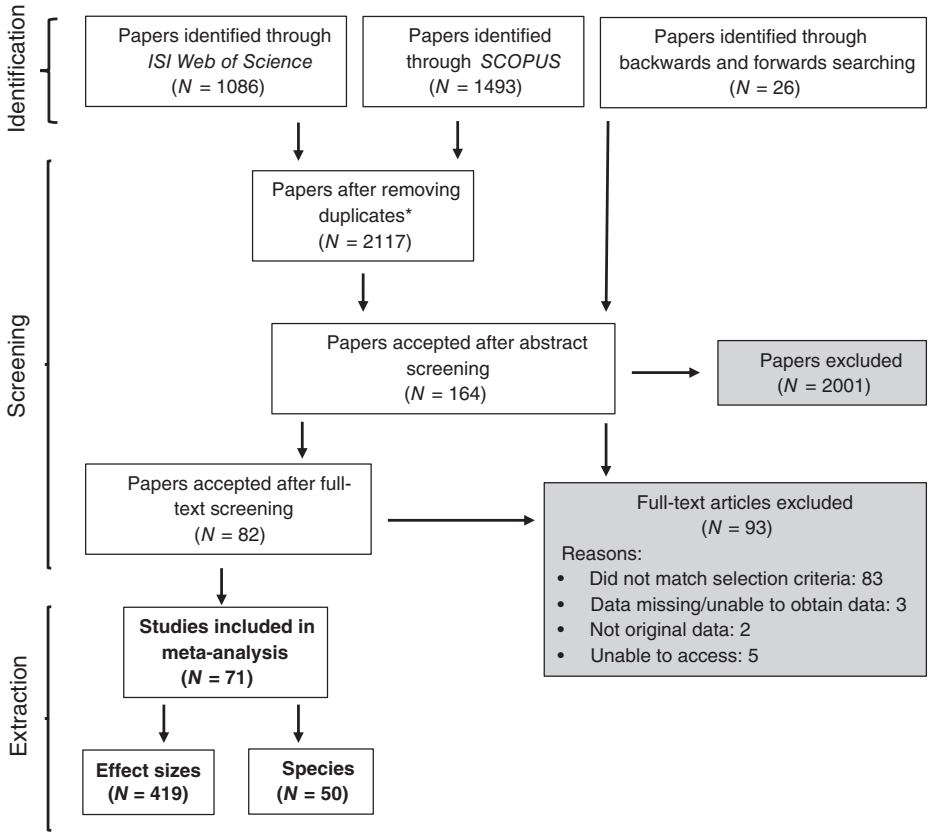

Fig. 1. PRISMA flow diagram of the data-collection process. The number of papers remaining after each stage of the selection process is shown in each identification box, as well as the number of papers excluded and the reasons for exclusion for the papers that remained until final screening. Effect-size number is after processing data (i.e. collapsing repeated measures). *Some duplicates were missed due to differences in title formatting between databases.

frequency and the per cent that were motile), sperm quantity (including total number or concentration of stored sperm or sperm in an ejaculate) (note that there were not enough data to separate sperm number traits into sperm stored within the male *versus* sperm transferred to the female within an ejaculate so these were pooled), sperm normality (if the sperm were viable and/or of normal morphology), seminal fluid quantity (including non-sperm ejaculate size and accessory gland size), and traits that encompass multiple ejaculate components that were not separated into individual components such as sperm and semen quantity (i.e. testes size and spermatophore size). The ontogenetic life stage when nutrients were limited was divided into two categories: before sexual maturity (juveniles) or after sexual maturity (adults). Diet manipulations were divided into six categories: total quantity of food (including manipulations of concentration), protein, carbohydrates, fats [polyunsaturated fatty acids (PUFAs) and monounstaturated fatty acids (MUFAs)], micronutrients (i.e. dietary carotenoids, amino acids, vitamins and minerals), and food quality (i.e. subjective manipulations of diet quality specific to the study species of interest). We excluded the effects of carbohydrates in all analyses except the sub-analyses on mammals as carbohydrate manipulations were only carried out in mammal studies. Where effects of diet manipulation on body size were reported along with effects on ejaculate traits, we used these body-size results to carry out parallel analyses of the condition dependence of body size for comparison with responses of ejaculate traits.

Moderator-variable levels required a sample size of >5 (k) observations to be included in analyses. Any moderator variables (i.e. a specific diet manipulation or trait) that were only investigated in one taxon were removed from the overall analysis to avoid confounding effects of moderator variables with effects of taxon, but these moderator variables were included in the taxonomic sub-analyses (Appendix S1). Sub-analyses of the overall effect of diet on ejaculate traits and meta-regression of moderator variables were also completed on arthropods, mammals and fish in order to assess if any effects differ among taxa. Sub-analyses were not carried out on other taxa (e.g. birds and reptiles) as studies on these taxa were too few for meaningful conclusions (see Fig. 2).

(4) Statistical analyses

(a) *Meta-analysis and meta-regression*

Meta-analyses and meta-regressions were carried out using multi-level, mixed-effects models in R version 1.1.447 using package metafor (Viechtbauer, 2010) as these models

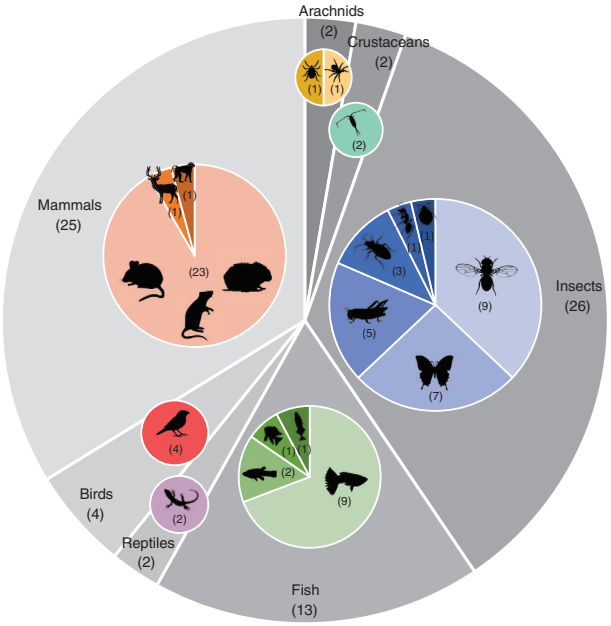

Fig. 2. Pie chart showing the number of studies included in this meta-analysis within each taxonomic group. The outer pie chart depicts the number of studies within large taxonomic groups used in this analysis: Arachnids, Crustaceans, Insects, Fish, Reptiles, Birds, and Mammals (note that Insects, Arachnids and Crustaceans were analysed together as Arthropods in the sub-analysis; results remain unchanged if these groups are analysed separately). The inner pie charts depict the number of studies conducted within each order. Moving clockwise from light to dark: Arachnids: Araneae, Trombiformes; Crustaceans: Copepoda; Insects: Diptera, Lepidoptera, Orthoptera, Coleoptera, Hymenoptera, Hemiptera; Fish: Cyprinodonteformes, Cypriniformes, Cichliformes, Gasterosteiformes; Reptiles: Squamata; Birds: Passeriformes; Mammals: Rodentia, Artiodactyla, Primates.

are generally required for ecological and multispecies meta-analyses (Nakagawa *et al.*, 2017). Initially, we ran models without any moderator variables to test for the overall mean effect of nutrient limitation on ejaculate trait expression and body size (separate models). We controlled for ejaculate trait co-linearity within study individuals (i.e. it would be expected that different ejaculate traits measured from the same study individuals would be correlated to some degree) by including a variance–covariance matrix for multiple traits measured from the same individuals in the model (see Nakagawa *et al.*, 2017; Noble *et al.*, 2017). We also originally included a variance–covariance matrix for phylogenetic relatedness of the included species as a random effect in the model (Chamberlain *et al.*, 2012), but this was later removed as its inclusion did not improve model fit and the phylogenetic signal was very weak (see Section III). Effects of nutrient restriction remained robust (Appendix S2). Animal group ID (i.e. the unique identifier for groups of animals within one set of SMD calculations), species (i.e. to control for differences between species, but not accounting for phylogenetic relatedness) and effect size ID (i.e. an observation-level unique identifier for each SMD calculated) were included as random-effects. I^2 was calculated as a measure of total heterogeneity between SMDs that is not attributed to sampling error (Higgins *et al.*, 2003). We expected high heterogeneity ($I^2 = 60–90\%$) as the current literature on the effects of diet on ejaculate traits suggest that effects vary considerably among studies, and high levels of heterogeneity are generally expected in ecological studies (Higgins *et al.*, 2003; Senior *et al.*, 2016).

Next, we added single moderator variables (see Section II.3) to the models to test for their effects in mediating the response of traits to nutrient limitation. We quantified how much variance in responses to nutrient limitation was explained by each individual moderator by calculating its marginal R^2, *sensu* Nakagawa & Schielzeth, 2013. We then tested a full model containing all moderator variables to calculate how much variance in responses to nutrient limitation is explained by all the moderator variables together.

In addition to the analysis of nutrient limitation effects on male body size (based on all effect sizes for body size from all papers included in this meta-analysis), we used studies that measured both ejaculate traits and body size to conduct a pair-wise comparison between effects of nutrient limitation on ejaculate traits *versus* body size. This model included animal group ID, species and effect-size ID as random effects, as well as the variance–covariance matrix to control for trait co-linearity within study individuals.

Pairwise comparisons were conducted using the package multcomp (Hothorn, Bretz, & Westfall, 2008) and the function glht.

All data and code can be found at https://osf.io/3sq5g/.

(*b*) *Taxonomic sub-analyses*

Finally, we completed sub-analyses on the taxonomic groups for which enough data were available from multiple studies (arthropods, mammals, and fish), to determine if ejaculate responses to nutrient limitation differed between these taxonomic groups. Separate sub-analyses were also completed on insects and rodents, but results were qualitatively similar, so taxonomic groups were expanded to 'mammals' (including studies on rodents, cervids and primates) and 'arthropods' (including studies on insects, crustaceans and spiders). Sperm movement was excluded from the arthropod sub-analysis, sperm size was excluded from the mammal sub-analysis, and testes size and seminal fluid quantity were excluded from the fish sub-analysis due to small sample sizes ($k \leq 5$). All diet manipulations except 'quantity' and 'micronutrient' (carotenoid) manipulations were excluded from the fish sub-analysis because few studies examined other types of dietary effects. All results are presented as SMD (Cohen's *d*) and 95% credible intervals (CI).

(*c*) *Publication bias and sensitivity analysis*

The R package MCMCglmm (Hadfield, 2010) was used for publication bias analyses. Publication bias was assessed

using multiple methods. First, we assessed funnel asymmetry of the 'meta-analytic' residuals (*sensu* Nakagawa & Santos, 2012), calculated from the full model and plotted against 'precision' (i.e. the inverse of standard error). Note that the use of the meta-analytic residuals fulfils the independence assumption, and residuals are less affected by heterogeneity; the funnel asymmetry can be due to true heterogeneity in data (i.e. unexplained variation). We then used Egger's regression tests for deviations in funnel asymmetry (i.e. using linear regression to test for a significant deviation of the *y*-intercept from 0) (Egger *et al.*, 2015), and a 'trim-and-fill' test to predict 'missing' (i.e. unpublished) studies from the literature based on funnel asymmetry (Duval & Tweedie, 2000). Next, we tested for a correlation of publication year with the size of the SMDs by including publication year as a single moderator variable in the model.

These assessments of publication bias on ejaculate traits were conducted with one low-precision (i.e. small sample size) study on the house mouse (*Mus musculus*) (Chinoy, Mehta, & Jhala, 2006) removed from the analyses, as they reported very large effects of nutrient limitation on ejaculate traits and this resulted in issues with running the Egger's regression test and trim-and-fill test on the meta-analytic residuals as suggested by Nakagawa & Santos (2012) as it skewed the mean meta-analytic residuals away from zero. However, this study had low weight in the meta-analysis, meta-regression and sub-analyses on mammals due to its small sample size; there was no substantial difference in the other reported results with this study removed (see Appendix S3). Additional sensitivity analyses ('leave-one-group-out') were conducted where one animal group at a time was taken out of the data set and a new SMD and 95% CI were calculated for the global meta-analytic model. Assessments of publication bias and sensitivity analyses were conducted separately for ejaculate traits and body size (Appendix S4).

III. RESULTS

(1) Does nutrient limitation cause an overall reduction in male ejaculate traits?

On average, across all ejaculate traits, taxa, diet manipulations, and ontogenetic life stages, a reduction in nutrient intake resulted in a significant and 'moderate' decrease in male ejaculate traits (SMD [non-phylo model] $= -0.525$; CI $= -0.684, -0.338$) (Fig. 3). Including phylogenetic relatedness as a random effect did not improve model fit according to the change (Δ) in Akaike Information Criterion (ΔAIC $= 1.210$). There was no evidence of a phylogenetic signal (Pagel's Lambda $= <0.001\%$), and effects of nutrient restriction on ejaculate traits remained moderate and robust with phylogeny omitted from the model (SMD [phylo model] $= -0.496$; CI $= -0.854, -0.138$) (see also Appendix S2). However, as expected, there was a high amount of heterogeneity in ejaculate responses to a reduced diet (I^2[total] $= 89.6\%$), with effect-size ID (i.e. observation-level SMD) accounting for 70.0% of the variance, different experimental animal groups (i.e. groups of animals from different experiments) accounting for 14.7% of the variance, and differences between species that were not accounted for by phylogenetic relatedness accounting for 5.0% of the variance.

The high amount of heterogeneity in male ejaculate traits to nutrient limitation suggests that other factors modulate the responses of such traits to a reduction in nutrient intake. Therefore, we attempted to explain this variation using meta-regression analyses of several moderator variables: the degree of nutrient reduction, the life stage at which nutrients were reduced, the type of nutrient manipulation, and the type of ejaculate trait measured. Overall, these moderator variables explained 19.0% (R^2) of the variance in ejaculate trait responses to nutrient limitation.

(2) Are ejaculate traits sensitive to the degree of nutrient limitation?

The degree of nutrient limitation did not affect ejaculate trait expression (SMD [degree nutrient limitation] $= -0.015$; CI $= -0.227, 0.198$, $R^2 = 0.6\%$, $k = 253$). However, this may be due to the limited range of reported diet reductions. Of the studies that reported the exact reduction in diets, 67.90% reported a diet reduction of greater than 50% compared to the control diet.

(3) Are certain ejaculate traits more sensitive to nutrient limitation?

Overall, seminal fluid quantity was reduced to a large extent when nutrients were limited.

Spermatophore/testes size and sperm quantity were moderately reduced when nutrients were limited. Sperm movement and sperm length had a small, negative response to nutrient limitation, and sperm normality was largely unaffected (Fig. 3). The type of ejaculate trait accounted for 8.3% of the variance in responses (R^2).

(4) Are ejaculate traits more sensitive to nutrient limitation at the juvenile or adult stage?

Nutrient limitation at both juvenile and adult life stages resulted in a moderate, significant decrease in ejaculate traits (Fig. 3). The mean effect of nutrient limitation in juveniles was slightly greater than the effect of nutrient limitation in adults, but this small difference was not significant (SMD [adult–juvenile comparison] $= -0.178$; CI $= -0.492, 0.167$). The life stage that nutrients were limited accounted for 0.8% of the variance in responses ($R^2 = 0.8\%$;).

(5) Are male ejaculate traits sensitive to specific dietary components?

A reduction in total food quantity and dietary protein resulted in a moderate, significant decrease in ejaculate trait expression. A reduction in PUFA and MUFA fats, micronutrients (e.g. dietary carotenoids, vitamins, minerals

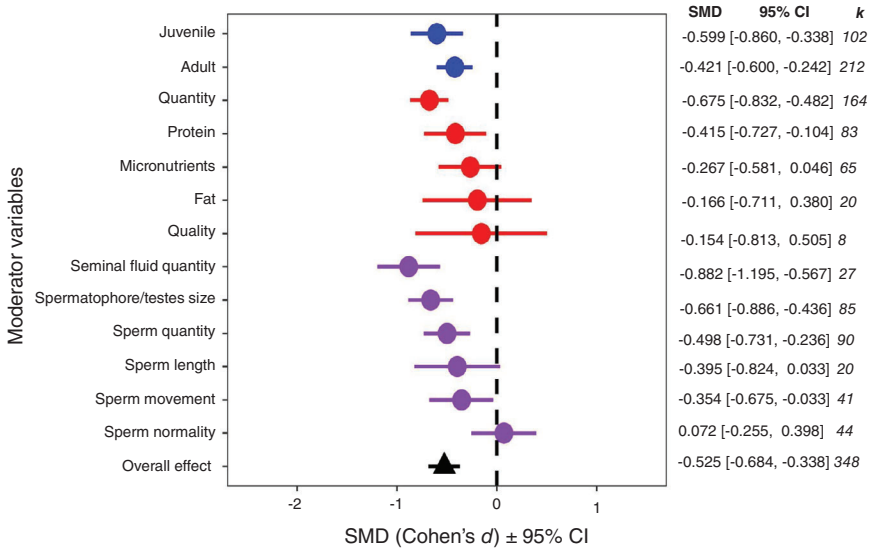

Fig. 3. Forest plot displaying the overall effect (black triangle) of nutrient limitation on male ejaculate traits, as well as the effects of ontogenetic life stage (blue), type of diet manipulation (red), and the effects on different ejaculate traits (purple). Text on the right displays the standardised mean difference (SMD, Cohen's *d*), 95% credible interval (CI), and the number of observations for each moderator variable (*k*).

and amino acids), and diet quality (i.e. reductions in diet quality specific to the study species) resulted in small, non-significant reductions in ejaculate trait expression (Fig. 3). The type of nutrient limitation accounted for 4.4% of the variance in responses (R^2).

(6) Are these results consistent across taxa?

Ejaculate trait expression was moderately and significantly reduced with nutrient limitation in arthropods (SMD [$\text{arthropod}_{\text{total}}$] = −0.442; CI = −0.662, −0.222) and strongly reduced in mammals (SMD [$\text{mammal}_{\text{Total}}$] = −0.859; CI = −1.155, −0.563). There was also a small and near-significant reduction in fish (SMD [$\text{fish}_{\text{total}}$] = −0.254, CI = −0.577, 0.067) (Fig. 4). Therefore, the strength of ejaculate condition dependence may be taxon specific, but the difference in effects may also reflect differences in sample size or be due to underlying differences in experimental design used within different taxa. Most ejaculate traits across taxa were reduced when diet was reduced (Fig. 4). However, our analysis also revealed some interesting exceptions. In particular, sperm and seminal fluid quantity were significantly reduced in arthropods and mammals but sperm quantity was not significantly reduced in fish (no studies measured seminal fluid quantity in fish). Sperm length appeared to be largely unaffected in arthropods but was significantly reduced in fish (few studies measured sperm length in mammals), and sperm normality was unaffected in both arthropods and mammals but was significantly reduced in fish (Fig. 4). Therefore, the condition-dependent trait responses of fish sperm quality and quantity were largely opposite to those of arthropods and mammals.

Most diet manipulations resulted in at least a slight decrease in ejaculate trait expression across arthropods, mammals, and fish. A reduction in the total quantity of food resulted in a consistently significant, moderate to large decrease in ejaculate traits across all taxa, and a reduction in protein resulted in a significant, moderate to large decrease in ejaculate traits in arthropods and mammals (protein was not included in the fish sub-analysis due to a lack of data). We also detected a large, significant negative effect of carbohydrates in mammals (carbohydrates were not manipulated in arthropod or fish studies), albeit with large variation. Diet quality (i.e. qualitative reductions in diet specific to the diet of the study species) did not result in a substantial decrease in ejaculate traits (specific to arthropod studies), and micronutrients such as carotenoids (specific to the fish studies), amino acids, vitamins and minerals do not appear to be important for arthropod or fish sperm and semen traits but resulted in a moderate, marginally non-significant reduction in ejaculate traits in mammals (Fig. 4).

Nutrient limitation as juveniles resulted in a significant decrease in ejaculate trait expression in both arthropods and mammals, and nutrient limitation as adults resulted in a relatively weaker, significant reduction in ejaculate traits in arthropods and a near-significant reduction in mammals. The effect of juvenile nutrient limitation was significantly larger than the effect of adult nutrient limitation in mammals (SMD [$\text{mammal}_{\text{adult–juvenile comparison}}$] = −0.962; CI = −1.845, −0.109), but the difference between adult and juvenile nutrient limitation was non-significant in arthropods (SMD [$\text{arthropod}_{\text{adult–juvenile comparison}}$] = −0.311; CI = −0.786, 0.163). By contrast, in fish, nutrient limitation as adults resulted in a significant reduction in ejaculate traits but nutrient limitation as juveniles largely unaffected ejaculate traits, however this difference was not significant (SMD [$\text{fish}_{\text{adult–juvenile comparison}}$] = 0.448; CI = −0.0410, 0.937).

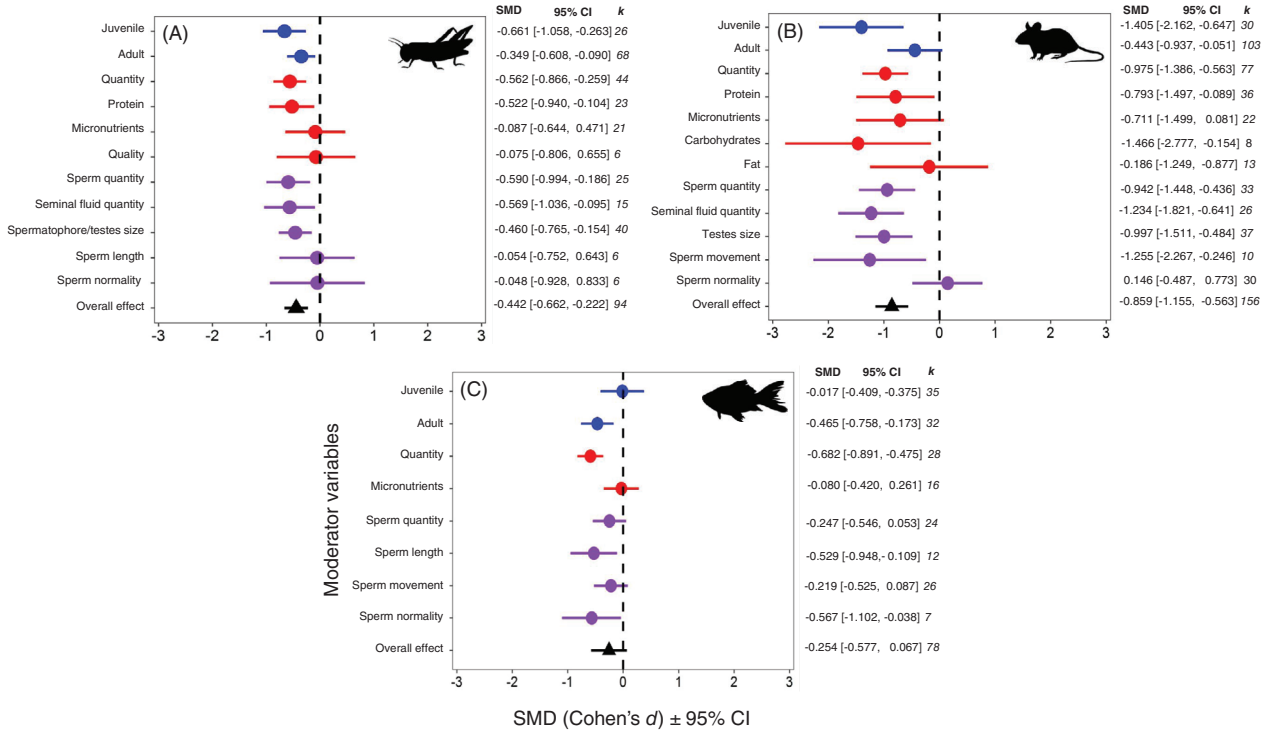

Fig. 4. Forest plots displaying the overall effect (black triangles) of nutrient limitation on male ejaculate traits across taxa (A, arthropods; B, mammals; C, fish), as well as the effects of ontogenetic life stage (blue), type of diet manipulation (red), and the effects on different ejaculate traits (purple). Text on the right displays the standardised mean difference (SMD, Cohen's *d*), 95% credible interval (CI), and the number of observations for each moderator variable (*k*).

(7) Effect of nutrient limitation on male body size

We carried out a parallel meta-analysis of nutrient effects on body size, using effects reported in the same studies that were included in our meta-analysis of effects on ejaculate traits. We found that a decrease in nutrient intake resulted in a large, significant reduction in body size (SMD [total] $= -1.359$; CI $= -1.779, -0.940$, $k = 71$, also see Appendix S4).

The estimated effect of nutrient limitation on body size substantially exceeded the estimated effect on ejaculate traits. To verify this difference, we analysed the subset of studies that reported effects of nutrient limitation on both ejaculate traits and body size within the same study. This pair-wise comparison showed that the difference in trait responses between ejaculate traits and body size was indeed significant (SMD [ejaculate–body size comparison] $= -0.708$; CI $= -0.955, -0.462$).

(8) Publication bias and sensitivity analysis

Statistical assessment of our ejaculate trait data, after controlling for heterogeneity using 'meta-analytic' residuals, suggests that there is some publication bias in the literature; this was confirmed with the Egger's regression test for funnel asymmetry (β [intercept] $= -0.621$, S.E. $= 0.248$, $t_{273} = -2.509$, $P = 0.013$), and the 'trim-and-fill' method added three points to the right side (positive effects) of the funnel plot (Fig. 5). Funnel asymmetry suggests that several low-precision studies (i.e. studies with small sample sizes) that report a strong increase in male ejaculate traits when fed a nutrient-reduced diet are missing from the published literature. There was also a significant effect of publication year on the SMDs of male ejaculate traits where the size of the SMD became smaller with publication year (β [publication year] $= -0.0003$, CI $= -0.0003, -0.0002$) (Fig. 6), and publication year explained 5.76% (R^2) of the variance in effect sizes (see Appendix S4 for publication bias analyses on body size).

'Leave-one-group-out' sensitivity analyses showed that effects of nutrient limitation on ejaculate traits (Fig. S6) and body size (Fig. S7) remain robust when any one group of study animals is omitted from the meta-analytic data set [also see Appendix S3 for analyses with the large outlier Chinoy *et al.*, 2006 removed].

IV. DISCUSSION

(1) Overall effect of nutrient limitation on ejaculate trait expression

Theory suggests that ejaculate trait expression should decrease when nutrients are limited, such that these traits should exhibit condition-dependent expression like that of other sexual traits. This is because male ejaculate

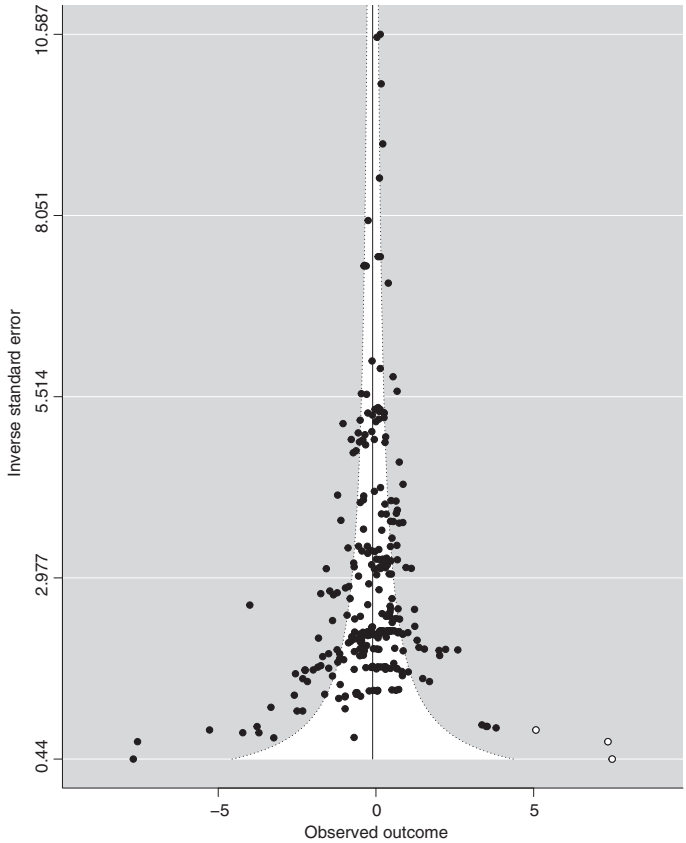

Fig. 5. Funnel plot to test for publication bias of nutrient limitation on male ejaculate traits. The *x*-axis indicates the meta-analytic residuals, and the *y*-axis indicates the inverse standard error (precision). The black dots represent published data, and the white dots represent 'missing' data as calculated by the trim-and-fill analysis.

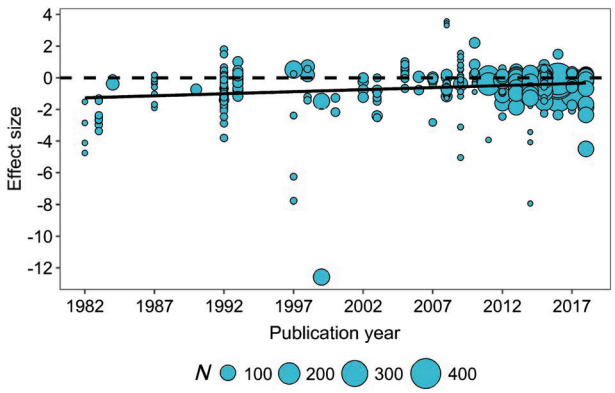

Fig. 6. Bubble plot showing the correlation of publication year and effect size. The size of the circles indicates the sample size for each effect size calculated.

traits can be under strong directional sexual selection due to their importance for male fitness, particularly under sperm competition, and selection is expected to promote costly exaggeration of at least some ejaculate traits (e.g. Linklater *et al.*, 2007; Crudgington *et al.*, 2009; Lüpold *et al.*, 2016; Godwin *et al.*, 2017). Although many studies have investigated the condition dependence of sperm and semen traits, variation in the nature and strength of responses remains poorly understood.

We carried out the first quantitative synthesis (a systematic review with a series of meta-analyses and meta-regression analyses), examining underlying sources of variation in responses of male ejaculate traits to nutrient limitation. Overall, the available empirical evidence indicates that male ejaculate traits show moderate but significant condition dependence (i.e. trait expression is reduced when males are nutrient limited). However, as expected, there is a high amount of heterogeneity in reported effects. Interestingly, differences between species (including both the phylogenetic signal and variation among species that is not explained by phylogeny) did not account for much of this variation. Rather, differences in observation-level effect sizes and between-study differences accounted for most of the variation in ejaculate responses to nutrient limitation, indicating that differences between other biologically relevant variables are modulating the effect of nutrient limitation on ejaculate trait expression. Through meta-regression analyses, we found that differences in the type of ejaculate trait, the type of nutrient limitation, and the ontogenetic life stage when nutrients were limited explained 19% of this variation. Sub-analyses on arthropods, mammals, and fish also suggested some inter-taxon differences in ejaculate condition dependence,

but the overall condition dependence of ejaculate traits appears to be a taxonomically widespread phenomenon. Therefore, while the prediction that male ejaculate traits are condition dependent holds true overall, the strength of this response depends substantially on other biological variables.

(2) Factors that explain variation in condition-dependent expression of ejaculate traits

We found that most ejaculate traits are likely to respond in a condition-dependent manner, but the strength of the response varied among traits, and some trait responses varied among taxa. Overall, seminal fluid quantity (i.e. non-sperm ejaculate size, and accessory gland size) decreased to the greatest extent with nutrient limitation, and seminal fluid quantity also showed substantial condition dependence in the sub-analyses on arthropods and mammals (we did not find any studies of seminal fluid quantity in fish). Sperm quantity also showed moderate condition dependence in arthropods and mammals (and approached a significant reduction in fish). This clear condition-dependent effect of nutrient limitation on sperm and seminal fluid quantity is consistent with expectations, given that selection can favour costly investment in these traits (e.g. Linklater *et al.,* 2007; Crudgington *et al.,* 2009). For example, the risk and intensity of sperm competition, as well as the likelihood of achieving a higher mating rate (normally in high-condition males) can favour increased expenditure on ejaculate production (Parker, 1982; Parker *et al.,* 1997; Gage & Morrow, 2003; Engqvist & Reinhold, 2005; Vahed & Parker, 2012; but see Simmons *et al.,* 2003). Additionally, testes size is correlated with sperm production (Parker *et al.,* 1997; Schärer, Ladurner, & Rieger, 2004; reviewed in Simmons & Fitzpatrick, 2012). Therefore, the condition dependence of sperm quantity, at least partly, likely results from the production of larger testes by high-condition males, but it must be noted that testes size often scales hypo-allometrically with body size, which may result in low-condition males producing more sperm relative to body size.

By contrast, measures of sperm quality (i.e. sperm length, sperm movement and sperm normality) showed less-consistent condition-dependent responses. Sperm normality (proportion of sperm that are alive or morphologically normal) did not show a condition-dependent response overall, in arthropods, or in mammals, even though the proportion of live sperm has been shown to be highly important in sperm competition and paternity, particularly in arthropods (e.g. Hunter & Birkhead, 2002; Fry & Wilkinson, 2004; Garcia-Gonzalez & Simmons, 2005). This suggests that, even in some taxa where selection acts on sperm viability, this does not necessarily exhibit strong condition dependence. Interestingly, sperm normality significantly decreased with nutrient limitation in fish, while sperm quantity did not, suggesting that selection may favour aspects of sperm quality over sperm quantity in fish. However, six out of the seven fish studies that measured sperm viability were on guppies (*Poecilia reticulata*). This suggests that guppies may experience stronger selection on sperm viability than do arthropods and mammals (see Fitzpatrick & Evans, 2014), but it is not clear whether this effect can be generalised across fish species, particularly to external fertilisersing fish species. Additionally, while sperm movement exhibited a small but significant reduction overall, and sperm length exhibited a small and near-significant reduction, there was substantial variation within and among taxa. Nutrient limitation strongly and significantly reduced sperm movement in mammals (albeit with substantial variation across studies) but did not significantly reduce sperm movement in fish, and nutrient limitation moderately reduced sperm length in fish but not in arthropods. This variation among taxa in trait responses to nutrient limitation likely reflects variation in sperm form and function, even between closely related species (reviewed in Reinhardt, Dobler, & Abbott, 2015), potentially resulting from differential patterns of selection on sperm morphology, movement and viability among taxa (see Snook, 2005; Simmons & Fitzpatrick, 2012) – a topic that warrants further research.

A reduction in total food quantity (i.e. reducing calories while maintaining nutrient ratios) resulted in a significant reduction in male ejaculate traits across taxa. Similarly, a reduction in ejaculate traits in response to protein limitation was observed in arthropods and mammals (the sample size was insufficient to test effects of protein limitation in fish). A reduction in total food quantity (i.e. calorie restriction) is known to induce condition-dependent responses in many reproductive traits and in male fitness (Kotiaho, 2000; Bonduriansky, 2007; Judge, Ting, & Gwynne, 2008; Fritzsche & Arnqvist, 2015). However, the effects of protein on male reproduction are less clear. Some recent studies have shown that effects of protein restriction on male fitness can be highly context dependent (e.g. Zajitschek *et al.,* 2012; Adler *et al.,* 2013; Macartney, Crean, & Bonduriansky, 2017), and effects of protein restriction are generally less pronounced in males compared to females (e.g. Chippindale & Leroi, 1993; Adler *et al.,* 2013; Le Couteur *et al.,* 2016). However, our results suggest that protein limitation can indeed reduce male ejaculate trait expression across a wide range of taxa. Protein has been shown to be important for normal testicular functioning, with protein-deficient male rats producing a reduced quantity of sex hormones, atrophied accessory glands, and abnormal sperm (Srebnik & Nelson, 1962; Vawda & Mandlwana, 1990). Therefore, protein limitation is likely to have a significant effect on male fitness. Perhaps reported effects of dietary protein on male fitness are relatively subtle (e.g. Zajitschek *et al.,* 2012; Adler *et al.,* 2013; Macartney *et al.,* 2017) because these studies assayed male performance in the absence of sperm competition. Protein-restricted males might suffer reduced fitness if forced to compete for fertilisations against other males.

Overall, significant reductions in ejaculate trait expression were observed with nutrient limitation in both juveniles (i.e. before sexual maturity) and in adults, and differences in life stage did not explain much of the total variance

in responses. However, there was some variation among taxonomic groups. In mammals, nutrient limitation in juveniles resulted in a significantly greater reduction in ejaculate traits compared to nutrient limitation in adults, and this also appeared to be the trend in arthropods. In comparison, juvenile nutrient limitation did not affect ejaculate trait expression in fish, but adult nutrient limitation resulted in a moderate and significant reduction in ejaculate traits. We predicted that adult nutrient limitation would reduce ejaculate trait expression because sperm and seminal products are produced by adults. This was the case overall, in arthropods and fish, and was nearing significance in mammals. However, it is interesting that juvenile nutrient limitation had an even stronger negative effect on ejaculate trait expression in arthropods and mammals, given that these traits are not yet fully developed in juveniles. This could reflect differences in how nutrients are mobilised in juveniles *versus* adults (Gheorghe *et al.,* 2010) or variation in the ontogenetic timing and condition dependence of epigenetic programming of cells involved in the synthesis of sperm and seminal fluid (Macartney *et al.,* 2018*a*). For example, the developmental environment can alter many epigenetic factors such as DNA methylation, chromatin structure and non-coding RNAs (reviewed in Burdge & Lillycrop, 2010; Lo, Simpson, & Sword, 2017), and such epigenetic factors have been shown to alter spermatogenesis (e.g. Song *et al.,* 2011; Wang *et al.,* 2017). Therefore, modifications of epigenetic factors in response to juvenile nutrient availability may alter the development and synthesis of adult ejaculate traits.

(3) The condition dependence of ejaculate traits is relatively weak compared to body size

While many male ejaculate traits exhibited moderate levels of condition dependence, male body size showed a more than twofold greater response to nutrient limitation. Body size responses were also substantially more variable than those of ejaculate traits, and the inclusion of the moderator variables (degree of nutrient limitation, type of nutrient manipulation, and age at nutrient limitation) accounted for considerably more of this variation (>35%). Therefore, while many ejaculate traits are expected to co-vary with body size (e.g. Gage, 1994; Locatello *et al.,* 2008; O'Dea *et al.,* 2014; Macartney *et al.,* 2018*b*) these results suggest that body size and ejaculate traits respond somewhat differently to nutrient limitation, and that the condition dependence of ejaculate traits is weak compared to the condition dependence of body size.

There are at least two plausible explanations for the relatively weak condition dependence of ejaculate traits (and apparent lack of condition dependence in some of these traits, such as sperm quality). One possibility is that sperm and semen traits are strongly canalised (buffered) against perturbations such as nutrient limitation (Wagner, Booth, & Bagheri-Chaichian, 1997). While reproduction may still be possible even with substantial reduction in body size and the expression of pre-copulatory secondary sexual traits (e.g. *via* sneak mating tactics), there may be a limit on the extent to which ejaculate quantity or quality can be reduced without suffering complete loss of reproductive capacity. Selection may therefore favour physiological mechanisms that maintain near-constant levels of resource allocation to ejaculate traits in order to ensure that ejaculate quantity and quality exceed the minimum threshold levels required to achieve fertilisation even when males are in low condition.

Alternatively, body size may exhibit stronger condition dependence because overall growth and maintenance of body size requires a much greater investment of resources compared to the production and maintenance of ejaculate traits. In other words, ejaculate traits may exhibit weaker condition dependence because they are metabolically 'cheap' by comparison with body size (and perhaps also by comparison with many exaggerated signal and weapon traits). Discriminating between these contrasting hypotheses will require a better understanding of the metabolic costs of ejaculate trait expression.

Whatever its cause, the relatively weak condition dependence of ejaculate traits may limit the potential for the ejaculate to provide honest signals of male condition. If body size and pre-copulatory display traits are more strongly condition dependent than ejaculate traits, then these pre-copulatory traits would provide more honest signals of male mate quality (Andersson, 1982; Rowe & Houle, 1996). Therefore, selection may favour female preferences based on such pre-copulatory signals, rather than cryptic female mate choice based on ejaculate traits. However, if females are unable to exercise pre-copulatory mate choice (e.g. because males can coerce matings), selection could favour cryptic female mate choice based on the most condition-dependent ejaculate traits, such as sperm and seminal fluid quantity (Eberhard & Cordero, 1995). While our results show that most ejaculate traits could serve as signals of environmental quality, it is less clear whether such traits could also serve as honest signals of genetic quality (although see Simmons & Kotiaho, 2002; Hosken *et al.,* 2003; Fisher *et al.,* 2006). If so, then sexual selection on ejaculate traits could contribute to the purging of deleterious mutations from populations (Rowe & Houle, 1996; Agrawal, 2001; Lorch *et al.,* 2003), although the contribution of ejaculate traits to purging may be relatively weak by comparison with the role of pre-copulatory traits such as body size that often experience strong viability and sexual selection and exhibit strong condition dependence.

(4) Publication bias and gaps in the literature

Our analyses suggest some publication bias and highlights clear gaps in the literature. Trim-and-fill analyses suggested that some low-precision studies reporting positive effects of nutrient limitation on ejaculate traits (i.e. effects in the opposite direction to predictions) are missing from the published literature. This may be because studies that contradict expectations are less likely to be published, or because some heterogeneity in the data is not accounted for in our analyses (Nakagawa & Santos, 2012).

There are also many taxonomic gaps in the literature. Insects and mammals make up the bulk of studies that have manipulated diet and examined ejaculate traits, and rodents (mainly laboratory-strain rats and mice) comprised over 90% of the mammal studies. Therefore, we may be limited in our ability to draw general conclusions about ejaculate condition dependence, particularly given the large variation in sperm form and function among species (Snook, 2005; Simmons & Fitzpatrick, 2012; Reinhardt *et al.*, 2015).

Even within taxonomic groups, there are inconsistencies in the types of traits that are measured, and the types of diet manipulations applied. For example, in arthropods, very few studies have examined condition dependence of sperm movement (but see Macartney *et al.*, 2018*b*) and we did not find any studies that manipulated male condition by reducing carbohydrates. In mammals, we did not find any studies that measured sperm length. In fish, few studies have quantified testes size (but see Sullivan *et al.*, 2014; Mehlis *et al.*, 2015), we did not find any studies that measured ejaculate/accessory gland size, and most diet manipulations consisted of a reduction in total food quantity or a reduction in dietary carotenoids. Also, only 65% of studies included in our analyses reported an effect of nutrient limitation on body size as well as on ejaculate traits. The need to report effects on body size in studies on the condition dependence of signal and weapon traits was emphasised by Cotton *et al.* (2004), and measuring effects on body size is equally important in studies of ejaculate traits.

Finally, we found that smaller effect sizes have been published in recent years. Surprisingly, the smaller effect sizes do not appear to be driven by sample size. This effect may be due to changes in research practices within the field (such as movements towards measuring sperm quality), or through a time-lag effect whereby the first papers to report an effect will publish larger effects compared to subsequent studies (Koricheva, Jennions, & Lau, 2013).

(5) Male nutrition and fitness

The available evidence suggests that nutrient-limited males often suffer reduced ejaculate quantity and sometimes quality. This pattern could have implications for our understanding of the evolution of mating systems. Low-condition males (which are typically smaller, and express reduced signal and weapon traits) are likely to be less successful in gaining access to females through combat or agonistic signalling (e.g. Rowe & Arnqvist, 1996; Sokolovska, Rowe, & Johansson, 2000; Danielsson, 2001). In many species, low-condition males therefore employ alternative tactics such as sneaking or satellite behaviour, and such tactics are expected to enable low-condition males to achieve higher fitness than they would otherwise be able to attain (e.g. Gross, 1996; Moczek & Emlen, 2000). However, if low-condition males are also disadvantaged in sperm competition relative to high-condition males because nutrient limitation depresses the quantity and/or quality of their sperm and seminal fluid, the reproductive potential of such males may be limited. For example, males that have fewer sperm are expected to be less competitive in 'raffle' competition based on sperm numbers (Parker, 1990), and a reduction in seminal fluid production can limit male mating rate (e.g. Reinhardt, Naylor, & Siva-Jothy, 2011) and reduce female fecundity (reviewed in South & Lewis, 2011). However, in some species, low-condition males may be able to allocate sufficient resources to ejaculate traits to overcome such disadvantages. Moreover, males that are more likely to gain multiple matings (i.e. as a result of their larger body size or exaggerated secondary sexual traits) may strategically allocate smaller ejaculates per mating in order to prevent ejaculate depletion. Such strategic ejaculate allocation has been demonstrated empirically in several species where it has been shown that larger males mate for a shorter time, transfer fewer sperm per mating, and may therefore fare poorly in sperm competition against smaller males (e.g. Rowe & Arnqvist, 1996; Danielsson, 2001; Fricke *et al.*, 2015; also see Pitnick, 1991). Unfortunately, our literature search found very few studies that measured the effect of nutrient limitation on the number of sperm transferred, so we were unable to test for differences in sperm production *versus* sperm transferred to the female based on nutrient availability. This would be especially interesting to address in the future.

Additionally, conditional tactics can be complex and subtle, and more research is needed to understand the nature of such tactics at the post-copulatory stage. Different ejaculate traits may interact to affect the outcome of sperm competition. For example, Lüpold *et al.* (2012) found that male *Drosophila melanogaster* that had slower-swimming sperm were at a competitive advantage when competing against males with faster sperm as the slow-sperm males produced longer sperm and transferred more sperm per mating. The slow sperm were then more likely to remain in the female sperm storage organ to be used for fertilisation. However, these interactions may be taxon specific as other studies have found positive correlations between sperm swimming speed and fitness under sperm competition (e.g. Birkhead *et al.*, 1999; Gasparini *et al.*, 2010; Boschetto *et al.*, 2011). Moreover, pre- and post-copulatory ejaculate traits could interact in their effects on male fitness. Tazzyman *et al.* (2009) used a model to show that the fitness effect of reduced ejaculate production under nutrient limitation could depend on the energetic cost and likelihood of gaining matings. Thus, our understanding of the condition dependence of male fitness would be enhanced by studies that consider both pre- and post-copulatory competitive environments, investigate how the condition dependence of body size interacts with the condition dependence of ejaculate traits to affect male reproductive success, and explore the specific roles of various ejaculate traits in the tactics employed by low- and high-condition males under sperm competition.

V. CONCLUSIONS

(1) As predicted by theory, we show that most male ejaculate traits exhibit condition-dependent expression by

reducing trait expression when males are nutrient limited, and this effect is conserved across broad taxonomic groups. The literature reports highly variable ejaculate responses, and we show that variation among ejaculate traits, and in the type of nutrient limitation and the ontogenetic life stage when nutrients are limited, jointly account for 19% of this variation.

(2) We show that sperm and semen quantity are consistently condition-dependent in fish, mammals and arthropods. By contrast, while some aspects of sperm quality are also condition-dependent, this effect is less consistent across taxa.

(3) A reduction in total food quantity and protein induce the strongest condition-dependent responses. Nutrient limitation at the adult stage affects male ejaculate traits across all major taxa. Nutrient limitation at the juvenile stage significantly affects ejaculate trait expression in arthropods and mammals, but not in fish, and nutrient limitation at the adult stage significantly affects ejaculate trait expression in arthropods and fish, but not in mammals.

(4) The condition dependence of male ejaculate traits was relatively weak compared with that of body size. This could reflect canalisation (buffering) of male ejaculate traits. Alternatively, the metabolic costs of ejaculate trait expression may be relatively low by comparison with those of body size. The relatively weak condition dependence of ejaculate traits limits their ability to serve as honest signals of male quality.

(5) A reduction in sperm and semen traits as well as in body size is likely to reduce male fitness, and these effects could interact. Future studies should aim to examine effects of both nutrient limitation and pre- and post-copulatory competition on male fitness (i.e. progeny sired).

(6) There are considerable gaps in the literature. Future studies should investigate ejaculate condition dependence in other taxonomic groups (especially birds, reptiles, and non-rodent mammals), as well as the condition dependence of less-studied traits within certain taxa (i.e. sperm movement in arthropods, sperm length in mammals, and semen traits in fish). Future studies should ensure they report effects on body size as well as on the ejaculate trait of interest.

VI. REFERENCES

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VII. SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Phylogenetic tree of the species included in the meta-analysis. Note that some species names differ in the Open Tree of Life from those used in their papers of origin due to recent taxonomic revisions.

Appendix S1. Factorial tables.

Appendix S2. Analyses accounting for phylogenetic relatedness.

Appendix S3. Results with outlier (Chinoy *et al.*, 2006) excluded.

Appendix S4. Meta-analysis and meta-regression on male body size.

Fig. S6. 'leave-one-group-out' sensitivity analysis on ejaculate traits.

Fig. S7. 'leave-one-group-out' sensitivity analysis on body size.

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