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# Parental dietary protein effects on offspring viability in insects and other oviparous invertebrates: a meta-analysis

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## ABSTRACT

Dietary protein is a key regulator of reproductive effort in animals, but protein consumption also tends to accelerate senescence and reduce longevity. Given this protein-mediated trade-off between reproduction and survival, how does protein consumption by parents affect the viability of their offspring? In insects, protein consumption by females enhances fecundity, but trade-offs between offspring quantity and quality could result in negative effects of protein consumption on offspring viability. Likewise, protein consumption by males tends to enhance the expression of sexual traits but could have negative effects on offspring viability, mediated by epigenetic factors transmitted via the ejaculate. It remains unclear whether dietary protein has consistent effects on offspring viability across species, and whether these effects are sex-specific. To address this, we conducted a meta-analysis of experimental studies that examined the effects of protein content in the maternal and/or paternal diet in insects and other oviparous invertebrates. We did not find consistent effects of paternal or maternal protein consumption on offspring viability. Rather, effects of dietary protein on offspring vary in both magnitude and sign across taxonomic groups. Further studies are needed to determine how the effects of dietary protein on offspring relate to variation in reproductive biology across species. Our findings also highlight important gaps in the literature and limitations in experiment design.

## Introduction

Many studies have investigated effects of parental overnutrition (typically, high fat/western diet manipulations) or undernutrition (typically, protein or caloric restriction) on offspring in insects, mammals, and other animals (Heilbronn and Ravussin, 2003, Soultoukis and Partridge, 2016, Lee, 2015). Interestingly, in addition to affecting the health of individuals directly exposed to these diets, both types of parental dietary imbalance can induce impaired metabolic function in offspring (Desai et al., 2014, Watkins et al., 2018, Jahan-Mihan et al., 2015, Dimofski et al., 2021). Such trans- or inter-generational effects of diet represent nongenetic parental effects, involving mechanisms of nongenetic inheritance such as modifications in nutrients, hormones or RNA in eggs, sperm, or seminal fluid (Watkins et al., 2018, Slyvka et al., 2015, Bell and Hellmann, 2019, Bonduriansky and Day, 2019). Thus, malnutrition can induce changes in the female or male reproductive system that influence the development of embryos, potentially affecting juvenile viability and, in some cases, impacting health- and fitness-related traits throughout life. Insects have provided an important model for research on effects of diet on reproduction, and a broad finding from this work is that dietary protein is a key regulator of female (and, to a lesser

extent, male) reproductive performance (Lee, 2015, Adler et al., 2013, Maklakov et al., 2008, Sultanova et al., 2021, Burger et al., 2007). A smaller number of studies have examined effects of protein consumption on offspring, but this literature has not been synthesized. This led us to ask whether the available evidence supports consistent effects of male and female dietary protein on offspring viability across species.

The effects of protein consumption on insect reproduction are complex and subject to strong trade-offs. Dietary protein enhances female fecundity (Sultanova et al., 2021, Burger et al., 2007, Carey et al., 2022) and affects some male mating and post-copulatory traits (Fricke et al., 2008, Morimoto and Wigby, 2016, Bunning et al., 2015). Likewise, dietary protein enhances ability to cope with cold- and heat stress (Sisodia and Singh, 2012, Lushchak et al., 2012), and can increase the ability to mount an immune response [(Lushchak et al., 2012), but see (Cotter et al., 2019)]. However, dietary protein also promotes senescence and reduces lifespan independent of caloric intake (Maklakov et al., 2008, Lushchak et al., 2012, Lee et al., 2008, Simpson et al., 2017). The most widely accepted explanation for this effect is a trade-off in resource allocation between somatic maintenance, which slows senescence and prolongs life and reproduction, imposing viability costs that shorten life (Kirkwood and Shanley, 2005, Food, 1989).

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A recent study suggests, instead, that protein consumption shortens life of *Drosophila melanogaster* by inducing the depletion of a specific compound – sterol – from somatic tissues (Zanco et al., 2021). The ecological and evolutionary interpretation of these effects of nutrition on physiology, life history and fitness remains controversial (Adler and Bonduriansky, 2014, Moatt et al., 2016, Piper et al., 2022).

But what effect does dietary protein have on offspring? If offspring quality is viewed as part of overall reproductive investment, then increased dietary protein might be expected to enhance offspring viability. For example, increased dietary protein could enable females to increase the amount of yolk per egg (Mirth et al., 2019), and might enable males to enhance the quality or quantity of their sperm or seminal fluid (Macartney et al., 2019). Increased dietary protein could also enable parents to fine-tune the epigenome of their gametes to maximize embryo survival (Macartney et al., 2018). Alternatively, if offspring quality is viewed as part of a parent's overall somatic state then increased protein consumption might be expected to reduce offspring viability. For example, increased dietary protein might accelerate reproductive senescence (Simpson et al., 2017), resulting in reduced quality of eggs and sperm, or in the transmission of dysregulated epigenetic factors to offspring. These opposing effects might balance differently within each sex, resulting in opposite effects of maternal versus paternal protein consumption on offspring viability. For example, if dietary protein is strongly limiting for yolk synthesis but less important for sperm epigenome regulation, offspring viability might be affected positively by maternal protein consumption but affected negatively by paternal protein consumption.

Studies examining the effects of dietary protein on offspring have indeed suggested that consequences for offspring viability could depend on parental sex, as well as parental age and aspects of the ambient environment. For example, using a nutritional geometry approach, we found opposite effects of protein content in the larval diet of mothers versus fathers on offspring viability in the neriid fly *Telostylinus angusticollis*: maternal dietary protein had a positive effect on egg hatching success, whereas paternal dietary protein had a negative effect (Bonduriansky et al., 2016). In a subsequent study, we manipulated dietary protein in the adult stage of the neriid flies and also found an effect of paternal dietary protein on egg hatching success, although the magnitude and sign of this effect was age-dependent (Macartney et al., 2017). Polak et al. (Polak et al., 2017) used nutritional geometry to investigate the effects of protein in the adult diet on offspring viability in *Drosophila melanogaster* and found a negative effect of paternal dietary protein on embryo survival, but only when the carbohydrate source was fructose (not sucrose). However, it is not clear whether effects of maternal and paternal dietary protein on offspring viability are consistent across species, or whether differences in reproductive biology might result in contrasting effects in different taxa.

Here, we addressed this question using a systematic review and meta-analysis of published studies of oviparous insects and other oviparous invertebrates that tested for effects of maternal and paternal dietary protein on early offspring developmental outcomes (i.e., embryo viability or egg hatching success). We searched for studies that manipulated the amount of protein in paternal and/or maternal diets while keeping other diet components constant. We excluded studies that simply diluted the diet or restricted feeding because we were interested in effects of the relative amount of protein in the parental diet.

## Methods

### Literature searching

We searched for relevant papers published up until December 2021 in which (1) dietary protein was manipulated prior to mating, (2) subsequent egg hatching success or embryo viability (hereafter “offspring viability”) was measured, and (3) was conducted on oviparous invertebrate species. We searched topics (titles, abstracts, and keywords) on the Web of Science Core Collection and Scopus using the following search string

(modified for each database): ((protein OR diet\* OR condition\* OR \*nutri\*) AND (\*male\* OR maternal OR paternal OR parental OR transgen\* OR transgen\* OR intergen\* OR inter-gen\* OR nongen\* OR nongen\*) AND (egg OR embryo) NEAR/3 (surviv\* OR mortality OR death OR dead OR hatching OR success) NOT (fish\* OR rat OR mouse OR mice OR rodent\* OR sheep OR cow\* OR pig\* OR mammal\* OR bird\* OR chicken\* OR rooster\* OR bull\* OR sow OR agri\* OR \*man\* OR infant\* OR child\* OR patient\* OR healthcare\*)). This resulted in 1753 search hits after duplicate removal.

AC and ELM then sorted through the titles, abstracts and keywords using Rayyan QCRI (Ouzzani et al., 2016). This resulted in 167 potentially relevant papers for full text screening. Of the 167 papers, we identified 31 papers that included a manipulation of dietary protein and measurement of offspring viability in oviparous invertebrates (see Fig. S1 for a PRISMA-type flow chart). We did not include studies that manipulated nutrient concentration rather than a direct manipulation of protein, as we were specifically interested in the effects of protein restriction, not caloric restriction.

From the 31 relevant papers, we were able to obtain 50 measures of offspring viability (percentages). If the paper reported multiple levels of a protein manipulation, we recorded offspring viability from the highest and lowest levels. We also recorded the sex of the dietary manipulated parent (males, females, or mixed sex populations), the life stage (development versus adult) when protein was manipulated, and the study species. We were unable to record the exact difference in protein between the high and low protein diets as many studies provided an additional protein source (e.g., yeast, pollen, meat, etc) that was otherwise absent in the low protein diet.

### Statistical analysis

All analyses were conducted using R version 1.4.1106 (RStudio Team 2021) and the metafor package (Viechtbauer, 2010).

Standard errors, confidence intervals, and percentiles (when reported; see below) were converted to standard deviations, and medians were converted to means (Hozo et al., 2005). Due to bounding between 0 and 100 with percentage data, we arcsine transformed each measure of offspring viability and the corresponding variance using the delta method [sensu (Ouzzani et al., 2016)]. We then calculated the log Response Ratio (lnRR) (Hedges et al., 1999, Lajeunesse, 2011) using Formula 1 and the sampling variance (if we were able to obtain SD; see below) using Formula 2.  $M$  denotes the mean,  $SD$  and  $N$  denote the sample standard deviation and sample size, and  $h$  and  $l$  correspond to the high protein and low protein diets respectively.

$$1) \ln RR = \ln(M_h) - \ln(M_l)$$

$$2) \text{var}(\ln RR) = \frac{SD_h^2}{N_h M_h^2} + \frac{SD_l^2}{N_l M_l^2}$$

Of the 50 measures of offspring viability (and thus, effect sizes), only 31 included a measure of error (e.g., SD). Therefore, we conducted two multilevel random-effects meta-analyses, one with all 50 effect sizes (“full dataset model”) where variance was held equal, and a second meta-analysis using the 31 effect sizes that also included a measure of error that allowed us to calculate variance (“reduced dataset model”). Both models included effect size ID, study ID, species ID, and a variance covariance matrix of phylogenetic relatedness as random effects (see Fig S2 for phylogenetic trees). We also calculated the heterogeneity ( $I^2$ ) for the reduced dataset model which quantifies unexplained variation after accounting for sampling variance (Nakagawa and Santos, 2012, Borenstein et al., 2021).

Additionally, we assessed the importance of sex (female, male, or mixed sex) and life stage (development versus adult) of the manipulated parent by conducting two uni-moderator meta-regressions on the reduced dataset. We then calculated marginal  $R^2$  which indicates how much of the total variation in the model is explained by each moderator (Nakagawa et al., 2017).

**Table 1**

Details of the relevant studies detected in our literature search. The references in bold represent the studies that were included in the reduced dataset analysis.

Paper	Species	Class, Order	Sex	Life stage	Protein manipulation
<b>Wilder and Schneider (2017)</b>	<b><i>Argiope bruennichi</i></b>	<b>Arachnida, Araneae</b>	<b>Female</b>	<b>Adult</b>	<b>Added essential amino acids to control diet</b>
Colloff (1987)	<i>Dermatophagoides pteronyssinus</i>	Arachnida, Sarcoptiformes	Mixed	Adult	House dust vs yeast
Hart et al (2007)	<i>Dermatophagoides pteronyssinus</i>	Arachnida, Sarcoptiformes	Mixed	Adult	Added liver & yeast to control diet
Hare and Bethke (1988)	<i>Panonychus citri</i>	Arachnida, Trombidiformes	Female	Adult	Added bovine serum albumin to control diet
Augustin and Boersma (2006)	<i>Acartia clausii</i>	Crustacea, Calanoida	Mixed	Adult	Nitrogen depleted algae to control diet
<b>Dam and Lopes (2003)</b>	<b><i>Temora longicornis</i></b>	<b>Crustacea, Calanoida</b>	<b>Mixed</b>	<b>Adult</b>	<b>Diatoms of different nitrogen content</b>
<b>Helland et al (2003)</b>	<b><i>Calanus finmarchicus</i></b>	<b>Crustacea, Calanoida</b>	<b>Female</b>	<b>Adult</b>	<b>Food suspensions with different protein content</b>
Jónasdóttir (1994)	<i>Acartia hudsonica</i>	Crustacea, Calanoida	Female	Adult	Algal cultures with different protein content
<b>Zhang et al., (2013)</b>	<b><i>Acartia tonsa</i></b>	<b>Crustacea, Calanoida</b>	<b>Mixed</b>	<b>Adult</b>	<b>Algal cultures with different protein content</b>
Fardisi et al., (2013)	<i>Tribolium castaneum</i>	Insecta, Coleoptera	Mixed	Adult	Extra amino acids to control diet
<b>Millar et al., (2003)</b>	<b><i>Phoracantha recurva</i></b>	<b>Insecta, Coleoptera</b>	<b>Mixed</b>	<b>Adult</b>	<b>Added dog chow to control diet</b>
<b>Rashed et al., (2016)</b>	<b><i>Coccinella septempunctata</i></b>	<b>Insecta, Coleoptera</b>	<b>Mixed</b>	<b>Larval</b>	<b>Added chicken liver to control diet</b>
<b>Berkebile et al., (2006)</b>	<b><i>Cochliomyia hominivorax</i></b>	<b>Insecta, Diptera</b>	<b>Mixed</b>	<b>Adult</b>	<b>Corn syrup vs blood</b>
<b>Bonduriansky et al., (2016)</b>	<b><i>Telostylinus angusticollis</i></b>	<b>Insecta, Diptera</b>	<b>Female Male</b>	<b>Larval</b>	<b>Nutritional geometry (calculated from the studies raw data)</b>
<b>Macartney et al., (2017)</b>	<b><i>Telostylinus angusticollis</i></b>	<b>Insecta, Diptera</b>	<b>Male</b>	<b>Adult</b>	<b>Added yeast relative to control diet</b>
<b>Dinh et al., (2021)</b>	<b><i>Bactrocera tryoni</i></b>	<b>Insecta, Diptera</b>	<b>Female</b>	<b>Adult</b>	<b>Altered protein to carbohydrate ratio</b>
<b>Hernández et al., (2014)</b>	<b><i>Anastrepha striata</i></b>	<b>Insecta, Diptera</b>	<b>Mixed</b>	<b>Larval</b>	<b>Synthetic diets with different yeast content</b>
<b>Nyasebe et al., (2021)</b>	<b><i>Aedes aegypti</i></b>	<b>Insecta, Diptera</b>	<b>Female</b>	<b>Adult</b>	<b>Frequency of blood meals</b>
<b>Panduranga et al., (2018)</b>	<b><i>Bactrocera cucurbitae</i></b>	<b>Insecta, Diptera</b>	<b>Mixed</b>	<b>Larval</b>	<b>Synthetic diets with varying amounts of yeast</b>
<b>Polak et al., (2017)</b>	<b><i>Drosophila melanogaster</i></b>	<b>Insecta, Diptera</b>	<b>Male</b>	<b>Adult</b>	<b>Nutritional geometry (calculated from the study's raw data)</b>
<b>Sagel et al., (2002)</b>	<b><i>Cochliomyia hominivorax</i></b>	<b>Insecta, Diptera</b>	<b>Mixed</b>	<b>Adult</b>	<b>Synthetic diets with varying amounts of blood</b>
<b>El-Wahab et al., (2016)</b>	<b><i>Bracon hebetor</i></b>	<b>Insecta, Hymenoptera</b>	<b>Mixed</b>	<b>Adult</b>	<b>Added pollen to control diet</b>
<b>Cahenzli and Erhardt (2012)</b>	<b><i>Coenonympha pamphilus</i></b>	<b>Insecta, Lepidoptera</b>	<b>Female</b>	<b>Larval Adult</b>	<b>Nitrogen enriched plants and Amino acid supplemented nectar to control diet</b>
Geister et al., (2008)	<i>Bicyclus anynana</i>	Insecta, Lepidoptera	Female	Adult	Added yeast to control diet
<b>Mevi-Schütz and Erhardt (2003)</b>	<b><i>Lasiommata megera</i></b>	<b>Insecta, Lepidoptera</b>	<b>Female</b>	<b>Adult</b>	<b>Added amino acids to control diet</b>
<b>Moghaddassi et al., (2019)</b>	<b><i>Ephestia kuehniella</i></b>	<b>Insecta, Lepidoptera</b>	<b>Mixed</b>	<b>Larval</b>	<b>Added yeast to control diet</b>
<b>Chalapathi Rao et al., (2022)</b>	<b><i>Pseudomallada astur</i></b>	<b>Insecta, Neuroptera</b>	<b>Mixed</b>	<b>Adult</b>	<b>Added yeast &amp; added protein to control diet</b>
<b>Salwa (2011)</b>	<b><i>Chrysoperla carnea</i></b>	<b>Insecta, Neuroptera</b>	<b>Mixed</b>	<b>Adult</b>	<b>Added pollen to control diet</b>
<b>Sattar (2017)</b>	<b><i>Chrysoperla carnea</i></b>	<b>Insecta, Neuroptera</b>	<b>Mixed</b>	<b>Adult</b>	<b>Different protein sources in different concentrations</b>
<b>Simmons et al., (2021)</b>	<b><i>Teleogryllus oceanicus</i></b>	<b>Insecta, Orthoptera</b>	<b>Male</b>	<b>Adult</b>	<b>Nutritional geometry (calculated from the study's raw data)</b>
<b>Auld and Henkel (2014)</b>	<b><i>Physa acuta</i></b>	<b>Gastropoda, Hygrophila</b>	<b>Female</b>	<b>Adult</b>	<b>Lettuce vs protein rich algae</b>

Lastly, using the reduced data set, we assessed publication bias by visually checking for funnel asymmetry. We also conducted a sensitivity analysis where we systematically removed one study at a time ('leave-one-group-out' analysis) to determine if a single study was having a disproportionate effect on the results.

#### Data availability

All data and code can be found on Github at [https://github.com/elmacartney/Protein\\_Viability](https://github.com/elmacartney/Protein_Viability).

#### Results

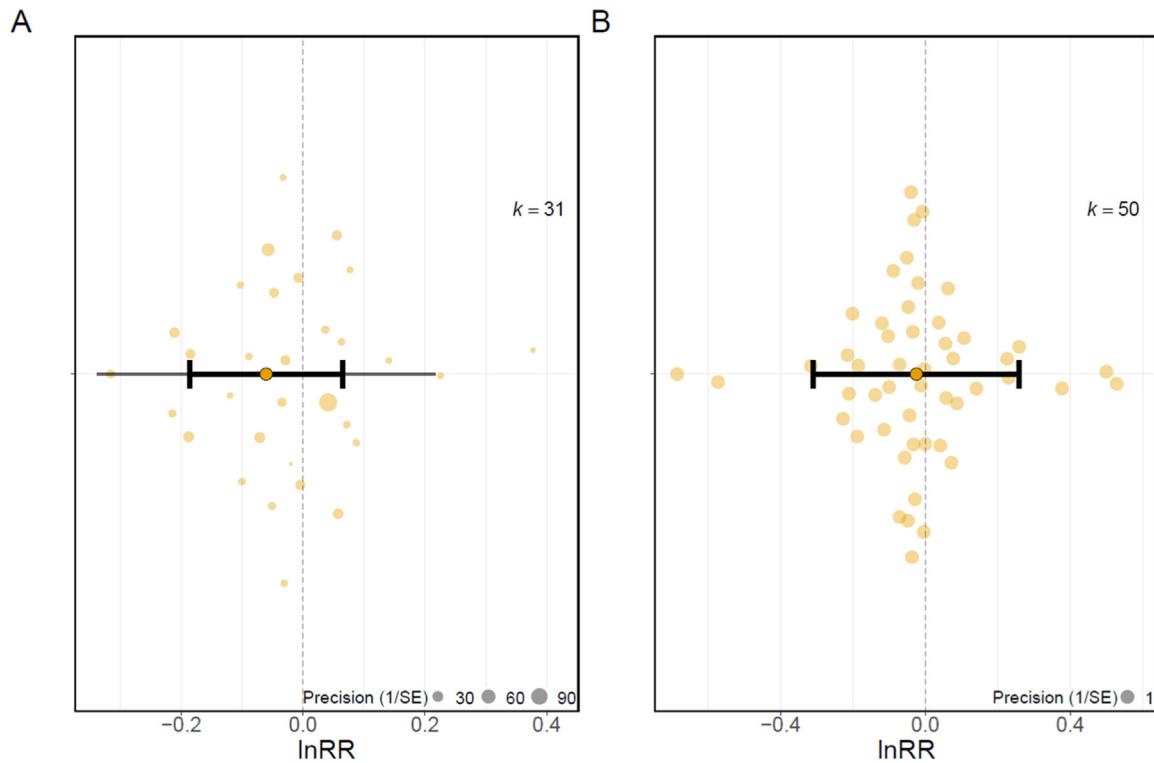
Out of the 50 effect sizes obtained in our systematic review, 72% were from insects, 16% from crustaceans, 10% from arachnids, and one effect size (2%) from a gastropod (Table 1). The majority (60%) of effect sizes were from studies of mixed sex populations where both parents were exposed to the same diet, 28% were from exclusively maternal dietary manipulations, and only 12% were from exclusively paternal dietary manipulations.

The models of the full dataset with equal variance ( $\text{estimate}_{[\text{full}]} = -0.02$ ; 95% Confidence Interval (CI)<sub>[full]</sub> = -0.31, 0.26) and of the reduced

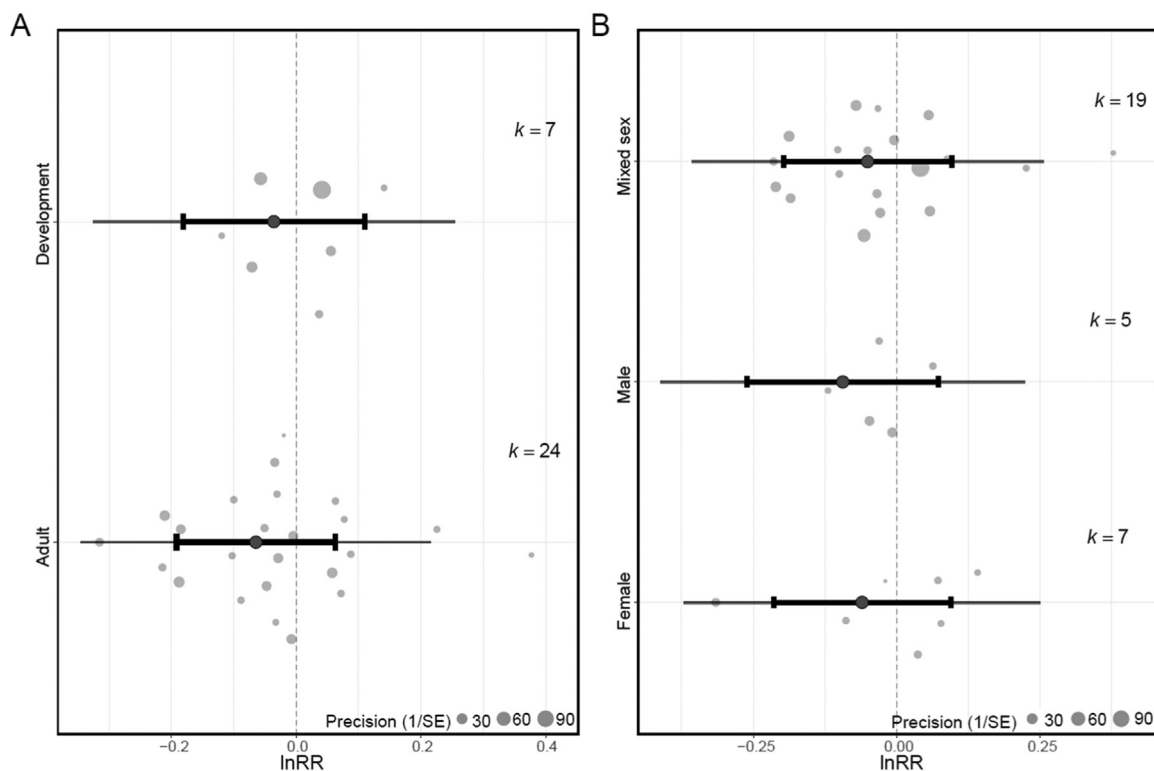
dataset for which variance could be calculated ( $\text{estimate}_{[\text{reduced}]} = -0.06$ , 95% CI<sub>[reduced]</sub> = -0.18, 0.06) both showed no significant effect of protein on offspring viability, although there was a slight trend towards reduced viability with high protein, particularly in the reduced dataset model (Fig. 1). Of note, the reduced dataset included 20 negative effects of dietary protein on offspring viability and 11 positive effects of dietary protein on offspring viability. However, higher-precision studies tended to show smaller responses (in both directions) to protein compared to low precision studies. Thus, the available evidence does not support an overall effect of protein on offspring viability.

Heterogeneity ( $I^2$ ) was 89.83% for the reduced dataset and was largely due to differences between species (species = 83.77%, paper ID = 6.06%, effect size ID = <0.01%). Parental sex and life stage explained minimal variation (1.40% and 0.9% respectively) (Fig. 2). Such small effects of parental sex and life stage may be expected given that paper ID and effect size ID accounted for little of the heterogeneity in the model.

Visual assessment of the funnel plot using the reduced data set suggests that there may be some low precision studies with negative effect sizes missing from the published literature (Fig. S3). In the sensitivity analysis on the reduced data set, we did not find any evidence that a single study was driving the non-significant effect of dietary protein on offspring viability (Fig. S4).



**Fig. 1.** Orchard plot of the two meta-analytic models. (A) Meta-analysis on the reduced dataset for which we were able to calculate variance. (B) Meta-analysis on the full dataset with equal variance for each effect size. Thick black lines are 95% confidence intervals and thin black lines are 95% precision intervals (only available for panel A). Each circle represents an individual effect size and the size of the circle represents the precision (precision only varies in panel A). Negative values along the x-axis indicate that offspring viability was lower on a high protein diet and positive values along the x-axis indicate that offspring viability was higher on a high protein diet. Points are randomly jittered along the y-axis to make each point visible.



**Fig. 2.** Orchard plots showing the two meta-regressions on the reduced dataset. (A) parental sex and (B) lifestage. Thick black lines are 95% confidence intervals and thin black lines are 95% precision intervals. Each circle represents an individual effect size and the size of the circle represents the precision. Negative values along the x-axis indicate that offspring viability was lower on a high protein diet and positive values along the x-axis indicate that offspring viability was higher on a high protein diet. Points are randomly jittered along the y-axis to make each point visible.

## Discussion

Our systematic review and meta-analysis found that effects of maternal or paternal dietary protein on offspring viability are consistent across species of oviparous invertebrates. Although protein is well known to enhance fecundity, we found no consistent effects on the proportion of eggs hatched or embryo survival. Interestingly, however, heterogeneity in our dataset was high, and much of the variation was related to species differences. This suggests that effects of dietary protein on offspring may be dependent on reproductive biology within species, highlighting interesting questions for future experimental research. Our findings also expose important taxonomic gaps in the empirical literature, and highlight limitations imposed by experiment design.

Although the majority (68%) of studies uncovered in our literature search reported non-significant results in their papers (see data at [https://github.com/elmacartney/Protein\\_Viability](https://github.com/elmacartney/Protein_Viability)), we note a bias in the direction of reported effect in the few studies where a significant effect was found (note that here we are referring to the reported effects in the papers and not the effect size directions shown in Fig. 1, although the same bias occurs). A significant positive effect of dietary protein on hatching success was only reported in four studies, one in which only the mother was manipulated and three in mixed sex populations. In contrast, 12 studies reported a significant negative effect of a high protein parental diet on egg hatching. Seven of these experiments were mixed sex, three were paternal manipulations and two were maternal manipulations. However, because we were unable to identify factors that could clearly account for variation in the direction of effects, we cannot conclude with confidence that the apparent variation in the direction of effects results from meaningful biological factors, rather than from unrepeatable variation between studies and samples. This bias in the direction of significant negative effects reported in the literature suggests a need for further research into the effects of dietary protein, particularly in males.

We limited our literature search to oviparous insects and other oviparous invertebrates to avoid the complicating factor of post-partum/hatching parental investment. Although some oviparous invertebrates also exhibit post-hatching parental care (Koch and Meunier, 2014, Smith, 1980, Kudo, 2002), to our knowledge this does not occur in any of the species included in our meta-analysis. Thus, in the included species, all maternal provisioning occurs prior to oviposition, and variation in the quality and quantity of nutrients and other substances (including small RNA and mRNA) transferred to eggs is likely to mediate the effects of maternal nutrition on offspring viability. In some species, maternal nutrition could also influence maternal oviposition behaviour and physiology (e.g. nest site choice, or the transfer of gut microbiota to eggs or larval food (Hosokawa and Fukatsu, 2020, Jaenike, 1983); and thus affect offspring viability. However, such effects are probably of limited importance under standardized lab culture conditions. Likewise, in the included species, males do not interact with or provision their offspring after hatching, and opportunity for paternal nutrition to influence offspring viability is therefore limited to compounds (such as small RNA) transferred to zygotes via the sperm or seminal fluid.

Our data set included 21 studies on insects (nearly half of these on Diptera), five studies on crustaceans (all copepods), four studies on arachnids (mites and spiders), and one study on a gastropod mollusc. This taxonomic distribution reveals a number of major gaps. For example, only six insect orders (Diptera, Lepidoptera, Neuroptera, Hymenoptera, Orthoptera and Coleoptera) have been used in studies on effects of parental protein consumption on offspring; no studies have been carried out on Hemiptera or other insect orders, and only one out of the six included insect Orders is hemimetabolous. Likewise, there is scope for much more research on other crustacean, arachnid, and mollusc species representing a greater diversity of reproductive traits, as well as on Annelida, Nematoda, and many other animal groups. A broader taxonomic coverage could illuminate the key reproductive vari-

ables responsible for the taxonomic heterogeneity revealed by our meta-analysis. For example, it is possible that variation in yolk investment per egg by females (Church et al., 2019) and in nutrient provisioning by males (Gilbert and Manica, 2015) could account for some of the variation in the effects of maternal and paternal dietary protein on offspring viability.

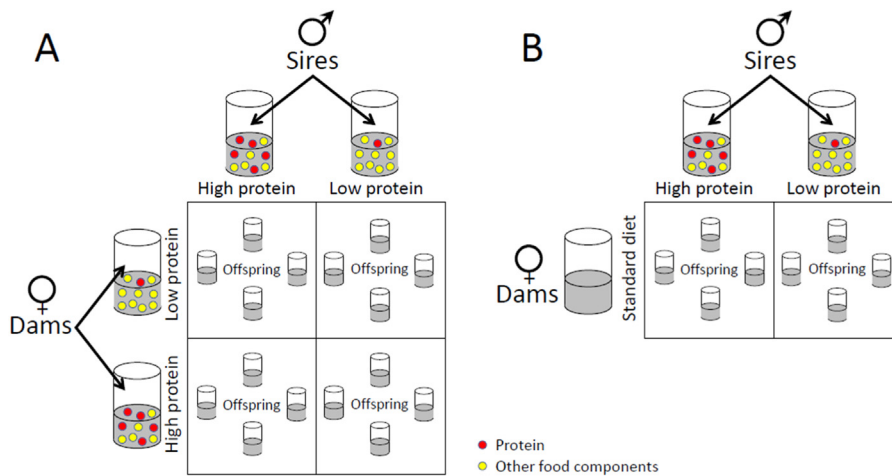
Our meta-analysis also highlighted some limitations that relate to experiment design. In particular, a number of studies ostensibly investigated maternal effects, but housed the females together with their male partners, such that both parents were exposed to the same diet treatment. This approach makes it impossible to determine whether any observed effects are maternal or paternal. Moreover, if no effect of dietary protein is observed, this could indicate that *neither* maternal nor paternal protein consumption affects offspring viability, or that *both* maternal and paternal effects occur but are of opposite sign and cancel out [e.g. see (Piper et al., 2022)]. This problematic experiment design reflects the long-standing assumption that paternal environment cannot affect offspring development in species lacking paternal care, but this assumption is untenable in light of the discovery of epigenetic mechanisms that can mediate paternal effects in any species, including animals lacking paternal nutritive investment or direct father-offspring interactions (Crean and Bonduriansky, 2014). In maternal effect studies, confounding effects should be minimised by exposing the non-focal parent to the treatment diet for the shortest time possible. Furthermore, mating protocols should be explicitly described in the methods for comparability across studies. The most informative design is to expose both parents separately to diet treatments prior to mating in a fully-crossed (or factorial) experiment design (Fig. 3). Such a design makes it possible to investigate maternal effects, paternal effects, and their interaction within the same experiment. To test for interactions between parental and offspring diets, it is also possible to rear offspring from each parental treatment combination on two or more different (e.g. high/low protein) diets.

We also note that the majority of studies manipulated food in the adult phase, and the effects of protein content in the parental developmental diet on offspring are therefore especially poorly known. It is important to investigate the effects of maternal and paternal diets across multiple life-history stages, preferably within the same experiment [e.g., see (Mirth et al., 2019)], in order to determine the ontogenetic stage when key reproductive traits are sensitive to dietary influence, and whether adult diet can modify or mitigate the effects of juvenile diet (Macartney et al., 2018). Such information would also help to develop dietary guidelines for animal producers seeking to optimise the health of males and females prior to conception.

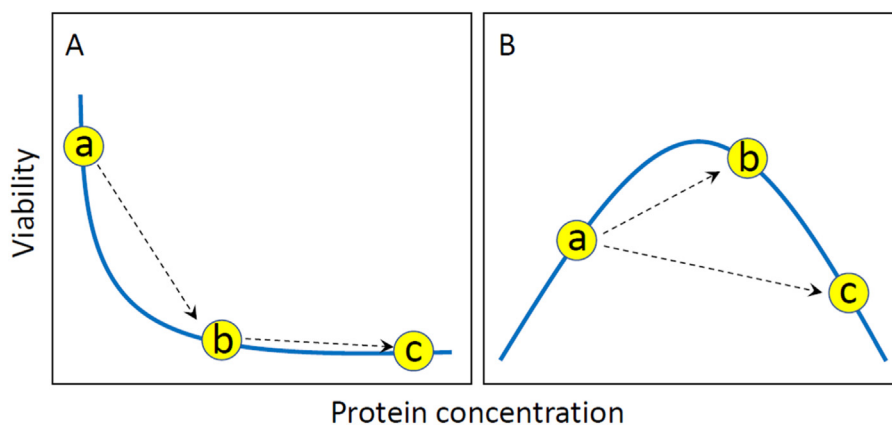
Another problem is the limited number of parental effect studies reporting hatching success, and the failure to report error estimates in several of the studies that did report hatching success. Although the focus of most dietary parental effects studies is changes in the body size and metabolic health of offspring, it is important to interpret these changes in the context of potential changes in the number of offspring produced. Protein often has a positive influence on fecundity, increasing the mating success of males and number of eggs laid by females (Sultanova et al., 2021, Burger et al., 2007, Carey et al., 2022). However, a diet-induced increase in fecundity can be associated with reduced investment per offspring (Guisande et al., 1996). While our meta-analysis did not find a significant effect of parental diet on offspring viability, the bias in direction and heterogeneity of results suggest that this question warrants further testing.

Another limitation of the literature is lack of information on the causes of differences in egg hatching success. In most studies, it was not determined whether eggs that failed to hatch had started developing. Hence, differences could be due to dietary effects on fertilization success (mediated, for example, by effects of male protein consumption on sperm quality or quantity) or, alternatively, to dietary effects on embryo viability (mediated by maternal nutrient investment in eggs, or the quality of egg- or sperm-borne epigenetic factors).





**Fig. 3.** Experiment designs to test for parental dietary protein effects on offspring: (A) Maternal (dam) and paternal (sire) diets can be manipulated independently prior to mating and manipulated sires and dams can be mated in a fully-crossed (factorial) design. This design makes it possible to test separately for maternal and paternal effects as well as their interaction on offspring. (B) In a simplified design, only sire (or dam) diet is manipulated while all dams (or sires) are provided with a standard diet (e.g. intermediate protein). In both cases, to test for effects of protein content in the diet, the amount of protein should be manipulated while holding the total amount of food constant. Offspring can be reared on a standard diet (e.g. intermediate protein), or on different diets (e.g. high/low protein) to investigate interactions between parental and offspring diets on offspring performance.



**Fig. 4.** Contrasts between just two levels of protein concentration can yield misleading results, especially if the biologically meaningful range of dietary protein concentrations is poorly known for the study species, and “high” and “low” treatment concentrations are therefore chosen arbitrarily. Panels (A) and (B) represent two hypothetical non-linear reaction norms representing effects of parental dietary protein concentration on offspring viability. Panel (A): If protein concentration (a) is chosen as the “low” protein treatment and (b) is chosen as the “high” protein treatment, a negative effect of parental dietary protein on offspring viability will be observed; however, if (b) is chosen as the “low” protein treatment and (c) is chosen as the “high” protein treatment, little or no effect will be observed. Panel (B): If (a) is chosen as the “low” protein treatment, the observed effect could be either positive or negative, depending on whether (b) or (c) is chosen

as the “high” protein treatment. Investigating more than two levels of protein concentration can enable more accurate characterisation of reaction norms.

A more complete understanding of the effects of parental protein consumption also requires extending studies beyond the offspring generation. None of the studies included in our meta-analysis tested for effects on grand-offspring, and it is therefore unclear whether such effects can persist over more than one generation. Many environmental factors can exert multi-generational effects (Bell and Hellmann, 2019, Bonduriansky and Day, 2019), so it is reasonable to investigate such effects for dietary protein as well.

Finally, because biological effects tend to be non-linear, studies that aim to characterise reaction norms relating environmental factors to developmental and phenotypic outcomes should generally examine more than two levels of the relevant environment (Rocha and Klaczko, 2012). Contrasts between effects of “high” vs. “low” parental dietary protein on offspring can give incomplete or even misleading clues to the shape and direction of effects, especially so if “high” and “low” protein treatments are chosen arbitrarily, without knowledge of the range of protein concentrations that the study organisms are able to tolerate, or normally experience in natural environments (Fig. 4). The most informative approach involves designs based on “nutritional geometry”, where protein and other diet components such as carbohydrates are manipulated along independent axes, resulting in multiple diet mixtures (Lee et al., 2008). Results from nutritional geometry studies can be represented as a response surface that reveals nonlinear and interactive effects of protein and other nutrients. Only two of the studies in our data set utilised the nutritional geometry framework to investigate effects of parental protein intake on offspring viability (Bonduriansky et al., 2016, Polak et al., 2017).

## Conclusions

In summary, by contrast with studies of high fat diets and dietary restriction, there are very few studies examining the intergenerational effects of a diet high in protein. Protein is generally regarded as beneficial for reproduction in animals, given the importance of protein as a building block for reproductive tissues, yolk synthesis, and male reproductive traits such as sperm production. Hence, it is vital to know if any gains in reproductive success may come at a cost to offspring health. We found no evidence of a consistent effect of either maternal or paternal protein consumption on offspring viability, but our analysis suggested that differences among species in reproductive biology represent a key source of variation in the effects of dietary protein. Further research is needed to better describe this taxonomic variation and uncover the key reproductive traits that determine how parental protein consumption affects offspring development and viability.

## Author contributions

ELM and AJC conceived the idea and found relevant literature, AJC extracted the data from the relevant papers, ELM conducted the analysis, and ELM, AJC and RB wrote the manuscript.

## Appendices

Supplementary material S1 with Fig. S1 (PRISMA-type diagram), Fig. S2 (phylogenetic trees) Fig. S3 (funnel plot) and Fig. S4 (leave-one-group-out sensitivity analysis).

Supplementary material S2: Raw data

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Russell Bonduriansky reports financial support was provided by Australian Research Council.

## CRedit authorship contribution statement

**Erin L. Macartney:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Angela J Crean:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Russell Bonduriansky:** Conceptualization, Funding acquisition, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing.

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## Supplementary materials

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