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Orientador: Prof. Dr. Eduardo da Silva Alves dos Santos

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Orientador

Dedicatória

À minha família,
que sempre me deu liberdade para fazer perguntas

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Resumo em português

A teoria prevê que em espécies sob maior risco de competição espermática, os machos irão investir mais em características do ejaculado. Em espécies com táticas alternativas de acasalamento (AMTs), machos de fenótipos diferentes estão sob diferentes riscos de competição espermática. Uma vez que machos *minors* (i.e., machos furtivos) se esgueiram para dentro do território de outros machos para acasalar, eles provavelmente sempre enfrentam competição espermática. Machos *major*, por outro lado, defendem territórios e possuem uma chance maior de acasalar exclusivamente com fêmeas. Para os *majors*, o risco de competição espermática é teoricamente menor. A principal previsão de modelos de teoria dos jogos para espécies com AMTs é que *majors* investem menos em características de ejaculado do que *minors*. Entretanto, quando a proporção de *minors* em uma população aumenta, os *majors* devem investir mais em características do ejaculado, alcançando um nível similar de investimento em ejaculado que os *minors*. Neste estudo, nós testamos essas previsões com uma meta-regressão de 29 espécies de peixes com AMTs. Como *proxy* para o risco de competição espermática, nós classificamos cada espécie de acordo com um *ranking* de competição espermática. Esse *ranking* utiliza características de história de vida e demografia de cada espécie, e possui cinco níveis, de 1 (baixo risco de competição espermática) até 5 (alto risco de competição espermática). De maneira geral, nós encontramos que *minors* investem mais em características de ejaculado do que *majors*. Nós também categorizamos o investimento em ejaculado dos machos de acordo com as variáveis originais quantificadas nos estudos que foram incluídos na nossa análise e encontramos o resultado de que *minors* investem mais na produção de gônadas para seu próprio tamanho do que *majors*. Além disso, *minors* e *majors* apresentam investimento similar em número de espermatozoides e qualidade espermática, mas *majors* alocam mais esperma para as fêmeas. Em geral, o *ranking* de competição espermática não influenciou a magnitude da diferença de investimento entre *majors* e *minors*. O investimento diferencial em massa gonadal entre *majors* e *minors* deveria representar um aumento no número de espermatozoides, porém nossos dados mostraram que *majors* e *minors* não estão produzindo quantidades diferentes de esperma. Assim, um investimento maior em massa gonadal pode estar relacionado aos *minors* acasalarem mais frequentemente que os *majors*. *Minors* não conseguem produzir esperma em maiores quantidades que os *majors*, mas eles provavelmente conseguem repor seu estoque de esperma mais rápido que os *majors*. Contrário às previsões teóricas, a qualidade espermática não responde às variações de competição espermática, provavelmente porque a qualidade espermática não está sob forte seleção como a

massa gonadal. Nossos resultados sugerem que, em peixes com táticas alternativas de acasalamento, tanto os *majors* como os *minors* estão sob forte seleção da competição espermática, mesmo quando o risco de poliandria é baixo.

Palavras-chave: táticas alternativas de reprodução. competição espermática. machos furtivos e guardiões. GSI. qualidade espermática.

Abstract

Theory predicts that in species with a greater risk of sperm competition, males will invest more in ejaculate traits. In species with alternative mating tactics (AMTs), males of different phenotypes will be under different sperm competition risk. Because minors sneak inside other males' territories to mate they should always face sperm competition. Major males, on the other hand, defend territories and have more chance of mating exclusively with females. For majors, the risk of sperm competition is theoretically lower. The main prediction from game theory models for species with AMTs is that majors invest less in ejaculate traits than minors. However, when the proportion of minors in the population increases, majors should invest more in ejaculate traits, reaching a similar level of ejaculate expenditure to minors. In this study, we tested these predictions with a meta-regression analysis of 29 fish species with AMTs. As a proxy for the risk of sperm competition, we ranked each species according to a sperm competition rank with five levels, from 1 (low risk of sperm competition) to 5 (high risk of sperm competition). Overall, we found that minors invest more in ejaculate traits than majors. We also categorized the ejaculate expenditure of males, according to the original variables quantified in the studies that were included in our analysis and found that minors invest more energy in the production of gonads than majors. Additionally, minors and majors have a similar investment in sperm number and sperm quality, but majors allocate more sperm to females. Overall, the sperm competition rank did not influence the magnitude of the difference in investment of majors and minors. The differential investment in gonad mass between majors and minors should represent an increase in sperm numbers, but our data showed that majors and minors are not producing different amount of sperm. Therefore, the higher investment in gonad mass can be related to minors mating more frequently than majors. Minors are not able to produce sperm in greater quantities than majors, but they probably can replenish sperm faster than majors. Against theoretical predictions, sperm quality does not respond to differences of sperm competition, probably because sperm quality is not under such strong selection as gonad mass. Our findings suggest that, in fishes with alternative mating tactics, both majors and minors are under strong selection from sperm competition, even when the risk of polyandry is low.

Keywords: alternative reproductive tactics. sperm competition. sneaks and guards. GSI. sperm quality.

Summary

Introduction

Males face several challenges in order find available mates and, consequently, sire offspring. For instance, males may have to spend considerable energy, and face large costs during the pre-copulatory phase of the competition for mates. Mate searching, and competing with same sex individuals for mates, for example, are among the challenges that demand energy and represent large costs for males (Andrade, 2003; Schärf *et al*., 2013). Even if a male is successful in acquiring a mate, there is no guarantee that he will sire all offspring of a given mate. Most of the challenges faced by males during the pre-copulatory phase of the competition to gain reproductive success (i.e., pre-copulatory sexual selection) have to be faced again by their own sperm after copula, particularly when their mates copulated with multiple males. Firstly, spermatozoa have to move towards females eggs in many species, a behavior that is energetic demanding (Perchec *et al*., 1995). Secondly, when females copulate with multiple males, sperm from these males may compete to fertilize each one of the female's eggs, a process known as sperm competition (Parker 1970, 1998).

Sperm competition is one of the processes that shape the mating behaviors across and within species. Over the years, models that use a game theory approach have been developed to predict how males will respond in ejaculate expenditure given different sperm competition scenarios (Parker, 1990a, 1990b; Parker, 1998; Parker *et al*. 2012). Sperm competition game models assume that the production of sperm is costly (Dewsbury, 1982; Wedell *et al*. 2002) and that males trade-off resource allocation between investing in ejaculate and in acquiring matings (Parker, 1998; Kvarnemo and Simmons, 2013). Another assumption of game theory models is that a higher expenditure in ejaculates increases male fertilization success (Parker, 1998; Martin *et al*., 1974). One of the predictions from game theory models is that, across species, males will invest more in ejaculates when the degree of sperm competition is higher (Parker, 1998). There is evidence in favor of this prediction in several taxa (Stockley *et al*., 1997; Byrne *et al*. 2002; Pitcher *et al*., 2005; Ramm *et al*., 2005). For example, in fishes, males of species with high communal spawning (e.g., *Meridia beryllina*: Middaugh and Hemmer, 1992) present greater values of the gonadosomatic index (i.e., GSI, often calculated as gonad mass divided by body or soma mass) and ejaculates with greater sperm numbers than males of species that generally mate in pairs rather than in groups (Stockley *et al*., 1997).

In game theory models, "ejaculate expenditure" is the response to sperm competition with other interacting components related to the life-history and the context of species or individuals (Parker, 1990a, 1990b, 1998; Parker *et al.*, 2012). Across the literature, "ejaculate

expenditure" is interpreted as different traits related to sperm and that can be under the pressure of sperm competition. As for the example cited above (Stockley *et al*., 1997), gonad size and sperm numbers can represent "ejaculate expenditure". By allocating energy to gonad mass and sperm numbers, males can increase their likelihood of fertilizing more ova than other males. In fact, ejaculates with more sperm are linked to a higher fertilization success and to higher proportion of paternity of offspring in a clutch of eggs (Casselman *et al*., 2006). Besides allocating energy to traits that increase the quantity of sperm in ejaculates, males can also invest in sperm quality to increase their chances of fertilizing more ova than other males. Sperm quality represents different traits that make sperm more fertile (Snook, 2005; Fitzpatrick & Lüpold, 2014). For example, males can produce more efficient sperm by increasing sperm metabolic performance (Perchec *et al*., 1995), sperm motility (Gage *et al*., 2004; Casselman *et al*., 2006; Rudolfsen *et al*., 2008), sperm longevity (García-González and Simmons, 2005; Smith, 2012) or by making the swimming environment more favorable (Lahnsteiner *et al.*, 1997). However, studies that investigated whether sperm quality traits consistently respond to sperm competition across different species had conflicting results (Snook, 2005). Within species, individuals can also respond to the immediate levels of sperm competition by allocating more or less sperm to females based on social contexts (Wedell and Cook, 1999; Pizzari *et al.*, 2003). Sperm allocation is also a type of ejaculate expenditure that is expected to increase with an increased risk of sperm competition (Wedell *et al*. 2002).

In species with alternative mating tactics, males face different risk of sperm competition because of their phenotypes (Parker, 1990b; Taborsky, 1998). Species with alternative mating tactics (AMTs) are characterized by two or more male showing different phenotypes that use different sets of behaviors in order to gain matings (Taborsky, 1994; Gross, 1996; Taborsky *et al*., 2008; Taborsky and Brockmann, 2010). Importantly, these alternative tactics may lead to similar reproductive success (Gross, 1996). In general, species with alternative mating tactics present two distinct male phenotypes. Males that usually defend territories or females are classified as majors (Taborsky *et al*., 2008). Majors sometimes perform courtship behaviors that makes them more attractive to females (Rios-Cardenas *et al*., 2007). Males that usually do not defend territories or females are *classified* as minors (Taborsky *et al*., 2008). Minor males are less exuberant than majors to females and use sneak copulations inside a major's territory to try to gain reproductive success.

Minors always face sperm competition, because they always mate with females in other males' territories (Parker, 1990b; Taborsky, 1998). Majors, however, face less sperm competition, as they defend their territories and prevent females from mating with other males.

Because minors face more sperm competition, they are expected to invest more in traits that can make them greater sperm competitors than majors. A game theory model of sperm competition in species with alternative mating tactics predicts this pattern (Parker, 1990b). Moreover, if the proportion of minors increases in a population, the model predicts that majors will also increase their investment in traits related to sperm competition (Parker, 1990b). The increase in the proportion of minors represents an increase in the level of sperm competition for majors, because the chances of a major mating with a female that will mate with another male will increase. As a result, majors will have an investment in ejaculate expenditure that approaches the investment made by minors, because the pressure of sperm competition will be similar to all individuals. When the proportion of minors is too low, it is predicted that sperm competition is not strong enough for majors and minors to make great investments in ejaculate expenditure (Parker, 1990b). Therefore, majors and minors should have similar ejaculate expenditure when sperm competition is too low and when it is too high. In intermediate sperm competition levels, the difference of ejaculate expenditure between majors and minors will be the greatest (Parker, 1990b). A comparative analysis of dimorphic dung beetles of the genus *Onthophagus* supports the prediction that minors will invest more in traits related to sperm competition, because minors of the species of *Onthophagus* had proportionally larger testis than majors (Simmons *et al*., 2007). However, the prediction that majors and minors will invest similarly in sperm competition when the proportion of minors increases was not supported in that study (Simmons *et al*., 2007).

Several fish species present alternative male mating tactics. In most fish species, fertilization occurs externally (Montgomerie & Fitzpatrick, 2009) and grouping spawning is also common. These characteristics not only favor the occurrence of alternative mating tactics, but also favor the occurrence of sperm competition (Taborsky, 2008). Thus, fish are an ideal group to investigate theoretical predictions of game theory models of how alternative male phenotypes should invest into traits to deal with differing levels of sperm competition. As far as we are aware, no study has investigated these predictions using an analysis across species with alternative male phenotypes.

In this study, we use a phylogenetic meta-regression analysis, using different fish species with male alternative mating tactics, to test the prediction proposed by Parker (1990a) that majors and minors will have different investment in sperm competition traits according to their phenotype and the level of sperm competition. The theoretical model by Parker predicts that minors will make greater investments in ejaculate expenditure than majors. Moreover, we will test the prediction that the difference between the investment of majors and minors will be

greater when species have an intermediate level of sperm competition than when levels of sperm competition are low and high.

Methods

Literature search

We conducted a systematic literature search using the platforms *Web of Knowledge* and *Scopus*. Our search was last updated on the 16th of May of 2017. We used the following combination of keywords at the basic search in all databases of the *Web of Knowledge* platform and at the advanced search of the *Scopus* platform: ("alternat* sex* behavio*r*" OR "alternat* sex* role*" OR "alternat* sex* phenotype*" OR "alternat* sex* tactic*" OR "alternat* sex* strateg*" OR "alternat* reproductive behavio*r*" OR "alternat* reproductive role*" OR "alternat* reproductive phenotype*" OR "alternat* reproductive tactic*" OR "alternat* reproductive strateg*" OR "alternat* mating behavio*r*" OR "alternat* mating role*" OR "alternat* mating phenotype*" OR "alternat* mating tactic*" OR "alternat* mating strateg*" OR "reproductive role*" OR "reproductive phenotype*" OR "reproductive tactic*" OR "reproductive strateg*" OR "mating role*" OR "mating phenotype*" OR "mating tactic*" OR "mating strateg*" OR "male* *morph*") AND ("sperm competition" OR "testis size" OR "ejaculate" OR "sperm allocation" OR "post*copulato*"). The search in *Web of Knowledge* returned 868 results and the search in *Scopus* returned 682 results. After removing duplicates from both searches, we obtained 1,103 distinct results in total (Fig. 1).

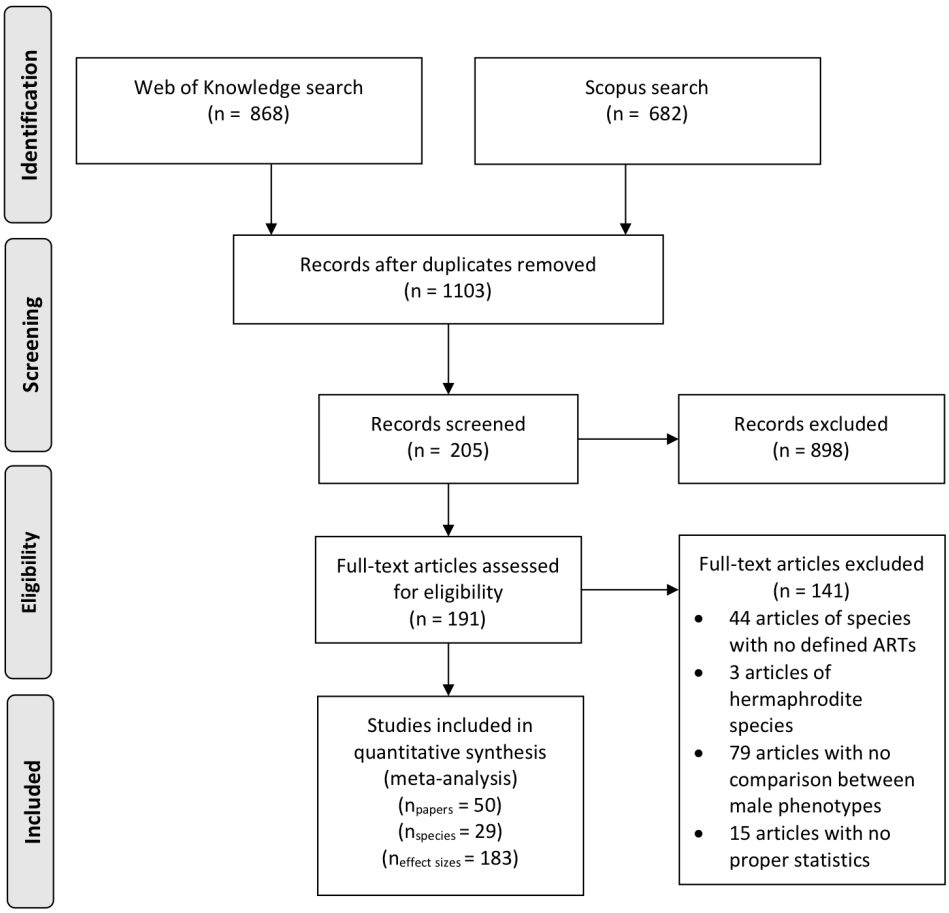

Fig 1. The screening process of exclusion and inclusion of articles following data extraction criteria, illustrated by a PRISMA flow diagram (Moher *et al*. 2009).

Data extraction

We read all the titles and abstracts from the results obtained in our searches in order to include articles that met the following criteria: (a) studied one or more fish species/populations, (b) had some indication that the males of the species have at least two distinct mating tactics (by mentioning different morphs or different specific behaviors), (c) abstract of the study presented some indication that both male phenotypes, if present, were compared in relation to characteristics involved in sperm competition and/or body size. After using these criteria, a total of 205 articles remained in our dataset (Fig. 1).

For the current version of this manuscript, we read 191 articles (of the 205 in our dataset; 14 articles were not screened because of time constraints) and applied the following, more detailed inclusion criteria: (a) confirmation that the study was about fish species, (b) species were not hermaphrodite, (c) confirmation that males have at least two described mating

tactics, (d) male phenotypes were compared in relation to characteristics involved in sperm competition (regardless of comparisons between body size), and (e) the presence of proper statistics. Proper statistics in this case means the study reports the mean estimate of the response variable, sample size, and S.D. or S.E. for descriptive analysis. In some cases, the relevant data was reported in figures and, in these cases, we extracted data using *GraphClick* (version 3.0.3; Arizona Software, 2012) or the *metaDigitise* package for R (Pick *et al.*, 2018; version 3.4.0, R Core Team, 2017). If no descriptive statistics were reported, we extracted data from inferential analyses (i.e., *t*-values, or *F*-values for one-way ANOVA, Wilcoxon matched pair signed-rank, Mann-Whitney U-test) only when authors reported the direction of the effect (i.e., which male phenotype presented the larger trait). Only if the study met all the criteria mentioned above it was included in our analyses (see Fig. 1). After reading the full text of 192 articles, we kept 50 studies in our dataset.

To understand how quality, quantity and allocation of sperm differ in relationship to alternative mating tactics and sperm competition, we categorized the response variables extracted from the original articles into three sperm expenditure categories. The first category is "Production" and we included in this category variables that represent investment in sperm number or sperm volume. This category was subdivided into "Quantity" and "GSI" subcategories. We classified as "Production/Quantity" variables such as sperm density, sperm count and sperm volume (Table 1). The only variable classified as "Production/GSI" was the Gonadal Somatic Index (GSI; either total body mass based, soma mass based and in one case energy based). We subdivided the "Production" category because the GSI represents investment in relation to individual body sizes, and other sperm traits represent the outcome of this investment in sperm numbers. For this version of the manuscript, we excluded all variables of gonad mass because they are not independent from GSI. The second category is "Quality" and we included in this category variables that represent investment in sperm quality. We included variables such as sperm velocity (average, straight and curvilinear velocities), sperm length (including midpiece, head and tail lengths), ATP concentration, motility, longevity, enzyme activity and seminal vesicle somatic index (SVSI) in the "Quality" category. All response variables used are in Table 1. When authors measured motility and velocity at multiple times post sperm activation, we extracted data only for the first measurement after activation. The third category is "Allocation" and we included in this category variables that comprehend facultative investment of males as an immediate response to sperm competition; i.e., variables that could be strategically modulated by males upon ejaculation. We included

variables such as sperm concentration in the water and number of ejaculations per spawning in the "Allocation" category.

The best proxy for the intensity of sperm competition faced by males in natural settings is, perhaps, the proportion of minor males in the population, when data was collected. Nevertheless, most of the studies in the dataset did not report the proportion of minors at their data collection sites. Thus, in order to make use of most of the data, we had to use a different proxy for the intensity of sperm competition. Therefore, we searched the literature of sperm competition in fishes and decided to choose a well-known index of the intensity of sperm competition. We classified each species in our dataset using life-history data from the literature to estimate the sperm competition rank proposed by Stockley *et al*. (2007). Similar ranks have been used in well-conducted comparative analyses (Byrne *et al*., 2002; Fitzpatrick *et al*., 2009). The sperm competition rank, as proposed by Stockley *et al*. (2007), ranges from 0 to 5 (in discrete categories). Species classified as level 0 have internal fertilization, including fertilization that occurs inside the mouth, and no polygamy or spawning in groups. Level 1 is composed of species that have internal fertilization with low group spawning or polygamy or external fertilization with pairing and no group spawning. On level 2, species have internal fertilization with high degree of spawning in groups or polygamy, or species have external fertilization with pairing and low incidence of spawning in groups. On level 3, all species have external fertilization with pairing and moderate spawning in groups, or species have no pairing and low spawning in groups. On level 4, all species have external fertilization, make pairings and there is a high incidence of spawning in groups, or species make no pairings and spawning in groups is low. On level 5, species have no pairing and spawning in groups has a high level. Therefore, level 5 corresponds to the species with higher levels of sperm competition in comparison to other levels in the rank, with level 0 the one with the least sperm competition.

Table 1. List of all response variables we collected to calculate Hedges' g separated in the three categories created for sperm expenditure

Category	Response variables
Production	sperm count Gonadal Somatic Index (GSI) sperm density relative testicular gland area sperm volume milt mass % glandular tissue

	stripped sperm mass
Quality	sperm velocity (total or of the 10% fastest sperm) average path velocity of sperm straight line velocity of sperm curvilinear sperm velocity of sperm loss of path velocity linearity % or proportion of motile sperm duration of motility sperm total length sperm flagellum length sperm end piece length sperm midpiece length sperm head length sperm head width ATP concentration Pyruvate kinase activity in sperm Citrate synthase activity in sperm longevity of sperm seminal vesicle mass Seminal Vesicle Somatic Index (SVSI) Testicular Gland Index (TGI) testicular gland area accessory gland mass accessory gland corrected for body mass energy charge values (based on ATP and ADP)
Allocation	number of ejaculations per spawning sperm concentration (sperm per mL of water) sperm per spawn proportion of sperm delivered in relation to sperm at rest

Effect size calculation

All the original studies in our dataset compared two groups of males in relation to different sperm expenditure traits. As we were interested in quantifying the magnitude of the difference between the investment of minor and major males, we chose a standardized effect size metric to represent this difference. To calculate the effect size of these differences we used Hedges' g. Hedges' g represents the standardized difference between the means of two groups and also corrects for small sample sizes (Hedges, 1981). For most of the papers we extracted data, we calculate Hedges' g using the means of major and minor groups and the corresponding standard deviation. If S.D. was not reported, we calculated S.D. based on the S.E. or the 95% confidence interval. When authors did not report any descriptive statistics or did not present data in plots,

we calculated effect size based on inferential statistics. We used the Practical Meta-Analysis Effect Size Calculator (Wilson, 2018) to calculate effect size based on inferential statistics.

We adjusted the directionality of our effect sizes so that positive effect sizes represent a greater mean value for majors when compared to minors. Negative effect sizes represent a greater mean value for minors when compared to majors. We corrected the direction of Hedges' g based on the criteria above when effect size was calculated from inferential statistics. We used the Hedges' g values of 0.2, 0.5 and 0.8 as benchmarks for small, medium and large effects (Cohen, 1969). In total, we estimated 183 effects sizes from 50 studies, with 29 fish species (Supplementary Table 1). Of all the effect sizes we calculated, 65 were calculated from variables associated to sperm production (35.5%), 31 for GSI and 34 for Quantity, 107 effect sizes to sperm quality (58.5%) and 11 effect sizes to sperm allocation (6%). Thus, given the small sample of sperm allocation effect sizes, we only included these data in a model to estimate the mean meta-analytic effect of each type of sperm expenditure variable.

Data analysis

First, we tested the prediction that minors invest more than majors in traits to deal with sperm competition. To test this prediction, we built a meta-regression model that included the type of investment variable as a categorical predictor. We chose to parameterize this model so that we obtained the mean meta-analytic estimate for each level of the investment variable categorical variable. This allowed us to test whether each level of this predictor variable differed from zero (0). The predictor, investment variable category was fit with four levels (allocation, production as sperm quantity, production as GSI, and quality). This model included three random effects to account for the hierarchical nature of the dataset. We fit paper identity, species identity and also a phylogeny term (Fig. S1).

Second, to test whether the level of investment made by minors and majors was influenced by the intensity of sperm competition, we fit an additional meta-regression model. In this second model, we used a subset of the data that consisted of effect sizes estimated for three levels of the investment variable category (production in quantity, production in GSI, and quality). We removed the allocation effect sizes as they had a small sample size. Moreover, as most of the effect sizes after this subset was created came from observational studies conducted in natural settings, we removed the small number of effect sizes from experimental/laboratory studies.

This second model included an interaction between the predictors: type of investment variable category and level of sperm competition. Level of sperm competition was fit as a categorical variable with five levels. As we were only interested in making inferences within each level of investment variable category, we parameterized the model so that the interactions were restricted to each level of this variable.

Based on our hypothesis, our predictions are: (1) there is a significant difference in investment in traits related to sperm competition between minor and major males, for all subtypes of investment (quantity, GSI, quality and allocation), with minors investing more than majors and (2) the difference in investment in traits related to sperm competition between minors and majors is higher in species classified as having an intermediate sperm competition rank and the difference in investment in traits related to sperm competition between majors and minors is smaller in species with a higher and lower sperm competition ranks, for all subtypes of sperm investment.

We estimated the effect size of the difference in sperm investment between majors and minors using a phylogenetic meta-analytic model using the function *rma.mv* in the package *metafor* (Viechtbauer, 2010) in R (version 3.4.0, R Core Team, 2017). Effect sizes were deemed to be statistically significant when 95% confidence intervals (CI) did not overlap zero.

Sensitivity analysis

As a form of sensitivity analysis, we used Egger's regressions (Egger *et al.*, 1997) to investigate if publication bias was present in our datasets. We also quantified the amount of heterogeneity explained by our models and by the random effects of each model. To quantify heterogeneity, we used a modified version of the I^2 metric (see Nakagawa and Santos 2012). For the phylogeny random effect, I^2 can be interpreted as analogous to phylogenetic signal (see Housworth *et al.*, 2004).

Results

We found that minors invested more than majors in all forms of sperm expenditure, as the overall meta-analytic mean effect was negative and moderate (-0.519, 95% CI: -1.317 to 0.278), but note that the 95% CI overlapped zero. This overall meta-analytic model presented high heterogeneity (83.21%; Table 2), as expected in biological meta-analyses.

Table 2. Summary of total heterogeneity value and random effects heterogeneity values of models used in this meta-analysis.

Model	Moderators	Random effects	$I^2_{study\ identity}$ % (95% CI)	$I^2_{species\ identity}$ % (95% CI)	$I^2_{phylogeny}$ % (95% CI)	I^2_{total} % (95% CI)
Null Model	NA	study identity + species + phylogeny	55.46 (53.54 to 57.38)	9.35 (7.43 to 11.28)	18.38 (16.46 to 20.30)	83.21 (81.60 to 85.44)
Meta-regression model 1	variable type	study identity + species	76.02 (74.10 to 77.94)	7.50 (5.58 to 9.42)	NA	83.52 (81.60 to 85.44)
Meta-regression model 2	variable type : sperm competition rank	study identity + species	46.24 (44.32 to 48.16)	36.33 (34.41 to 38.25)	NA	82.57 (80.65 to 84.50)

In our first meta-regression analysis, we tested whether the type of expenditure investment variable influenced the magnitude of the effect size. We found that majors invested significantly more in allocation traits than minors, and that the effect size was large (2.732, 95% CI: 1.476 to 3.989; Fig. 2). Minors, on the other hand, invested significantly more in gonad mass in relation to their own body size (*i.e.*, GSI) than majors (-2.638, 95% CI: -3.105 to -2.177; Fig. 2). However, we found small and non-significant effect sizes for sperm expenditure traits related to sperm quality (-0.251, 95% CI: -0.699 to 0.197; Fig. 2) and absolute sperm quantity (-0.384, 95% CI: -0.845 to 0.076; Fig. 2). We found high heterogeneity in this meta-regression model (83.52%; Table 2). A large proportion of the heterogeneity was explained by the study identity (76.02%; Table 2) and the species identity (9.35%; Table 2) random effects. Phylogeny explained virtually no variation and was removed from the model.

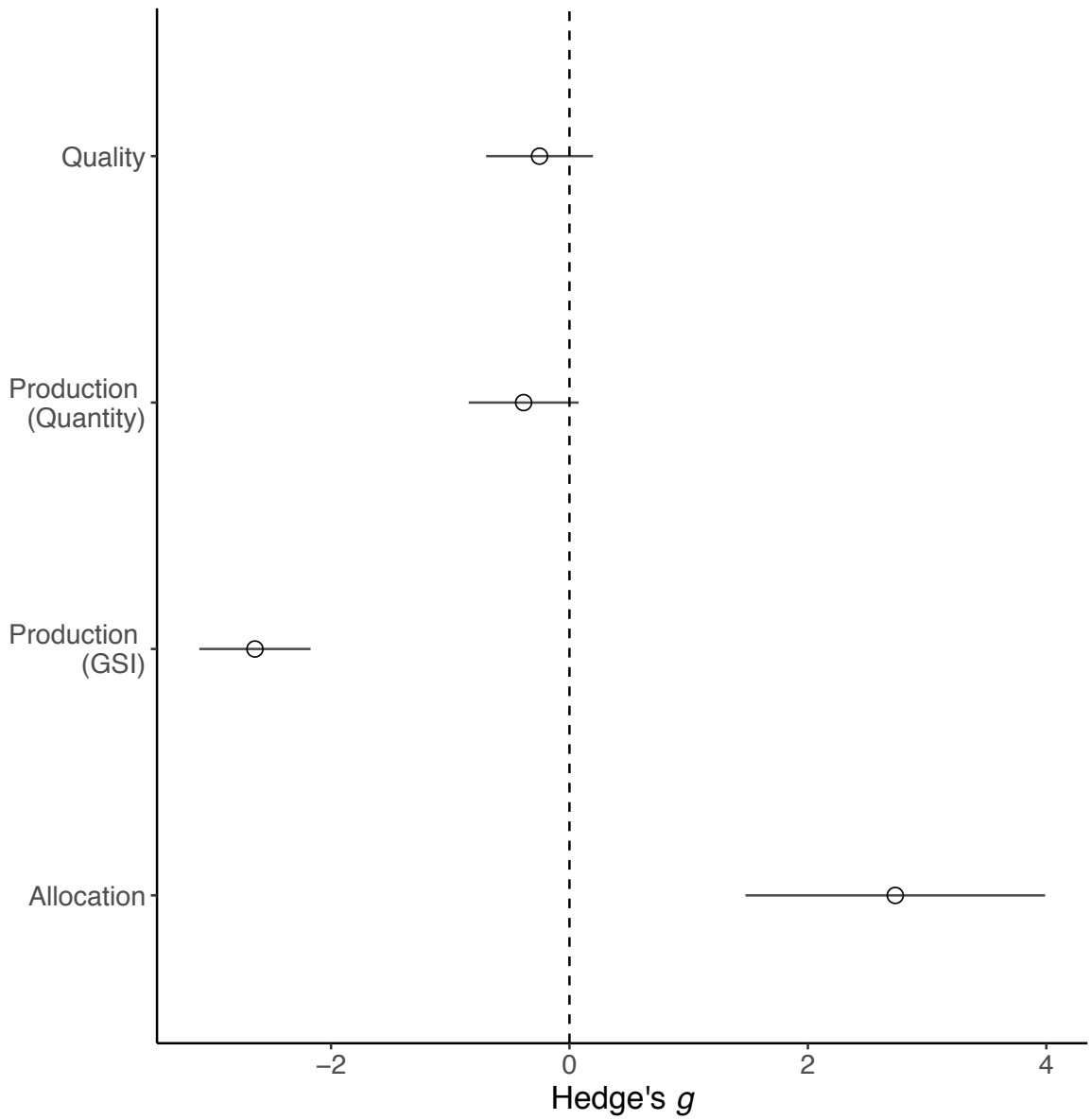

Figure 2. Standardized mean Hedge's g effect sizes ± 95% CIs of the differences between majors and minors in investment in traits related to sperm competition by the type of sperm investment. Vertical dashed line represents Hedge's g equal to zero.

Finally, we built a meta-regression model using only the effect sizes obtained from observational studies and excluding sperm allocation traits (due to small sample sizes, see Methods, above). In this model, we included, in addition to the type of sperm investment variable, the level of sperm competition as a predictor. Within each type of investment variable, we found little evidence that the level of sperm competition influenced the difference in investment between minors and majors (Table 3; Fig. 3). That is, the magnitude of the difference in investment between minors and majors was similar across the range of sperm competition ranks. This meta-regression model also presents high heterogeneity (82.57%;

Table 2). For this meta-regression model, a large proportion of the heterogeneity was also explained by the study identity (46.24%; Table 2) and the species identity random effects (36.33%; Table 2). Phylogeny was also removed from this model because it did not explain variation.

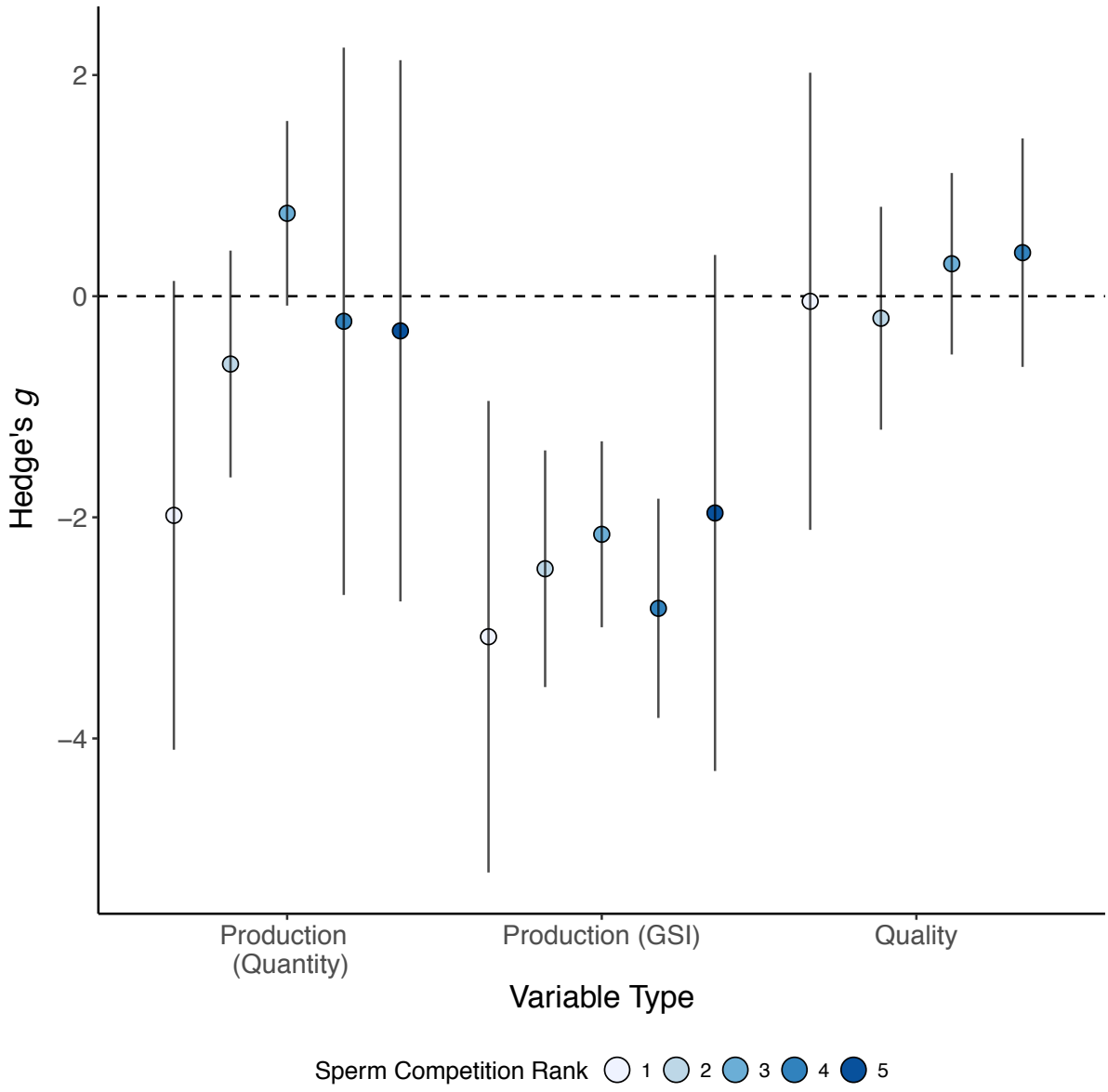

Figure 3. Standardized mean Hedge's g effect sizes ± 95% CIs of the differences between majors and minors in investment in traits related to sperm competition by the type of sperm investment under different sperm competition ranks. Dashed horizontal line represents Hedge's g equal to zero. See methods for more details.

We found little evidence of publication bias in our models, as indicated by the intercept terms of the Egger's regressions not being statistically different from zero (Table 3).

Table 3. Results of the Egger's regressions for the models used in this study.

Model	Moderators	Random effects	Intercept	*t*-value	p-value
Null Model	NA	study identity + species + phylogeny	-0.323	-0.633	0.528
Meta-regression model 1	variable type	study identity + species	-0.338	-0.664	0.507
Meta-regression model 2	variable type : sperm competition rank	study identity + species	-0.610	-1.067	0.288

Discussion

In this study, we investigated the hypothesis that, in fishes with male alternative mating tactics, minor males invest more in sperm traits related to sperm competition than major males. Overall, we found that minors invest more in sperm traits than majors, yet this overall result was not statistically significant. We then tested whether different categories of sperm traits modulated how majors and minors invest into dealing with sperm competition. Interestingly, we found that minors invest significantly more in relative gonad size than majors and, contrary to our general prediction, majors allocate significantly more sperm than minors during ejaculation bouts. We found that minors invested more than majors in “quantity” and “quality” traits, but the effect sizes were small, and the confidence intervals overlapped zero. We also tested the prediction that the level (intensity) of sperm competition would influence the investment in traits related to sperm competition; that is, the differences in sperm expenditure between majors and minors would be higher on intermediate levels and decrease as sperm competition became stronger. We found that the level of sperm competition had very little influence on the magnitude of the difference between majors and minors. Below, we discuss the findings of our

meta-analysis with regards to theoretical implications for the understanding of sperm competition.

Selection through sperm competition should favor larger testes because this can directly increase sperm quantity and, therefore, the likelihood of fertilization. Several studies that investigated the relationship between testes size and sperm competition in species with alternative tactics have found conflicting patterns (see, for example, Smith & Ryan, 2010; Simmons *et al*., 1999; Byrne, 2004; Kelly, 2008). Our study provides robust overall evidence that in fishes with male alternative tactics, minors make greater investment in relative gonad size. Interestingly, our meta-analytic approach allowed us to gain a more refined understanding of how sperm competition influences the investment in sperm production by the alternative male phenotypes. While minors make large investments into relative gonad size, we found the sperm quantity *per se* is similar between majors and minors. Combined, these two findings provide some important insights. First, finding evidence that majors and minors exhibit similar amount of sperm in their testes suggests that both tactics are experiencing similar levels of sperm competition risk per spawning event. Both tactics, according to these findings, have similar amount of sperm available at their disposal for a spawning event. Second, the larger investment into relative testes size by minors indicate that these males are able to replenish their sperm stocks faster than majors. This is likely a consequence of the different behavioral phenotypes exhibited by these tactics in order to obtain fertilizations. While majors possess similar number of sperm cells available, the probably copulate in lower frequencies than minors. Minors, probably need to replenish their sperm stocks much faster in order to try and sneak copulations with different females. Thus, it seems that sperm competition in fishes with male alternative tactics acts similarly in these phenotypes on the investment into sperm quantity but given the different routes to achieve fertilizations by majors and minors, gonad size is possibly a result of a combination of factors other than just sperm competition.

The percentage of paternity males have over offspring is related to the number of sperm males allocate to females (Martin *et al.*, 1974; Simmons, 1987; Wedell, 1991; Wedell and Cook, 1998). But there is a share of the resulting paternity that is not explained by the number of sperm males allocate (Snook, 2005; Parker and Pizzari, 2010). There are two main non-exclusive factors that can explain the share of paternity not directly related to the number of sperm. One of the factors is that females have mechanisms to favor specific sperm or sperm of specific males (i.e. cryptic female choice; Birkhead, 1998). For example, in Chinook salmon, a species with external fertilization, males that are less related to females sire more offspring when fertilization occurs in the presence of ovarian fluid than when it occurs in water (Lehnert

et al., 2017). The other factor that explains unexpected shares of paternity is the variance in sperm quality between males. For example, in Atlantic salmon, sperm quality was positively correlated to fertilization success (Vladić and Järvi, 2001). Our data show that the investment in sperm quality is not different between majors and minors, regardless of sperm competition levels. Therefore, sperm competition does not seem to affect sperm quality as strongly as it affects other types of investment, such as gonad mass. How traits related to sperm quality respond to sperm competition is still a less understood question (Snook, 2005). As discussed by Smith and Ryan (2010), the evolution of sperm quality traits can be dissociated from the evolution of traits that enhance the number of sperm. There is a theoretical prediction that some sperm quality traits, such as sperm size and longevity, should respond more to the risk sperm competition when there are constraints to the evolution of sperm numbers (Parker, 1993).

Our results indicate that majors allocate more sperm per spawning event that minors, despite the lack of difference in the sperm production in their gonads (see above). This result is the opposite of what is predicted by game theory models and counterintuitive when considering that majors theoretically face less sperm competition, and that there a large number of studies that support the increase of sperm allocation when the risk of sperm competition is experimentally increased (Gage and Barnard, 1996; Pizzari *et al.*, 2003; for a meta-analysis see: Kelly and Jennions, 2011). As our sample size is small (n = 11) for allocation traits, more data on this type of investment is needed so that a robust conclusion can be inferred. Therefore, we encourage researchers to conduct more experiments manipulating the number of sneakers and comparing sperm allocation between majors and minors.

One prediction from Parker's theoretical model of sperm competition between minors and majors is that, across species, males will invest more into sperm traits as the likelihood of sneak mating (Parker 1990b), or the frequency of minors in the population (Gage *et al*., 1995) increases. Our meta-regression analysis with fishes with alternative male phenotypes showed that investment in sperm traits was not influenced by increases in the level of sperm competition (as measured by the sperm competition rank). Our finding is, thus, not consistent with Parker's (1990b) prediction. Similar to our results, species of *Onthophagus* beetles that have a higher proportion of minors did not have greater difference of testes size between mating tactics (Simmons *et al*., 2007). It is possible that our results and Simmons *et al.* (2007) results occur due to sperm competition faced by majors being greater than expected. This means that the probability of females mating with more than one male is higher than expected. The similar expenditure in sperm numbers between majors and minors (Fig. 2) is evidence of majors facing similar sperm competition than minors. Parker's (1990b) model predicts a curvilinear

relationship between the increasing probability of sneak matings and expenditure in sperm traits. The model predicts that at moderate levels of sperm competition risk (in our case, a sperm competition rank of 3), the disparity between the investment of majors and minors should be at its maximum. Yet, even if we consider the tendency of the average effect size coefficients (not considering their 95% CIs) it seems that the sperm trait expenditure is behaving contrary to Parker's prediction. As Simmons *et al*. (2007) have stated, discrepancies between model predictions and empirical findings can be attributed to violations of the model's assumptions. In this sense it is worth noting, as Simmons *et al*. (2007) did, that Parker's (1990b) sneak-guard model assumes majors only face sperm competition from one minor male at a time. Most of the fish species included in our meta-analysis are external fertilizers. This natural history fact means that the sneak-guard model assumption is most likely violated in this case. Majors of these fish species will most likely have to deal with several minors trying to sneak matings with the female being courted. Therefore, it seems reasonable to expect that the risk of sperm competition, even at low levels (such as a sperm competition rank of 1), should be strong enough to cause the investment patterns we observe in this meta-analysis.

In conclusion, we have conducted the first meta-analysis testing Parker's (1990b) predictions about sperm competition in species with male alternative phenotypes. Using a sample of 29 fish species, we found that relative gonad size was larger in minors than in majors, supporting one of Parker's predictions. However, contrary to the theoretical predictions, we did not find support for the influence of the increase in sperm competition risk on the magnitude of the difference in investment by minors and majors. Taken together, our findings suggest that, in fishes with alternative mating tactics, both majors and minors are under strong selection from sperm competition, even when the risk of polyandry is low.

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*Represents references from which effect sizes were estimated.

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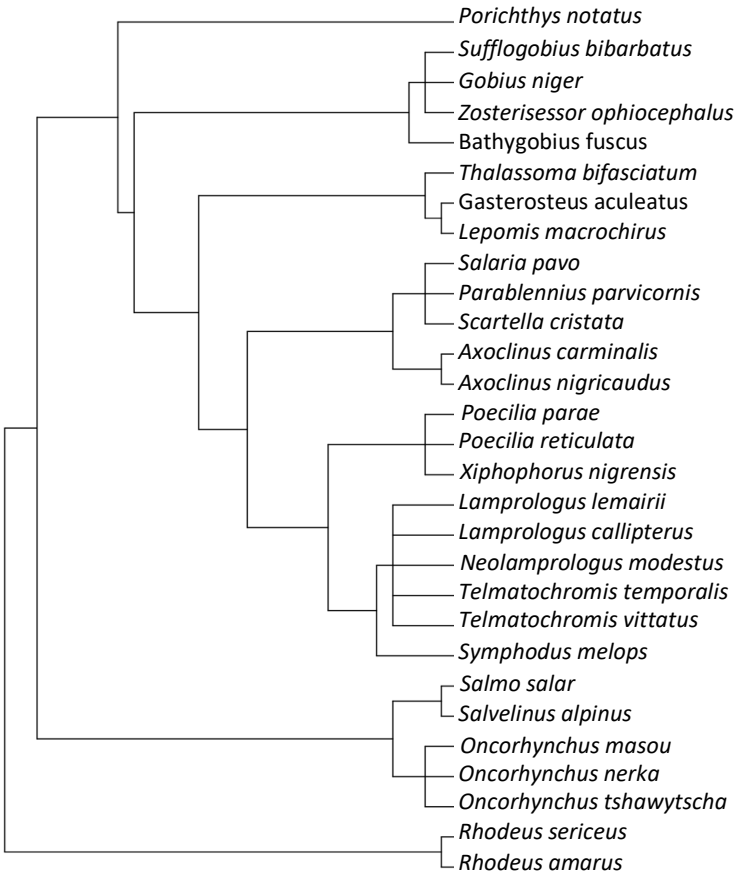
Supplementary Material

Supplementary Figure 1. Phylogeny of species included in this meta-analysis.



Supplementary Table 1. List of effect sizes used in this meta-analysis. *N* corresponds to sample sizes (number of males investigated) reported in the original articles, and *SCR* to the sperm competition rank.

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
Axoclinus carminalis	0.134	48	2	GSI	production	Neat (2001)
Axoclinus carminalis	1.396	24	2	% glandular tissue	production	Neat (2001)
Axoclinus nigricaudus	-0.440	45	5	GSI	production	Neat (2001)
Axoclinus nigricaudus	-0.841	49	5	GSI	production	Neat (2001)
Axoclinus nigricaudus	1.002	24	5	% glandular tissue	production	Neat (2001)
Bathygobius fuscus	0.037	71	3	testes mass (g)	production	Takegaki *et al*. (2012)
Gasterosteus aculeatus	1.254	75	3	gonad mass (g)	production	Côte *et al.* (2009)
Gasterosteus aculeatus	0.491	75	3	average path velocity (μm s^{-1})	quality	Côte *et al.* (2009)
Gasterosteus aculeatus	0.581	75	3	straight line velocity (μm s^{-1})	quality	Côte *et al.* (2009)
Gasterosteus aculeatus	-0.111	75	3	VCL (μm s^{-1})	quality	Côte *et al.* (2009)
Gasterosteus aculeatus	0.077	75	3	motile percentage (%)	quality	Côte *et al.* (2009)
Gasterosteus aculeatus	0.576	75	3	rapid percentage (%)	quality	Côte *et al.* (2009)
Gasterosteus aculeatus	0.293	75	3	estimated number of sperm (10^6 sperm)	production	Côte *et al.* (2009)
Gasterosteus aculeatus	-0.078	75	3	sperm concentration (10^6 sperm/g of gonad)	production	Côte *et al.* (2009)
Gasterosteus aculeatus	-1.198	75	3	loss of path velocity (μm/s^2)	quality	Côte *et al.* (2009)

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
Gasterosteus aculeatus	-0.769	75	3	PK activity in sperm (U/10^6 sperm)	quality	Côte *et al.* (2009)
Gasterosteus aculeatus	0.067	75	3	CS activity in sperm (U/10^6 sperm)	quality	Côte *et al.* (2009)
Gobius niger	0.405	21	1	sperm concentration (sperm per mL of water)	allocation	Pilastro *et al*. (2002)
Gobius niger	0.158	21	1	sperm concentration (sperm per mL of water)	allocation	Pilastro *et al*. (2002)
Gobius niger	0.922	21	1	sperm concentration (sperm per mL of water)	allocation	Pilastro *et al*. (2002)
Gobius niger	1.057	21	1	sperm concentration (sperm per mL of water)	allocation	Pilastro *et al*. (2002)
Gobius niger	-1.916	59	1	GSI	production	Rasotto & Mazzoldi (2002)
Gobius niger	1.335	59	1	SVSI	quality	Rasotto & Mazzoldi (2002)
Gobius niger	-1.501	59	1	sperm number	production	Rasotto & Mazzoldi (2002)
Gobius niger	-0.168	59	1	sperm density (per mm^2)	production	Rasotto & Mazzoldi (2002)
Gobius niger	-2.203	18	1	GSI	production	Scaggiante *et al.* (2006)
Gobius niger	0.206	18	1	SVSI	quality	Scaggiante *et al.* (2006)
Gobius niger	-0.322	26	1	proportion of live sperm postactivation	quality	Locatello *et al.* (2007)
Gobius niger	-1.366	24	1	average path velocity (µm s-1) postactivation	quality	Locatello *et al*. (2007)

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
Gobius niger	-1.303	24	1	VCL (μm s^{-1}) postactivation	quality	Locatello *et al*. (2007)
Gobius niger	-1.428	24	1	straight line velocity (μm s^{-1}) postactivation	quality	Locatello *et al*. (2007)
Gobius niger	-1.152	15	1	ATP concentration	quality	Locatello *et al*. (2007)
Lamprologus callipterus	4.708	161	4	gonad weight (mg)	production	Sato *et al.* (2004)
Lamprologus callipterus	-2.579	161	4	GSI (%)	production	Sato *et al*. (2004)
Lamprologus callipterus	-2.344	80	4	GSI (%)	production	Schültz *et al.* (2010)
Lamprologus lemairii	-0.182	23	2	testes mass (g)	production	Ota *et al.* (2014)
Lamprologus lemairii	-0.364	21	2	spermatocrit (%)	production	Ota *et al*. (2014)
Lamprologus lemairii	0.088	21	2	sperm flagellum length (μm)	quality	Ota *et al*. (2014)
Lamprologus lemairii	-0.444	23	2	sperm longevity (s)	quality	Ota *et al*. (2014)
Lamprologus lemairii	-0.336	22	2	sperm velocity after 10s (μm/s)	quality	Ota *et al*. (2014)
Lepomis macrochirus	0.399	22	2	sperm length (μm)	quality	Leach & Montgomerie (2000)
Lepomis macrochirus	-1.061	21	2	sperm concentration (milion sperm/μl)	production	Leach & Montgomerie (2000)
Lepomis macrochirus	1.376	21	2	number of sperm	production	Leach & Montgomerie (2000)
Lepomis macrochirus	2.591	15	2	sperm velocity 45s postactivation (μm/s)	quality	Leach & Montgomerie (2000)
Lepomis macrochirus	1.401	22	2	ejaculate volume (μl)	production	Leach & Montgomeri

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
						e (2000)
Lepomis macrochirus	3.650	114	2	gonad mass (g)	production	Neff *et al.* (2003)
Lepomis macrochirus	-2.482	114	2	GSI	production	Neff *et al.* (2003)
Lepomis macrochirus	-3.855	98	2	sperm density (per mm^3)	production	Neff *et al.* (2003)
Lepomis macrochirus	1.886	17	2	sperm longevity (s)	quality	Neff *et al.* (2003)
Lepomis macrochirus	-0.120	22	2	straightness after 5s	quality	Burness *et al.* (2004)
Lepomis macrochirus	-1.460	22	2	average path velocity (µm s^{-1}) after 5s	quality	Burness *et al.* (2004)
Lepomis macrochirus	-0.483	22	2	motility (%) after 5s	quality	Burness *et al.* (2004)
Lepomis macrochirus	-1.364	22	2	sperm tail length (µm)	quality	Burness *et al.* (2004)
Lepomis macrochirus	-1.023	22	2	ATP concentration	quality	Burness *et al.* (2004)
Lepomis macrochirus	54.723	25	2	sperm density (spermatozoa/µl milt)	production	Burness *et al.* (2005)
Lepomis macrochirus	-0.160	25	2	sperm average swimming speed 10s postactivation	quality	Burness *et al.* (2005)
Lepomis macrochirus	0.396	25	2	percentage sperm showing motility 10s postactivation	quality	Burness *et al.* (2005)
Lepomis macrochirus	-0.642	25	2	ATP levels 10s postactivation	quality	Burness *et al.* (2005)
Neolamprologus modestus	-1.030	33	3	gonad mass (mg)	production	Hellmann *et al.* (2015)
Neolamprologus modestus	-2.113	33	3	GSI (%)	production	Hellmann *et al.* (2015)
Oncorhynchus masou	1.569	182	3	GSI (energy based)	production	Koseki & Maekawa

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
						(2002)
Oncorhynchus masou	1.591	182	3	GSI	production	Koseki & Maekawa (2002)
Oncorhynchus masou	2.235	99	3	GSI (energy based)	production	Koseki & Maekawa (2002)
Oncorhynchus masou	2.287	99	3	GSI	production	Koseki & Maekawa (2002)
Oncorhynchus nerka	-2.000	40	3	sperm concentration	production	Hoysak & Liley (2001)
Oncorhynchus nerka	-1.700	9	3	motility (ranked proportion)	quality	Hoysak & Liley (2001)
Oncorhynchus nerka	0.511	9	3	duration of motility (s)	quality	Hoysak & Liley (2001)
Oncorhynchus tshawytscha	1.672	54	3	gonad mass (g)	production	Butts *et al.* (2012)
Oncorhynchus tshawytscha	-1.233	54	3	GSI	production	Butts *et al.* (2012)
Oncorhynchus tshawytscha	-0.858	54	3	average path velocity (μm s^{-1})	quality	Butts *et al.* (2012)
Oncorhynchus tshawytscha	-0.857	54	3	straight line velocity (μm s^{-1})	quality	Butts *et al.* (2012)
Oncorhynchus tshawytscha	-0.818	54	3	curvilinear velocity (μm s^{-1})	quality	Butts *et al.* (2012)
Oncorhynchus tshawytscha	-0.260	54	3	motility (% after 5s activation)	quality	Butts *et al.* (2012)
Oncorhynchus tshawytscha	-0.163	54	3	sperm density	production	Butts *et al.* (2012)
Oncorhynchus tshawytscha	1.798	64	3	testes mass (g)	production	Flannery *et al.* (2013)
Oncorhynchus tshawytscha	-1.203	64	3	GSI (%)	production	Flannery *et al.* (2013)

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
Oncorhynchus tshawytscha	-0.598	64	3	sperm velocity (µm/s) 5s postactivation	quality	Flannery *et al*. (2013)
Oncorhynchus tshawytscha	-0.353	64	3	sperm motility (%) 5s postactivation	quality	Flannery *et al*. (2013)
Oncorhynchus tshawytscha	-0.376	64	3	sperm longevity (s)	quality	Flannery *et al*. (2013)
Oncorhynchus tshawytscha	-0.297	64	3	sperm density (sperm/mL)	quality	Flannery *et al*. (2013)
Oncorhynchus tshawytscha	-0.251	30	3	sperm head length	quality	Flannery *et al*. (2013)
Oncorhynchus tshawytscha	-0.305	30	3	sperm head width	quality	Flannery *et al*. (2013)
Oncorhynchus tshawytscha	0.561	30	3	flagellum length	quality	Flannery *et al*. (2013)
Oncorhynchus tshawytscha	-0.275	36	3	ATP (nM ATP/10^9 sperm)	quality	Flannery *et al*. (2013)
Oncorhynchus tshawytscha	0.345	10	3	sperm count (percent packed sperm)	production	Young *et al.* (2013)
Oncorhynchus tshawytscha	-0.414	28	3	sperm velocity (µm/s)	quality	Lehnert *et al.* (2017)
Oncorhynchus tshawytscha	0.086	28	3	sperm velocity (µm/s)	quality	Lehnert *et al*. (2017)
Parablennius parvicornis	-1.543	20	4	GSI	production	Miranda *et al.* (2003)
Parablennius sanguinolentus parvicornis	-1.984	17	4	GSI (%)	production	Oliveira *et al.* (2001)
Parablennius sanguinolentus parvicornis	0.487	15	4	TGI (%)	quality	Oliveira *et al*. (2001)
Poecilia parae	-0.691	37	2	sperm count	production	Hurtado-Gonzales & Uy (2009)
Poecilia parae	-0.165	159	2	gonad mass (mg)	production	Hurtado-Gonzales &

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
						Uy (2009)
Poecilia parae	-0.054	37	2	sperm head length (µm)	quality	Hurtado-Gonzales & Uy (2009)
Poecilia parae	1.022	37	2	sperm midpiece length (µm)	quality	Hurtado-Gonzales & Uy (2009)
Poecilia parae	-1.381	37	2	sperm flagellar length (µm)	quality	Hurtado-Gonzales & Uy (2009)
Poecilia parae	-1.149	37	2	sperm total length (µm)	quality	Hurtado-Gonzales & Uy (2009)
Poecilia reticulata	-0.172	39	2	stripped sperm	production	Pilastro & Bisazza (1999)
Poecilia reticulata	7.943	39	2	proportion of sperm delivered (in relation to sperm at rest)	allocation	Pilastro & Bisazza (1999)
Porichthys notatus	0.357	35	3	absolute mass of testes	production	Fitzpatrick *et al.* (2016)
Porichthys notatus	1.000	35	3	absolute accessory gland mass	quality	Fitzpatrick *et al.* (2016)
Porichthys notatus	1.163	26	3	sperm head length (µm)	quality	Fitzpatrick *et al.* (2016)
Porichthys notatus	-0.835	26	3	sperm midpiece length (µm)	quality	Fitzpatrick *et al.* (2016)
Porichthys notatus	0.376	26	3	sperm flagella length (µm)	quality	Fitzpatrick *et al.* (2016)
Porichthys notatus	-0.044	26	3	sperm swimming speed (µm/s) 10s postactivation	quality	Fitzpatrick *et al.* (2016)
Porichthys notatus	-1.423	35	3	accessory gland corrected for body mass	quality	Fitzpatrick *et al.* (2016)
Porichthys notatus	-0.787	35	3	testes corrected for body mass	production	Fitzpatrick *et al.* (2016)

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
Rhodeus amarus	0.356	26	2	VCL: curvilinear velocity (μm s^{-1})	quality	Smith & Reichard (2013)
Rhodeus amarus	0.249	26	2	straight line velocity (μm s^{-1})	quality	Smith & Reichard (2013)
Rhodeus amarus	0.413	26	2	linearity (%)	quality	Smith & Reichard (2013)
Rhodeus amarus	-0.167	26	2	motility (%)	quality	Smith & Reichard (2013)
Rhodeus sericeus	-0.075	80	2	number of ejaculations per spawning	allocation	Reichard *et al*. (2004)
Salaria pavo	-2.997	45	3	GSI	production	Saraiva *et al*. (2010)
Salaria pavo	-3.619	60	3	GSI	production	Saraiva *et al*. (2010)
Salaria pavo	4.163	59	3	Relative testicular gland area	production	Saraiva *et al*. (2010)
Salaria pavo	1.785	58	3	Relative testicular gland area	production	Saraiva *et al*. (2010)
Salaria pavo	0.000	57	3	testes weight (g)	production	Fagundes *et al.* (2012)
Salaria pavo	0.364	57	3	testicular gland area (mm2)	quality	Fagundes *et al*. (2012)
Salmo salar	-2.222	23	3	GSI	production	Gage *et al*. (1995)
Salmo salar	14.018	77	3	stripped sperm volume (ml)	production	Gage *et al*. (1995)
Salmo salar	10.219	77	3	stripped sperm number	production	Gage *et al*. (1995)
Salmo salar	-48.947	77	3	relative sperm volume (ml kg^{-1})	production	Gage *et al*. (1995)
Salmo salar	-23.531	77	3	relative sperm number (kg^{-1})	production	Gage *et al*. (1995)

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
Salmo salar	-1.855	23	3	motility (%) (4-6h after stripping)	quality	Gage *et al*. (1995)
Salmo salar	-1.596	23	3	motility (%) (24-26h after stripping)	quality	Gage *et al*. (1995)
Salmo salar	-1.438	23	3	duration of motiliy (s) (4-6h after stripping)	quality	Gage *et al*. (1995)
Salmo salar	-2.005	23	3	duration of motiliy (s) (24-26h after stripping)	quality	Gage *et al*. (1995)
Salmo salar	-0.298	10	3	sperm head length (µm)	quality	Gage *et al*. (1995)
Salmo salar	-1.439	25	3	sperm flagellar length (µm)	quality	Gage *et al*. (1995)
Salmo salar	2.425	23	3	gonad mass	production	Gage *et al*. (1995)
Salmo salar	-0.371	24	3	sperm total length (µm)	quality	Gage *et al*. (1998)
Salmo salar	2.326	40	3	total gonad weight (g)	production	Vladić & Järvi (2001)
Salmo salar	-3.038	40	3	GSI (%)	production	Vladić & Järvi (2001)
Salmo salar	-1.952	40	3	spermatocrit	production	Vladić & Järvi (2001)
Salmo salar	-6.873	34	3	ATP (nmol ml^{-1})	quality	Vladić & Järvi (2001)
Salmo salar	-0.526	34	3	ATP residual spermatocrit	quality	Vladić & Järvi (2001)
Salmo salar	1.034	29	3	longevity (s)	quality	Vladić & Järvi (2001)
Salmo salar	-1.473	29	3	proportion of motile sperm after 10s	quality	Vladić & Järvi (2001)
Salmo salar	0.000	24	3	sperm velocity (mm s^{-1}) after 10s	quality	Vladić & Järvi (2001)

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
Salmo salar	0.433	14	3	sperm flagellum length (μm)	quality	Vladić *et al.* (2002)
Salmo salar	-0.242	14	3	sperm end piece length (μm)	quality	Vladić *et al.* (2002)
Salmo salar	-0.375	14	3	sperm midpiece length (μm)	quality	Vladić *et al.* (2002)
Salmo salar	-0.993	14	3	energy charge values	quality	Vladić *et al.* (2002)
Salmo salar	-2.242	14	3	spermatocrit	quality	Vladić *et al.* (2002)
Salmo salar	-0.354	14	3	sperm longevity (s)	quality	Vladić *et al.* (2002)
Salmo salar	2.320	22	3	stripped sperm mass (g)	production	Vladić *et al.* (2010)
Salmo salar	-1.798	22	3	spermatocrit	production	Vladić *et al.* (2010)
Salmo salar	-2.132	22	3	GSI	production	Vladić *et al.* (2010)
Salmo salar	-2.436	22	3	ATP (nmol ml^{-1})	quality	Vladić *et al.* (2010)
Salvelinus alpinus	0.125	39	4	testis mass (g)	production	Liljedal & Folstad (2003)
Salvelinus alpinus	0.297	40	4	milt mass (g)	production	Liljedal & Folstad (2003)
Salvelinus alpinus	-1.133	40	4	sperm density (%)	production	Liljedal & Folstad (2003)
Salvelinus alpinus	-0.387	40	4	sperm number	production	Liljedal & Folstad (2003)
Salvelinus alpinus	0.042	46	4	mean sperm velocity of the 10% fastest sperm (μm/s)	quality	(Serrano *et al.*, 2006)
Scartella cristata	-0.142	20	4	mass of testis (mg)	production	Neat *et al.* (2003)

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
Scartella cristata	-4.053	20	4	adjusted IG	production	Neat *et al.* (2003)
Sufflogobius bibarbatus	-3.156	116	3	GSI	production	Seivåg *et al.* (2016)
Sufflogobius bibarbatus	2.887	116	3	SVSI	quality	Seivåg *et al.* (2016)
Sufflogobius bibarbatus	-17.716	116	3	testes mass	production	Seivåg *et al.* (2016)
Sufflogobius bibarbatus	78.624	116	3	seminal vesicle mass	quality	Seivåg *et al.* (2016)
Symphodus melops	-8.088	165	2	GSI	production	Uglem *et al.* (2000)
Symphodus melops	-4.979	138	2	GSI	production	Uglem *et al.* (2000)
Symphodus melops	-4.766	49	2	GSI	production	Uglem *et al.* (2001)
Symphodus melops	-0.483	49	2	spermatrocrit level	production	Uglem *et al.* (2001)
Symphodus melops	-0.478	44	2	proportion of motile sperm at 1 min	quality	Uglem *et al.* (2001)
Symphodus melops	-0.897	49	2	gonad wet weight (g)	production	Uglem *et al.* (2001)
Symphodus melops	-1.147	40	2	gonad wet mass (g)	production	Uglem *et al.* (2002)
Symphodus melops	-4.510	40	2	GSI	production	Uglem *et al.* (2002)
Symphodus melops	0.100	37	2	sperm motility	quality	Uglem *et al.* (2002)
Telmatochromis temporalis	-5.231	30	4	GSI	production	Katoh *et al.* (2005)
Telmatochromis temporalis	0.130	30	4	gonad mass (g)	production	Katoh *et al.* (2005)
Telmatochromis vittatus	2.459	47	4	gonad mass (mg)	production	Ota & Kohda (2006)

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
Telmatochromis vittatus	-1.094	47	4	GSI	production	Ota & Kohda (2006)
Telmatochromis vittatus	-0.547	18	4	sperm swimming speed (μm s^{-1}) after 0.5s	quality	Fitzpatrick *et al.* (2007)
Telmatochromis vittatus	0.138	18	4	median sperm length (μm)	quality	Fitzpatrick *et al.* (2007)
Telmatochromis vittatus	0.096	18	4	sperm longevity (s)	quality	Fitzpatrick *et al.* (2007)
Telmatochromis vittatus	2.338	22	4	gonad mass (g)	production	Ota *et al.* (2010)
Telmatochromis vittatus	2.103	20	4	gonad mass (g)	production	Ota *et al.* (2010)
Telmatochromis vittatus	-0.258	21	4	sperm flagellar length (μm)	quality	Ota *et al.* (2010)
Telmatochromis vittatus	1.271	18	4	sperm flagellar length (μm)	quality	Ota *et al.* (2010)
Telmatochromis vittatus	-0.320	22	4	sperm longevity (s)	quality	Ota *et al.* (2010)
Telmatochromis vittatus	-1.712	20	4	sperm longevity (s)	quality	Ota *et al.* (2010)
Thalassoma bifasciatum	-2.034	60	3	sperm per spawn	allocation	Warner *et al.* (1995)
Thalassoma bifasciatum	-1.063	76	3	sperm concentration in the milt of spawn males (μl^{-1})	production	Schärer & Robertson (1999)
Thalassoma bifasciatum	0.205	76	3	average volumes of individual sperm cells produced by spawn males (μm^3)	production	Schärer & Robertson (1999)
Xiphophorus nigrensis	1.773	37	2	gonad mass (g)	production	Smith & Ryan (2010)
Xiphophorus nigrensis	-0.328	38	2	average path velocity (μs^{-1})	quality	Smith & Ryan (2010)
Xiphophorus nigrensis	-0.282	38	2	curvilinear velocity (μs^{-1})	quality	Smith & Ryan (2010)

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
Xiphophorus nigrensis	-0.595	38	2	straight line velocity (μs^{-1})	quality	Smith & Ryan (2010)
Xiphophorus nigrensis	0.070	40	2	sperm head shape (μm)	quality	Smith & Ryan (2010)
Xiphophorus nigrensis	-0.921	40	2	sperm midpiece length (μm)	quality	Smith & Ryan (2010)
Xiphophorus nigrensis	-0.087	40	2	sperm tail length (μm)	quality	Smith & Ryan (2010)
Xiphophorus nigrensis	-0.347	40	2	sperm total length (μm)	quality	Smith & Ryan (2010)
Xiphophorus nigrensis	-0.935	36	2	sperm viability	quality	Smith & Ryan (2010)
Xiphophorus nigrensis	-0.759	30	2	sperm longevity	quality	Smith & Ryan (2010)
Xiphophorus nigrensis	0.587	38	2	sperm count	production	Smith & Ryan (2010)
Zosterisessor ophiocephalus	0.284	36	4	sperm concentration (sperm per mL of water)	allocation	Pilastro *et al*. (2002)
Zosterisessor ophiocephalus	0.085	36	4	sperm concentration (sperm per mL of water)	allocation	Pilastro *et al*. (2002)
Zosterisessor ophiocephalus	0.418	36	4	sperm concentration (sperm per mL of water)	allocation	Pilastro *et al*. (2002)
Zosterisessor ophiocephalus	0.531	36	4	sperm concentration (sperm per mL of water)	allocation	Pilastro *et al*. (2002)
Zosterisessor ophiocephalus	-1.878	27	4	GSI	production	Scaggiante *et al.* (2006)
Zosterisessor ophiocephalus	2.708	27	4	SVSI	quality	Scaggiante *et al*. (2006)
Zosterisessor ophiocephalus	0.241	30	4	proportion of live sperm postactivation	quality	Locatello *et al.* (2007)
Zosterisessor ophiocephalus	0.000	28	4	average path velocity ($\mu m\ s^{-1}$) postactivation	quality	Locatello *et al*. (2007)

Species	*Hedges' g*	*N*	SCR	Original response variable	Variable type	Source
Zosterisessor ophiocephalus	0.044	28	4	curvilinear velocity (μm s^{-1}) postactivation	quality	Locatello *et al*. (2007)
Zosterisessor ophiocephalus	-0.130	28	4	straight line velocity (μm s^{-1}) postactivation	quality	Locatello *et al*. (2007)
Zosterisessor ophiocephalus	0.000	22	4	ATP concentration	quality	Locatello *et al*. (2007)
Zosterisessor ophiocephalus	-0.086	126	4	gonad mass (g)	production	Pujolar *et al.* (2012)