



**Male alternative reproductive tactics and sperm competition: A meta-analysis**

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4 **Male alternative reproductive tactics and sperm competition: a**  
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6 **meta-analysis**  
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45 7771).  
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49 1 ABSTRACT  
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51 2 In many animal species, males may exhibit one of several discrete, alternative ways of  
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53 3 obtaining fertilisations, known as alternative reproductive tactics (ARTs). Males exhibiting  
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55 4 ARTs typically differ in the extent to which they invest in traits that improve their mating  
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57 5 success, or the extent to which they face sperm competition. This has led to the widespread  
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3 6 prediction that males exhibiting ARTs associated with a high sperm competition risk, or  
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6 7 lower investment into traits that improve their competitiveness before mating, should invest  
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8 8 more heavily into traits that improve their competitiveness after mating, such as large  
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10 9 ejaculates and high-quality sperm. However, despite many studies investigating this question  
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12 10 since the 1990s, evidence for differences in sperm and ejaculate investment between male  
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14 11 ARTs is mixed, and there has been no quantitative summary of this field. Following a  
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16 12 systematic review of the literature, we performed a meta-analysis examining how testes size,  
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18 13 sperm number and sperm traits differ between males exhibiting ARTs that face either a high  
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20 14 or low sperm competition risk, or high or low investment in traits that increase mating  
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22 15 success. We obtained data from 92 studies and 67 species from across the animal kingdom.  
23  
24 16 Our analyses showed that male fish exhibiting ARTs facing a high sperm competition risk  
25  
26 17 had significantly larger testes (after controlling for body size) than those exhibiting tactics  
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28 18 facing a low sperm competition risk. However, this effect appears to be due to the  
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30 19 inappropriate use of the gonadosomatic index (GSI) as a body-size corrected measure of  
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32 20 testes investment, which overestimates the difference in testes investment between male  
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34 21 tactics in most cases. We found no significant difference in sperm number between males  
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36 22 exhibiting different ARTs, regardless of whether sperm were measured from the male sperm  
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38 23 stores or following ejaculation. We also found no significant difference in sperm traits  
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40 24 between males exhibiting different ARTs, with the exception of sperm ATP content in fish.  
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42 25 Finally, the difference in post-mating investment between male ARTs was not influenced by  
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44 26 the extent to which tactics were flexible, or by the frequency of sneakers in the population.  
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46 27 Overall, our results suggest that, despite clear theoretical predictions, there is little evidence  
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48 28 that male ARTs differ substantially in investment into sperm and ejaculates across species.  
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50 29 The incongruence between theoretical and empirical results could be explained if (a)  
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52 30 theoretical models fail to account for differences in overall resource levels between males  
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3 31 exhibiting different ARTs or fundamental trade-offs between investment into different  
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5 32 ejaculate and sperm traits, and (b) studies often use sperm or ejaculate traits that do not  
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8 33 reflect overall post-mating investment accurately or affect fertilisation success.  
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11  
12 35 *Key words:* alternative strategies, sperm competition, testes, spermatozoa, gonadosomatic  
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14 36 index, ejaculate allocation, sperm quality, sneaky mating, sperm velocity, sperm motility.  
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21 64 **I. INTRODUCTION**  
22

23  
24 65 **(1) Background**  
25

26 66 The plainfin midshipman *Porichthys notatus* is a species of toadfish native to the Eastern  
27 Pacific Ocean. Males alone care for the offspring; females deposit their eggs into nests built  
28 67 by males, who defend the eggs from predators and keep them oxygenated by fanning them  
29 68 with their fins (Brantley & Bass, 1994). Parental males spend a significant amount of time  
30 69 defending their nests from rival males, and court females by producing low-frequency hums  
31 70 (Brantley & Bass, 1994). However, not all males in the population pursue this parental tactic.  
32 71 A small proportion of males exhibit a ‘sneaking’ tactic (Brantley & Bass, 1994; Fitzpatrick *et al.*,  
33 72 2016). Sneaker males patrol the nests of parental males, waiting for new females to  
34 73 spawn there. At the exact moment of spawning, when both the female and parental male  
35 74 release their gametes into the nest, sneaker males attempt to ‘steal’ fertilisations by  
36 75 simultaneously releasing their sperm into the nest (Fitzpatrick *et al.*, 2016).  
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39

40 77 Plainfin midshipman males provide a striking example of alternative reproductive tactics  
41 78 (ARTs). ARTs are discrete tactics or strategies performed by individuals within a sex, usually  
42 79 males, to obtain fertilisations (Gross, 1996; Brockmann, 2001; Oliveira, Taborsky &  
43 80 Brockmann, 2008), which may also involve discontinuous variation in physiological and  
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3 81 morphological traits among individuals. For example, male ARTs often involve a dominant  
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5 82 morph that invests heavily into attracting females and competing to repel rivals, and a  
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7 83 sneaker morph that is much smaller and attempts to avoid such competition (Gross, 1996).  
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10 84 ARTs are predicted to arise for one of two reasons. First, males can often benefit from  
11  
12 85 avoiding the costs associated with sexual competition, or by parasitising the reproductive  
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14 86 efforts of other males (Taborsky, 1994). In such cases, ARTs persist because males exhibiting  
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16 87 different tactics have roughly equal fitness payoffs at equilibrium, with each tactic  
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18 88 maintained in the population through negative frequency-dependent selection (e.g. Gross,  
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20 89 1991; Shuster & Wade, 1991). There are, however, very few robust examples of tactics with  
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22 90 equal fitness being maintained by negative frequency-dependent selection (Gross, 1996;  
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24 91 Oliveira *et al.*, 2008). By contrast, there is strong evidence for a second explanation that  
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26 92 males may often be unable to breed in the conventional way, for example because they are  
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28 93 small or in poor condition and so are unlikely to outcompete rivals in a straight competition  
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30 94 (Gross, 1996). Here, males may employ ARTs because they are ‘making the best of a bad  
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32 95 job’ (Dawkins, 1980). In such cases, ARTs can persist in a population even if the fitness  
33  
34 96 payoffs of the different tactics are not equal. Indeed, a common source of variation in  
35  
36 97 competitive ability is age, especially in fishes which grow continuously throughout their life  
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38 98 (Taborsky, 2008). Here, males may sneak when they are young and small, and switch  
39  
40 99 strategies after they reach a threshold body size and become competitive (Oliveira *et al.*,  
41  
42 100 2008).  
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44  
45 101 Males exhibiting ARTs often face different levels of sperm competition. Sperm competition  
46  
47 102 is competition between the sperm from different males for access to a female’s eggs (Parker,  
48  
49 103 1970; Simmons, 2001). For species that exhibit external fertilisation (sperm and eggs meet  
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51 104 outside of the body), sperm competition occurs when multiple males spawn with a female at  
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53 105 the same time. For internally fertilising species (sperm and eggs meet inside the female  
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3 106 reproductive tract), sperm competition occurs when females mate with more than one male  
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5 107 before producing offspring. The externally fertilising plainfin midshipman males face  
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7 108 fundamentally differing risks of sperm competition (the proportion of fertilisation  
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10 109 opportunities in which they compete directly with a rival's sperm) depending on which ART  
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12 110 they adopt: whereas parental males only spawn with rivals in a minority of cases, sneaker  
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14 111 males *always* spawn in the presence of at least one parental male (Fitzpatrick *et al.*, 2016).  
15  
16 112 This asymmetry in the risk of sperm competition occurs in many species with sneak-mating  
17  
18 113 males (Parker, 1990*b*; Taborsky, 1998; Kustra & Alonzo, 2020) as well as species with other  
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20 114 types of alternative reproductive tactics (see Section II.2). For species without ARTs, game-  
21  
22 115 theoretical models predict that males should increase their investment into sperm production  
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24 116 and ejaculate size as the risk of sperm competition increases (Parker & Pizzari, 2010), and  
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26 117 this is well supported empirically (Gage & Baker, 1991; Kelly & Jennions, 2011; Lüpold *et*  
27  
28 118 *al.*, 2020). These observations have led to the prediction, supported by formal models, that  
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30 119 males exhibiting tactics that elevate the risk of sperm competition should invest more into  
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32 120 sperm production, and produce larger ejaculates (Parker, 1990*a,b*; Gage, Stockley & Parker,  
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34 121 1995; Ball & Parker, 2003; Parker & Pizzari, 2010). Male ARTs may also influence  
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36 122 investment into sperm production and ejaculates in two other important ways. First, an ART  
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38 123 might cause a male to occupy a non-favoured role, which will reduce his fertilisation success  
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40 124 for reasons other than the competitiveness of his ejaculate (Parker, 1990*a*). For example,  
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42 125 males in non-favoured roles may be forced to spawn at a greater distance from females, or  
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44 126 find that females discriminate against using their sperm (Parker, 1990*a*; Ball & Parker, 2003).  
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46 127 These males can benefit by increasing the competitiveness of their ejaculate to compensate  
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48 128 for this disadvantage. Second, males exhibiting ARTs often show reduced investment into  
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50 129 secondary sexual traits that are used in fighting for access to females, and/or during courtship  
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52 130 to attract females and persuade them to mate (Gross, 1996; Brockmann, 2001; Oliveira *et al.*,  
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3 131 2008). By forgoing such investment into pre-mating traits, males may free up resources that  
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5 132 can be invested into post-mating traits instead (Parker, Lessells & Simmons, 2013; Lüpold *et*  
6  
7 133 *al.*, 2014; Simmons, Lüpold & Fitzpatrick, 2017).

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10 134 Males can increase their post-mating competitiveness by producing more sperm at each  
11  
12 135 mating, in order to outnumber the sperm of their rivals (Parker, 1970; Simmons, 2001;  
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14 136 Simmons & Fitzpatrick, 2012). Increasing ejaculate size is especially beneficial when  
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16 137 fertilisation follows the principle of a 'fair raffle'. In such species, any given sperm has an  
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18 138 equal chance of fertilisation, so that the more sperm that a male ejaculates, the greater the  
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20 139 chance that one will reach an egg first (Parker & Pizzari, 2010). This principle applies to the  
21  
22 140 majority of externally fertilising aquatic species, because here sperm and eggs meet randomly  
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24 141 in the water column. In internally fertilising species, sperm may not have an equal chance of  
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26 142 fertilisation, because the positioning of the ejaculate within the female reproductive tract can  
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28 143 influence sperm uptake and utilisation (Simmons, 2001; Section II.2). However, in such cases  
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30 144 males may still benefit from producing large ejaculates if this enables them to displace sperm  
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32 145 from previous males (Parker & Simmons, 1991). A common metric used to infer investment  
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34 146 in sperm number is testes size: larger testes have more seminiferous tissue, and so produce  
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36 147 sperm at a greater rate (e.g. Ramm & Stockley, 2010). Indeed, there is good evidence that  
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38 148 males in species that face a greater level of sperm competition have relatively larger testes  
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40 149 (after controlling for body size), and that males that produce larger ejaculates tend to have  
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42 150 greater fertilisation success (Simmons, 2001; Kelly & Jennions, 2011; Simmons &  
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44 151 Fitzpatrick, 2012; Lüpold *et al.*, 2020).

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46 152 It is important to note that theoretical models of sperm competition typically distinguish  
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48 153 between sperm/ejaculate 'expenditure' *versus* 'allocation' (Parker & Pizzari, 2010). In this  
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50 154 context, sperm/ejaculate expenditure typically refers to long-term investment into sperm  
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52 155 production or sperm-producing organs (Parker, 2016). By contrast, sperm/ejaculate allocation  
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3 156 typically refers to investment into a single ejaculate. In other words, males produce sperm  
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5 157 (expenditure), which are then allocated to individual matings. This distinction is important,  
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7 158 because models suggest that optimal evolutionary strategies may differ for sperm expenditure  
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9 159 and allocation (Parker & Pizzari, 2010), and we expand on this point in Section I.2. However,  
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11 160 these terms may have different meanings in other fields; for example, the term ‘allocation’ is  
12  
13 161 often used in life-history theory (Van Noordwijk & de Jong, 1986). Therefore, in this review  
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15 162 we refer to specific traits whenever possible (e.g. investment into sperm production, ejaculate  
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17 163 size, or sperm traits) in order to avoid confusion.

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21 164 Males can also increase their post-mating competitiveness by producing sperm with high  
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23 165 fertilisation ability [i.e. high-‘quality’ sperm (Snook, 2005; Simmons & Fitzpatrick, 2012)].  
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25 166 Comparative studies typically find that species with higher levels of sperm competition  
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27 167 produce sperm that are longer and swim faster, and have ejaculates with a higher proportion  
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29 168 of viable sperm (Snook, 2005; Simmons & Fitzpatrick, 2012; Lüpold *et al.*, 2020). Within  
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31 169 species, sperm fertilisation ability has been shown to be influenced by sperm length (Lüpold  
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33 170 *et al.*, 2012; Bennison *et al.*, 2015), swimming speed (Birkhead *et al.*, 1999; Gage *et al.*,  
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35 171 2004), and viability (García-González & Simmons, 2005), but the direction of these effects is  
36  
37 172 inconsistent. For example, in some species longer sperm are better at fertilisation, whereas in  
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39 173 other species shorter sperm are better (Simmons & Fitzpatrick, 2012). Other traits that have  
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41 174 been suggested to affect fertilisation ability include sperm longevity (Snook, 2005), and ATP  
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43 175 content [ATP is produced by the mitochondria of sperm and provides the energy for sperm  
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45 176 motility (Werner & Simmons, 2008; Tourmente, Varea-Sánchez & Roldan, 2019)], with  
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47 177 high-quality sperm assumed to be motile for longer and with a higher ATP content. One  
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49 178 important point to note is that sperm traits are often significantly correlated with each other,  
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51 179 and are unlikely to evolve independently (Snook, 2005; Simmons & Fitzpatrick, 2012). These  
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3 180 correlations may partly explain the mixed results seen in intraspecific studies (see Section IV  
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5 181 for more discussion).

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8 182 Species with male ARTs may provide the best opportunity to examine intraspecific variation  
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10 183 in sperm and ejaculate investment, given the clear differences in post-mating competition  
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12 184 experienced by males using each tactic. Since this question was first investigated in the 1990s  
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14 185 (e.g. Jennings & Philipp, 1992; Stockley *et al.*, 1994; Gage *et al.*, 1995), a large number of  
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16 186 studies have compared differences in investment into sperm production and ejaculates  
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18 187 between ARTs. A recent narrative review summarising the findings of these studies  
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20 188 concluded that sneaker males have relatively larger testes (after controlling for body size) and  
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22 189 produce ejaculates with a higher density of sperm when compared to non-sneaker males, but  
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24 190 there was no clear relationship between ARTs and any morphological sperm traits (Kustra &  
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26 191 Alonzo, 2020). Importantly, these conclusions were based on counting the number of  
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28 192 significant and non-significant results reported from each study. An alternative approach is  
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30 193 formally to quantify the direction and magnitude of statistical effects using meta-analysis  
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32 194 (Arnqvist & Wooster, 1995; Koricheva, Gurevitch & Mengersen, 2013). This approach has  
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34 195 several benefits, including: (a) a focus on effect sizes rather than *P* values; (b) weighting of  
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36 196 studies based on their sample size; (c) formal methods to account for potential publication  
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38 197 bias in the literature; (d) the ability to test statistically for the effect of continuous or  
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40 198 categorical moderating factors; and (e) the ability to control for phylogenetic non-  
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42 199 independence (Koricheva *et al.*, 2013).

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## 50 51 201 **(2) Factors influencing the relationship between ARTs and sperm investment**

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53 202 The recent review by Kustra & Alonzo (2020) found that the relationship between ARTs and  
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55 203 investment into sperm production and ejaculates is variable across species, especially for  
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57 204 sperm traits. Part of this variation may be due to the action of moderating factors that have  
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3 205 not been investigated quantitatively. One of the strengths of meta-analysis is the ability  
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5 206 formally to test how potential moderators influence the differences between ARTs. In this  
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7 207 section, we review several factors that might affect the relationship between ARTs and sperm  
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9 208 investment.

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12 209 One important consideration is the extent to which ARTs are flexible (Kustra & Alonzo,  
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14 210 2020). The framework of Taborsky (1998) considers three main types of ART. First, fixed  
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16 211 tactics arise following distinct developmental trajectories, and are non-reversible at  
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18 212 adulthood. In this case, male expression of a tactic is based either on inherited genetic  
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20 213 differences (e.g. Lank *et al.*, 1995; Sandkam *et al.*, 2021), or conditions experienced during  
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22 214 early development. The major and minor morphs in dung beetles (Emlen, Hunt & Simmons,  
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24 215 2005a) and the alternative male morphs in salmonids (Gross, 1985) are examples of ARTs  
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26 216 that are fixed early in life. However, such fixed tactics are probably the exception rather than  
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28 217 the rule (Gross, 1996; Oliveira *et al.*, 2008). Second, and probably more commonly, state-  
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30 218 dependent (also known as sequential) tactics are conditional tactics which typically change  
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32 219 with an individual's age, body size or condition (Gross, 1996). Males may exhibit more than  
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34 220 one state-dependent tactic over their lifetime, but typically only switch once, and usually in  
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36 221 one direction (for example, from sneaking when young/small to guarding when old/large).  
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38 222 State-dependent tactics are common in fish, often because they grow continuously throughout  
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40 223 their life (Taborsky, 1998). Both fixed and state-dependent tactics are often associated with  
41  
42 224 distinct male morphs. Finally, plastic (or simultaneous) tactics are fully flexible, and their use  
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44 225 is typically unrelated to morphological differences. Males can switch tactics rapidly, and  
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46 226 usage is often based on the immediate social or environmental context. For example, poeciliid  
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48 227 males often show a mix of consensual matings where they court females, and non-consensual  
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50 228 matings where they attempt to force copulations (e.g. Hurtado-Gonzales & Uy, 2009; Smith  
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52 229 & Ryan, 2010). Fixed tactics show the least flexibility and the highest potential for  
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3 230 differential expenditure, and so are expected to show the greatest difference in sperm  
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5 231 production, ejaculate size or sperm traits between ARTs. State-dependent tactics have  
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8 232 moderate amounts of flexibility, but the potential for specialisation in sperm production (e.g.  
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10 233 testes size) may be limited by canalisation of gonadal traits early in life. However, state-  
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12 234 dependent tactics still allow for the possibility of differences in the allocation of sperm into  
13  
14 235 each ejaculate. Finally, the high flexibility of plastic tactics means the potential for shifts in  
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16 236 investment into some traits is unlikely, but still possible for others. Clearly, investment into  
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18 237 sperm production (either through changes in testes size or morphology) cannot be  
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20 238 significantly altered minute-to-minute. However, sperm traits such as motility or longevity  
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22 239 may show more potential for flexibility over minutes or hours, especially if these effects are  
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24 240 mediated by seminal fluid composition (e.g. Locatello, Poli & Rasotto, 2013; Poli, Locatello  
25  
26 241 & Rasotto, 2018), and ejaculate size can also be modulated depending on the context (Kelly  
27  
28 242 & Jennions 2011).

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31 243 Fertilisation mode could also influence investment into sperm production, ejaculate size, or  
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33 244 sperm traits, for several reasons (Fitzpatrick, 2020). First, sperm limitation may be more of a  
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35 245 problem for aquatic external fertilisers, because ejaculates can rapidly be diluted (Liao *et al.*,  
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37 246 2018). Therefore, external fertilisers may be more likely to increase investment into sperm  
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39 247 production, and produce larger ejaculates. Second, strong sperm precedence or cryptic female  
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41 248 choice in internal fertilisers can weaken the relationship between sperm number and  
42  
43 249 fertilisation success (Simmons, 2001), thus reducing the benefits of sneaking. Third, the  
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45 250 sperm of internal and external fertilisers encounter different environments, which may favour  
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47 251 different sperm traits. For example, faster, short-lived sperm may be more important for some  
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49 252 external fertilisers where sperm only need to survive for a short period, and dilution effects  
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51 253 and water flow are more important determinants of male fertilisation success (Liao *et al.*,  
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3 254 2018). By contrast, slower, longer-lived sperm may be more important in internal fertilisers  
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5 255 where sperm storage is more prevalent (Snook, 2005).  
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8 256 Theoretical models also highlight two important cases where ARTs should not lead to  
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10 257 differential post-mating investment, even when tactics differ in sperm competition risk. First,  
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12 258 evolutionarily stable strategy (ESS) models predict that males facing a greater risk of sperm  
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14 259 competition should increase their investment into sperm production (sperm expenditure), but  
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16 260 not ejaculate allocation (Parker & Ball, 2005; Parker & Pizzari, 2010). ESS models predict  
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18 261 that ejaculate allocation (i.e. ejaculate size) should be dynamically adjusted according to the  
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20 262 immediate social environment (Parker & Pizzari, 2010). As such, the number of rivals present  
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22 263 during a spawning is expected to be a stronger determinant of ejaculate allocation than a  
23  
24 264 male's ART (Parker *et al.*, 1996). This difference is not typically discussed in reviews of  
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26 265 ARTs and sperm competition, probably because few studies in this area consider the size of,  
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28 266 or number of sperm present in, single ejaculates (Section III.2). Another insight from game-  
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30 267 theoretical models is that the difference in post-mating investment between guarders and  
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32 268 sneakers should depend on the relative frequency of sneakers in the population (Parker,  
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34 269 1990*b*; Gage *et al.*, 1995). When sneakers are rare, guarders should expend very little on  
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36 270 sperm because they rarely face sperm competition, and sneakers should invest minimally  
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38 271 because of the low expenditure by guarders. However, when sneakers are as common as  
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40 272 guarders, or sneaking is involved in almost all guard matings, guarders will face as high a  
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42 273 sperm competition risk as sneakers, and males exhibiting both tactics are expected to invest  
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44 274 equally into sperm and ejaculates. These models lead to the prediction that the disparity in  
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46 275 post-mating investment between guarders and sneakers should be highest when the risk of  
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48 276 sneaking is at an intermediate level (Parker, 1990*b*; Gage *et al.*, 1995). However, empirical  
49  
50 277 support for this prediction is lacking: while a comparison of 16 dung beetle species showed  
51  
52 278 that species with a larger proportion of minor males had relatively larger testes (after  
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3 279 correcting for body size), the disparity in relative testes size between major and minor males  
4  
5 280 did not relate to minor male frequency (Simmons, Emlen & Tomkins, 2007).  
6  
7  
8 281 Finally, methodological issues can complicate measurement of the relationship between  
9  
10 282 ARTs and investment into sperm production, ejaculate size or sperm traits. For example,  
11  
12 283 testes size is often compared between ARTs using the proportion of body tissue accounted for  
13  
14 284 by the testes, especially in fishes. This measure is known as the gonadosomatic index (GSI),  
15  
16 285 and is calculated as  $100 \times (\text{testes mass}/\text{soma mass})$  (Devlaming, Grossman & Chapman,  
17  
18 286 1982). This metric has been criticised as inappropriate for comparing males exhibiting  
19  
20 287 different ARTs, because it only ‘controls’ for male body size when testes size scales  
21  
22 288 isometrically with body size (the slope of the relationship between testes size and body size is  
23  
24 289 exactly 1; Tomkins & Simmons, 2002). When the relationship between body size and testes  
25  
26 290 size is not isometric (either because the slope differs from 1, the intercept differs from 0, or  
27  
28 291 both), spurious results will be obtained. For example, a slope of less than 1 (negative  
29  
30 292 allometry) will result in smaller individuals having a higher GSI, independent of any  
31  
32 293 investment differences between male tactics (Simmons, Tomkins & Hunt, 1999; Tomkins &  
33  
34 294 Simmons, 2002). This approach is further problematic because it assumes that testes  
35  
36 295 allometry is the same for each male tactic, which is unlikely in species with clear  
37  
38 296 morphological differences between tactics (Tomkins & Simmons, 2002). For both of these  
39  
40 297 reasons, the use of GSI is likely to overestimate differences in investment into sperm  
41  
42 298 production between male tactics.  
43  
44  
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50

### 51 300 **(3) Meta-analysis overview**

52  
53 301 We systematically searched the literature for studies comparing sperm investment or sperm  
54  
55 302 traits between males of the same species exhibiting two or more ARTs that are expected to  
56  
57 303 differ in (a) sperm competition risk, or (b) the degree of investment into traits that increase  
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2  
3 304 mating success. Our searches resulted in three separate data sets, consisting of effect sizes  
4  
5 305 examining the relationship between male ARTs and: (a) testes size; (b) sperm quantity; and  
6  
7 306 (c) sperm traits. Notably, the sperm quantity data set included estimates representing both  
8  
9 307 sperm expenditure (the number of sperm present in dissected testes) and sperm allocation (the  
10  
11 308 number of sperm present in ejaculates). For each data set we performed a phylogenetically  
12  
13 309 controlled meta-analysis comparing males exhibiting tactics that face a high or a low sperm  
14  
15 310 competition risk, or have a high or low investment into secondary sexual traits that are used  
16  
17 311 in fighting for access to females, and/or during courtship to attract females and persuade them  
18  
19 312 to mate. We also use this framework to test quantitatively for factors moderating the strength  
20  
21 313 and direction of the relationship between sperm investment and ARTs. We have six main  
22  
23 314 predictions:

24  
25  
26 315 (1) Males exhibiting ARTs that elevate sperm competition risk, or who invest less into  
27  
28 316 traits that increase mating success, will invest more into sperm production, produce larger  
29  
30 317 ejaculates per mating, and produce more competitive sperm (sperm that are longer, swim  
31  
32 318 faster, stay motile for longer or have a higher ATP content) or ejaculates (containing a  
33  
34 319 high proportion of viable or motile sperm).

35  
36 320 (2) ARTs will differ in the average number of sperm present in the testes (sperm  
37  
38 321 expenditure) but not in the average number of sperm ejaculated (sperm allocation),  
39  
40 322 because the latter is likely to be more strongly influenced by the immediate social  
41  
42 323 environment.

43  
44 324 (3) The difference in investment into sperm production (sperm expenditure) between  
45  
46 325 ARTs will be greater for species in which male tactics are fixed for life than those in  
47  
48 326 which male tactics are sequentially or fully flexible.

49  
50 327 (4) The difference in sperm investment into sperm production, ejaculate size and sperm  
51  
52 328 traits between ARTs will be greater for external fertilisers than internal fertilisers because  
53  
54  
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1  
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3 329 fertilisation is likely less constrained by interactions between sperm and the female  
4  
5 330 reproductive tract.  
6  
7  
8 331 (5) The difference in investment into sperm production, ejaculate size and sperm traits  
9  
10 332 between ARTs will be negatively related to the proportion of sneakers in the population.  
11  
12 333 (6) The difference in testes size between ARTs will be greatest for studies measuring the  
13  
14 334 gonadosomatic index (GSI) than those using other metrics.  
15  
16  
17 335

## 19 336 **II. METHODS**

21 337 Throughout we follow the recent extension to the PRISMA reporting guidelines for ecology  
22  
23 338 and evolutionary biology by (O'Dea *et al.*, 2021). See the online Supporting Information,  
24  
25 339 Appendix S1, for a completed PRISMA checklist.  
26  
27  
28 340

### 31 341 **(1) Systematic searches**

32  
33 342 We focused our searches on published, peer-reviewed studies. We searched for published  
34  
35 343 papers in three ways. First, we searched the online database *Web of Science* for papers using a  
36  
37 344 variety of key words relating to ARTs and sperm investment. We searched all years and all  
38  
39 345 databases available in the *Web of Science* Core Collection. Nineteen separate searches were  
40  
41 346 performed, using the following terms:  
42  
43

- 44 347 (1) "alternative mating" AND (sperm\* OR ejaculat\*);
  - 46 348 (2) "alternative mating" AND (testes OR testis OR gonad\*);
  - 48 349 (3) "alternative reproductive" AND (sperm\* OR ejaculat\*);
  - 50 350 (4) "alternative reproductive" AND (testes OR testis OR gonad\*);
  - 52 351 (5) (sneak\* OR satellite\* OR helper OR guard\*) AND (sperm\* OR ejaculat\*);
  - 54 352 (6) (sneak\* OR satellite\* OR helper OR guard\*) AND (testes OR testis OR gonad\*);
  - 56 353 (7) (parr\* OR jack\*) AND (sperm\* OR ejaculat\*);
- 58  
59  
60



1  
2  
3 354 (8) (parr\* OR jack\*) AND (testes OR testis OR gonad\*);

4  
5 355 (9) sneak\* AND (sperm\* OR ejaculat\*);

6  
7 356 (10) guard\* AND (sperm\* OR ejaculat\*);

8  
9 357 (11) satellite\* AND (sperm\* OR ejaculat\*);

10  
11 358 (12) helper AND (sperm\* OR ejaculat\*);

12  
13 359 (13) parr\* AND (sperm\* OR ejaculat\*);

14  
15 360 (14) jack\* AND (sperm\* OR ejaculat\*);

16  
17 361 (15) sneak\* AND (testes OR testis OR gonad\*);

18  
19 362 (16) guard\* AND (testes OR testis OR gonad\*);

20  
21 363 (17) helper AND (testes OR testis OR gonad\*);

22  
23 364 (18) parr\* AND (testes OR testis OR gonad\*);

24  
25 365 (19) jack\* AND (testes OR testis OR gonad\*).

26  
27  
28  
29  
30 366 Second, we conducted reverse searches of papers citing nine influential articles in this area,  
31  
32  
33 367 again using *Web of Science*. We searched for papers citing Gage & Baker (1991), Gage *et al.*  
34  
35 368 (1995), Neff, Fu & Gross (2003), Parker (1990*b*), Parker *et al.* (2013), Simmons *et al.* (2007),  
36  
37 369 Simmons *et al.* (1999), Taborsky (1994) and Taborsky (1998). Third, we read all the papers  
38  
39  
40 370 identified in the recent narrative review of male ARTs and sperm competition (Kustra &  
41  
42 371 Alonzo, 2020). We also obtained one data set prior to publication (Loveland, Lank & Küpper,  
43  
44 372 2021) after contacting the authors regarding another paper.

45  
46  
47 373 Searches were performed in two stages. Initially we conducted key word searches on  
48  
49 374 07/12/2018 and reverse searches on 15/01/2019. In the second stage, both key word and  
50  
51 375 reverse searches were conducted on 22/10/2020, in order to cover 2019 and 2020 (only six  
52  
53 376 new papers were found in the second stage). All searches during the first stage were  
54  
55 377 performed by M.J.A.S., and in the second stage by L.R.D. The results of the searches, plus  
56  
57  
58 378 the screening process, are outlined in Fig. S1. In total, our literature searches identified 3861  
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60

1  
2  
3 379 studies. Search results were imported into the web application Rayyan (Ouzzani *et al.*, 2016),  
4  
5 380 and the titles and abstracts screened for eligibility. Title and abstract screening identified 263  
6  
7  
8 381 potentially eligible studies, which were then downloaded and read in their entirety.  
9

10 382

## 11 12 383 **(2) Study inclusion criteria**

13  
14 384 To be considered eligible for inclusion, a study had to compare sperm traits between males of  
15  
16  
17 385 the same species exhibiting discrete ARTs. We did not consider female ARTs. To be  
18  
19 386 considered an ART, males had to show discrete reproductive tactics or morphs (e.g. a  
20  
21 387 bimodal distribution in body size), or exhibit behaviours that could be assigned to mutually  
22  
23 388 exclusive categories (e.g. consensual *versus* coercive mating). We excluded studies relating  
24  
25  
26 389 sperm traits to continuous variation in any male phenotype (e.g. body size, ornament/weapon  
27  
28 390 size). We also excluded studies of species where subordinates are reproductively suppressed  
29  
30 391 by dominants (e.g. Fitzpatrick *et al.*, 2006; Kustan, Maruska & Fernald, 2012), and studies of  
31  
32  
33 392 sequential hermaphrodites.

34  
35 393 We included three types of ARTs, based on the categorisation by Taborsky (1998):

36  
37 394 (1) Fixed tactics. Tactics were assigned to this category if they have distinct  
38  
39 395 developmental trajectories, and are non-reversible at adulthood.

40  
41 396 (2) Sequential (state-dependent) tactics. Tactics were assigned to this category if their  
42  
43 397 expression is conditional on any aspect of individual state, such as age, body size or  
44  
45 398 condition. Tactics were also assigned to this category if they are associated with clear  
46  
47 399 morphological differences, but cannot be linked either to genetic differences or distinct  
48  
49 400 developmental trajectories between male morphs.

50  
51 401 (3) Flexible tactics. Tactics were assigned to this category if they are fully reversible, and  
52  
53  
54 402 not associated with alternative morphologies.  
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3 403 We focused only on ARTs that could potentially influence male sperm competition risk, or  
4  
5 404 that differed clearly in investment into traits that increase mating success. The actual risk of  
6  
7 405 sperm competition is rarely quantified for either male tactic, so we primarily relied on  
8  
9 406 behavioural observations or assertions made by the study authors. We excluded species with  
10  
11 407 ARTs that are unlikely to differ in sperm competition risk, such as the burrowing bee  
12  
13 408 *Amegilla dawsoni* for which observational and genetic data suggest that females only ever  
14  
15 409 mate once (Simmons, Tomkins & Alcock, 2000). We collected data for 18 types of ARTs  
16  
17 410 (Table 1).

18  
19  
20  
21 411 We considered three categories of post-mating traits.

22  
23 412 (1) *Testes size*. We included studies estimating both the mass and volume of the sperm-  
24  
25 413 producing organs, as a proxy for investment into sperm production. Ideally, we only  
26  
27 414 included data on relative testes size, after controlling for body size. However, we also  
28  
29 415 used absolute testes size as a metric when there was no significant difference in body size  
30  
31 416 between male tactics (Stockley *et al.*, 1994; Peer, Robertson & Kempnaers, 2000;  
32  
33 417 Olsson *et al.*, 2009). Studies controlled for body size using (a) the GSI, (b) the residuals  
34  
35 418 of a regression of body size against testes size (e.g. Simmons *et al.*, 2007), or (c) analysis  
36  
37 419 of covariance (Tomkins & Simmons, 2002). Most studies used body mass as a measure of  
38  
39 420 body size, although we also included studies in insects using pronotum or leg length as a  
40  
41 421 proxy for body size (Kelly, 2008; Rosa *et al.*, 2019).

42  
43 422 (2) *Sperm quantity*. We included data on the number of sperm cells present in the  
44  
45 423 ejaculate or packaged into a spermatophore (sperm allocation), or present in the testes  
46  
47 424 after stripping of live males or dissection of dead males (sperm expenditure). Ejaculates  
48  
49 425 were stripped from live males either by applying gentle pressure to the abdomen or testes,  
50  
51 426 or by electrostimulation (e.g. Sasson, Johnson & Brockmann, 2015; Meniri *et al.*, 2019).  
52  
53 427 After collection of the ejaculate, sperm quantity was estimated by counting the number or  
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3 428 density of sperm cells in a given volume of ejaculate, calculating the volume of the  
4  
5 429 ejaculate (e.g. Simmons *et al.*, 1999), or measuring the length of the spermatophore  
6  
7  
8 430 (Apostólico & Marian, 2017).

9  
10 431 (3) *Sperm traits*. We collected sperm traits (morphology, physiology, or behaviour) which  
11  
12 432 are purported to relate to sperm competitiveness. In all but two cases (Simmons *et al.*,  
13  
14 433 1999; Apostólico & Marian, 2018) sperm traits were measured using sperm that had not  
15  
16  
17 434 been ejaculated or packaged into a spermatophore.

18  
19 435 (a) Average sperm length. All identified studies focused on flagellate sperm,  
20  
21 436 which swim using a 'tail', or 'flagellum'. The flagellum is usually the longest  
22  
23 437 component of the sperm cell, so in all cases we used data on either total cell length  
24  
25  
26 438 or flagellum length. When multiple components were reported, we used flagellum  
27  
28 439 length only.

29  
30 440 (b) Average sperm swimming speed. Speed is estimated using either manual or  
31  
32 441 automated [computer-assisted sperm analysis (CASA)] video analysis. There are  
33  
34 442 multiple ways to estimate swimming speed provided by common video analysis  
35  
36 443 packages (Sloter *et al.*, 2006), with the most common being curvilinear velocity  
37  
38 444 ( $V_{CL}$ , the velocity across the track taken by the cell between each frame). Other  
39  
40 445 measures include linear velocity ( $V_{SL}$ : velocity in a straight line between the first  
41  
42 446 and last frame), and average path velocity ( $V_{AP}$ ; a smoothed version of  $V_{CL}$ ).

43  
44  
45 447 These measures are usually highly correlated within studies. One study also used  
46  
47 448 flagellum beat frequency to calculate swimming speed (Butts *et al.*, 2017). When  
48  
49 449 multiple speed estimates were available, we used  $V_{CL}$ .

50  
51 450 (c) Sperm longevity. Studies measured sperm longevity as either: (i) the time until  
52  
53 451 all (or a high proportion of) sperm stopped moving forward; (ii), the time when  
54  
55 452 the average swimming speed of sperm fell below some defined value (Taborsky *et*  
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1  
2  
3 453 *al.*, 2018); (iii) the proportion of sperm still swimming after a defined duration  
4  
5 454 (e.g. Hettyey & Roberts, 2005, 2007); or (iv) the slope in the decline in sperm  
6  
7  
8 455 motility over time (e.g. Fasel *et al.*, 2017).

9  
10 456 (d) Sperm ATP content. ATP content is estimated by measuring the amount of  
11  
12 457 light produced by the bioluminescent luciferin–luciferase reaction, which only  
13  
14  
15 458 occurs in the presence of ATP (Lundin, 2000).

16  
17 459 (e) The proportion of sperm in the ejaculate that are motile or alive. Motile sperm  
18  
19 460 are those that show some degree of forward movement, and viable sperm are  
20  
21 461 determined using a range of methods which differentially stain alive *versus* dead  
22  
23 462 sperm (Holman, 2009). Given that relatively few studies measured sperm viability  
24  
25 463 (Locatello *et al.*, 2007; Smith & Ryan, 2010; Rowe *et al.*, 2010; Smith, 2012;  
26  
27 464 Schrepf *et al.*, 2016; Green *et al.*, 2020), we combined these two measures into a  
28  
29  
30  
31 465 single category.

32  
33 466 We excluded studies presenting other reproductive traits that do not relate directly to sperm  
34  
35 467 investment, such as spermatophore morphology (e.g. Iwata, Sakurai & Shaw, 2015) or male  
36  
37 468 internal reproductive anatomy (e.g. accessory gland size: Barni, Mazzoldi & Rasotto, 2001).  
38  
39 469 We also excluded estimates of the fertilisation success of different male ARTs (e.g. Carroll,  
40  
41 470 1993; Adreani, 2012).

42  
43  
44 471 Finally, to be included in the data set a study had to present sufficient data (including sample  
45  
46 472 sizes for each male tactic) for an effect size and its variance to be calculated (Section II.3).

47  
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49 473

### 50 51 474 **(3) Effect size calculations**

52  
53 475 We used the standardised mean difference, also known as Hedges' *d*, as our measure of effect  
54  
55 476 size (Hedges & Olkin, 1985). This is very commonly used as an effect size when the aim is to  
56  
57  
58 477 compare average values between two groups (Nakagawa & Santos, 2012), and is especially  
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2  
3 478 appropriate when the two groups come from observational data (i.e. there are no control and  
4  
5 479 treatment groups). We assigned effect sizes a positive direction when investment into sperm  
6  
7 480 traits was higher for males exhibiting tactics associated with a greater sperm competition risk  
8  
9 481 or a reduced investment into pre-mating sexual traits (Table 1). The latter condition was  
10  
11 482 relevant for males that engage in coercive matings (Pilastro & Bisazza, 1999; Hurtado-  
12  
13 483 Gonzales & Uy, 2009; Smith & Ryan, 2010; Smith, 2012) and males that exhibit female-  
14  
15 484 mimicking plumage (Loveland *et al.*, 2021), which either have reduced sexual ornaments or  
16  
17 485 do not court females. Following the sperm competition literature, we assumed that higher  
18  
19 486 investment into post-mating traits should result in larger testes, more sperm in the testes,  
20  
21 487 more sperm in the ejaculate, a higher proportion of motile sperm in the ejaculate, and sperm  
22  
23 488 that are longer, swim faster, stay motile for longer or have a higher ATP content. We note  
24  
25 489 that there may be functional or resource-allocation trade-offs among sperm traits. For  
26  
27 490 example, studies have recorded a negative within-species correlation between sperm  
28  
29 491 swimming speed and sperm longevity (Levitan, 2000; Yamamoto *et al.*, 2017; Taborsky *et*  
30  
31 492 *al.*, 2018), and between sperm length and sperm longevity (Gage *et al.*, 2002). However, such  
32  
33 493 trade-offs are far from universal (Snook, 2005), and the traits that are important for male  
34  
35 494 fertilisation success differ across species (Simmons & Fitzpatrick, 2012). For both of these  
36  
37 495 reasons we did not attempt to model trade-offs directly; rather we assumed that all sperm  
38  
39 496 traits could potentially differ between ARTs. However, we also test for widespread trade-offs  
40  
41 497 in the analysis, by comparing the average effect size for each sperm trait separately.  
42  
43 498 We obtained effect sizes from papers in one of three ways. First, we calculated the  
44  
45 499 standardised mean difference directly from reported means and variances (standard deviation  
46  
47 500 or standard error), using the equations in Koricheva *et al.* (2013; p. 200). These data were  
48  
49 501 either taken directly from values reported in the text or tables, or extracted manually from bar  
50  
51 502 plots using the online tool WebPlotDigitizer v4 (Rohatgi, 2019). Second, we converted the  
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3 503 results of appropriate statistical tests into the standardised mean difference using the  
4  
5 504 conversion equations in Koricheva *et al.* (2013, pp. 200–201). We used results from *t*-tests,  
6  
7 505 paired *t*-tests, and Mann-Whitney *U* tests. Finally, we performed supplementary analyses  
8  
9 506 when we had access to the raw data. Raw data were either obtained from available online  
10  
11 507 supplementary material, extracted manually from scatter plots using WebPlotDigitizer, or  
12  
13 508 obtained by contacting the study authors (we received data from five studies in this way). In  
14  
15 509 species with more than two ARTs, we performed multiple pairwise comparisons. Full  
16  
17 510 information regarding effect size calculations is provided in Table S1. In cases where sperm  
18  
19 511 traits (e.g. motility) were measured at multiple time points, we only considered the first time  
20  
21 512 point. We extracted all available effect sizes from a study. This often resulted in multiple  
22  
23 513 effect sizes per study, especially when studies reported multiple sperm traits from the same  
24  
25 514 sample of individuals, which we controlled for statistically (Section II.6). All data extraction  
26  
27 515 was performed by L.R.D.

28  
29 516 Testes size is often compared between ARTs using the proportion of body tissue accounted  
30  
31 517 for by the testes, especially in fishes. This measure is known as the gonadosomatic index  
32  
33 518 (GSI). This metric has been criticised as not accounting fully for body size (see Section I.2).  
34  
35 519 Therefore, whenever possible we re-analysed raw data on testes mass using the analysis of  
36  
37 520 covariance (ANCOVA) method suggested by Tomkins & Simmons (2002). For this method,  
38  
39 521 we performed an ANCOVA with testes mass as the dependent variable, male tactic as the  
40  
41 522 independent variable, and soma mass (body mass – testes mass) as a covariate. If body mass  
42  
43 523 was measured before testes were dissected, we calculated soma mass manually. For the  
44  
45 524 ANCOVA, we first ran a full model testing the effect of soma mass, male tactic, and their  
46  
47 525 interaction, on testes mass. If the interaction term was not significant, this suggests that testes  
48  
49 526 allometry does not differ between the male tactics. This was the case in 39 out of 44 analyses.  
50  
51 527 When the interaction term was not significant, we dropped it from the model, and calculated  
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3 528 partial eta-squared for the fixed effect of male tactics using the EtaSq function in the R  
4  
5 529 package DescTools. Partial eta-squared was then converted to Cohen's  $d$  using the equation  
6  
7 530 in Cohen (1988, p. 284), and Cohen's  $d$  was converted into Hedges'  $d$  using the equation in  
8  
9 531 Borenstein *et al.* (2009). We used this ANCOVA approach on approximately half of the  
10  
11 532 studies reporting GSI (34 of 64 effect sizes).  
12  
13  
14 533 Studies sometimes reported non-significant results without providing information about the  
15  
16 534 direction of the effect. These effect sizes are traditionally excluded from meta-analysis;  
17  
18 535 however, this systematically biases the data set against non-significant results. Therefore, we  
19  
20 536 assigned relevant directionless effect sizes a value of zero (15 effect sizes: one testes size  
21  
22 537 trait, four sperm quantity traits, 10 sperm traits), and ran the analyses with and without  
23  
24 538 including these extra data points as a form of sensitivity analysis (Harts, Booksmythe &  
25  
26 539 Jennions, 2016; Booksmythe *et al.*, 2017; Dougherty, 2021).  
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#### 33 541 **(4) Phylogeny**

34  
35 542 We estimated the phylogenetic relationships among the species in our data set in order to  
36  
37 543 control for the potential non-independence of effect sizes due to shared evolutionary history  
38  
39 544 (Hadfield & Nakagawa, 2010; Koricheva *et al.*, 2013). As no single phylogenetic tree was  
40  
41 545 available that included all species, we constructed a supertree from available phylogenetic  
42  
43 546 and taxonomic information using the Open Tree of Life (OTL) database (Hinchliff *et al.*,  
44  
45 547 2015), and the rotl (Michonneau, Brown & Winter, 2016) and ape (Paradis, Claude &  
46  
47 548 Strimmer, 2004) R packages. We also manually searched for phylogenetic information for  
48  
49 549 species or taxa not listed in the OTL database. For the position of Opilliones in relation to  
50  
51 550 arthropods, we used Giribet, Edgecombe & Wheeler (2001). The relationships among the 15  
52  
53 551 *Onthophagus* species was found in Emlen *et al.* (2005b). We were unable to find information  
54  
55 552 about the phylogenetic position of two species: *Onthophagus nodulifer* and *O. rupicapra*.  
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57  
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3 553 Based on the geographic distribution of these species, and the tree in Emlen *et al.* (2005b),  
4  
5 554 we added both species as a polytomy at the base of the Australian *Onthophagus* clade. These  
6  
7 555 two species were only present in the testes size data set. We therefore tested the sensitivity of  
8  
9 556 the overall meta-analytic mean estimate by running this model with and without the inclusion  
10  
11 557 of these two species. As the supertree lacks accurate branch lengths, lengths were first set to 1  
12  
13 558 and then made ultrametric using Grafen's method (Grafen, 1989). The tree was then  
14  
15 559 converted into a variance–covariance matrix for incorporation into the meta-analysis models.  
16  
17 560 For analyses including subsets of the data, we used an appropriately pruned tree (Figs S2–  
18  
19 561 S4).  
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21  
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## 26 563 **(5) Moderator variables**

27  
28 564 For each study, we collected data on a range of moderator variables predicted to influence the  
29  
30 565 mean effect size (see Section I.2 for discussion):  
31  
32

33 566 (1) *Taxonomic group*. We obtained data from nine taxonomic groups: cephalopods,  
34  
35 567 chelicerates, arachnids, insects, fish, amphibians, reptiles, birds, and mammals. However,  
36  
37 568 over 70% of effect sizes came from fish (182 out of 251), and most of the remaining  
38  
39 569 groups contained few examples. Therefore, to increase our statistical power, we sorted  
40  
41 570 species into three categories: invertebrates (arachnids, cephalopods, chelicerates, and  
42  
43 571 insects), fish, and other vertebrates (amphibians, birds, mammals, and reptiles). We had  
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45 572 no directional prediction based on this categorisation.  
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49 573 (2) *Mode of fertilisation*. We obtained data for both externally and internally fertilising  
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51 574 species. We predicted that the difference in sperm traits would be greatest for externally  
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53 575 fertilising species, primarily because strong sperm precedence or cryptic female choice in  
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55 576 internal fertilisers might weaken the relationship between sperm number and fertilisation  
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57 577 success, thus reducing the benefits of sneaking.  
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3 578 (3) *Tactic type*. We classified ARTs into one of three categories: fixed, state-dependent,  
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5 579 or plastic. However, for all three data sets we obtained very few estimates for plastic  
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7 580 tactics (1–10 effect sizes per data set). Therefore, for two of the data sets (testes size and  
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9 581 sperm quantity) we only compared fixed and state-dependent categories (five effect sizes  
10  
11 582 removed in total). We predicted that the difference in post-mating investment would be  
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13 583 greatest for species with fixed ARTs, because fixed tactics are set early in life and so  
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15 584 show the highest potential for differences in post-mating investment.

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19 585 (4) *Measurement*. For the testes and sperm quantity data sets, we tested whether the mean  
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21 586 effect size differed depending on the measurement method used. For testes size, we  
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23 587 compared estimates obtained using the GSI and relative testes size (controlling for body  
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25 588 size; we excluded three effect sizes derived from absolute testes size for this comparison).  
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27 589 We predicted that studies using the GSI would result in a larger effect size than those  
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29 590 using other measures of testes investment, because this method inadequately controls for  
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31 591 testes allometry and could lead to a spurious difference between alternative male tactics.  
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33 592 For sperm quantity, we compared measures of sperm number, sperm volume and sperm  
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35 593 density (we excluded a single study measuring spermatophore size from this comparison).  
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37 594 We had no directional prediction for this category.

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42 595 (5) *Sperm trait*. For the sperm traits data set, we compared measures of sperm length,  
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44 596 sperm swimming speed, sperm longevity, sperm ATP content, and the proportion of  
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46 597 motile sperm in the ejaculate. While some studies have suggested the presence of trade-  
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48 598 offs between different sperm traits (e.g. between swimming speed and longevity: Levitan,  
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50 599 2000), such trade-offs are not ubiquitous (Snook, 2005), and there is evidence that all of  
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52 600 the traits may positively influence fertilisation success (Snook, 2005; Simmons &  
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54 601 Fitzpatrick, 2012). Therefore, we had no clear directional prediction for whether some  
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56 602 sperm traits would differ more strongly between ARTs than others.  
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3 603 (6) *Sneaker frequency*. We searched for published estimates of the frequency of sneaker  
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5 604 males for species showing fixed or state-dependent tactics (the frequency of sneakers is  
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7 605 not relevant for species exhibiting fully flexible tactics). We excluded estimates when  
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9 606 sampling was not random with respect to male tactic. Ideally, we used demographic data  
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11 607 from the same experimental population as the effect size. When this was unavailable, we  
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13 608 used estimates taken from the same population, location or species (listed in order of  
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15 609 priority). The sources for these data are listed in Table S2. We obtained data on sneaker  
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17 610 frequency for 54 of the 67 species in our data set (Fig. S5). Following the models by  
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19 611 Parker (1990*b*) and Gage *et al.* (1995), we predicted that the difference in post-mating  
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21 612 investment between sneaker and non-sneaker males would be greatest when the  
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23 613 proportion of sneakers in the population was intermediate. This is because males  
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25 614 exhibiting both tactics are expected to invest little into sperm traits when the risk of sperm  
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27 615 competition is very low (when there are few sneakers), and to invest highly when the risk  
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29 616 of sperm competition is high (when there are many sneakers). In other words, we predict  
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31 617 the average effect size to be significantly positive at intermediate sneaker frequency, and  
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33 618 close to zero when the proportion of sneakers in the population is very high or very low.  
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40 619 (7) *Sperm allocation versus expenditure*. For the sperm quantity data set, we compared  
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42 620 estimates obtained from sperm in the ejaculate or packaged into a spermatophore (sperm  
43  
44 621 allocation), or in the testes after stripping from live males or dissection of dead males  
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46 622 (sperm expenditure). We predicted that sperm expenditure would be significantly greater  
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48 623 for sneaker males (a significantly positive effect size), but that ARTs would not differ in  
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50 624 terms of sperm allocation (effect size does not differ from zero) as this is more strongly  
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52 625 influenced by the immediate social environment during mating (see Section IV.3).  
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## 627 (6) Statistical analysis

628 Our systematic searches resulted in three data sets (all data and code used in the analysis are  
629 available at 10.6084/m9.figshare.19174604), focusing on: (a) testes size; (b) sperm quantity;  
630 and (c) sperm traits, which we analysed separately using R v4.0.3 (R Development Core  
631 Team, 2020) and the Metafor package v2.4 (Viechtbauer, 2010). We first determined the  
632 overall mean effect size estimate using multi-level random effects models (Nakagawa &  
633 Santos, 2012) using the `rma.mv` function. Each model included phylogeny, species, study ID,  
634 and observation ID as random factors. Observation ID represents the observational or  
635 residual variance, and needs to be explicitly modelled in a meta-analytic model (Moran *et al.*,  
636 2020). Study ID was included because some studies provided multiple effect sizes (especially  
637 for the sperm traits data set). Species was included because estimates were available from  
638 more than one study for some species. The phylogeny was incorporated into all models using  
639 a variance–covariance matrix. We considered an effect size to differ significantly from zero  
640 when the 95% confidence intervals do not overlap zero. We ran these models with and  
641 without inclusion of directionless effect sizes (Section II.3). We calculated heterogeneity  
642 across each data set using the  $I^2$  statistic (Higgins *et al.*, 2003). We also partitioned  
643 heterogeneity with respect to each of the four random factors, using the method of Nakagawa  
644 & Santos (2012).  $I^2$  values of 25, 50 and 75% are considered low, medium and high,  
645 respectively (Higgins *et al.*, 2003).

646 Studies often presented measures of multiple sperm traits using the same sample of males. If  
647 these traits are correlated the effect size estimates are not independent, and a meta-analysis  
648 that does not take this into account can underestimate the uncertainty in the overall effect size  
649 estimate (Noble *et al.*, 2017). We attempted to control for this potential non-independence  
650 statistically by using a variance–covariance matrix to specify the correlation between effect  
651 sizes from the same experiment (Noble *et al.*, 2017). To do this, we first created a new factor

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2  
3 652 called ‘experiment ID’, with effect sizes derived from the same sample of males given the  
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5 653 same ID code. We then produced a variance–covariance matrix specifying the correlation  
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7 654 between each effect size in the data set. When the correlation between traits is unavailable,  
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9 655 studies typically assume a correlation of 0.5, which is halfway between no correlation and a  
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11 656 perfect correlation of 1 (e.g. Moran *et al.*, 2020; Dougherty, 2021). Therefore, to test the  
12  
13 657 sensitivity of our analysis (e.g. Bishop & Nakagawa, 2021) we produced three matrices, with  
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15 658 effect sizes from the same experiment assumed to have a correlation of 0.25, 0.5 or 0.75. We  
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17 659 then ran the same multi-level random effects model as above, with the addition of experiment  
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19 660 ID as a random effect, and study variance specified by one of the covariance matrices. We  
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21 661 only used this approach for the sperm traits data set, because presentation of multiple  
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23 662 correlated traits is not a feature of the testes size or sperm quantity data sets.

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25 663 We used meta-regression models to examine the effect of our moderator variables on the  
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27 664 mean effect size (Nakagawa & Santos, 2012). Each model included phylogeny, species, study  
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29 665 ID, and observation ID as random factors as before, but now also included one of the seven  
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31 666 moderator variables listed in Section II.5 as a categorical (taxonomic group, mode of  
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33 667 fertilisation, tactic type, measurement, sperm trait, and sperm allocation *versus* expenditure)  
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35 668 or continuous (sneaker frequency) fixed effect. We first tested for a quadratic relationship  
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37 669 between sneaker frequency and the difference between ARTs, as theory predicts the  
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39 670 difference between tactics should be greatest at intermediate sneaker frequencies (Parker,  
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41 671 1990b; Gage *et al.*, 1995). If there was no significant quadratic effect, we also tested for a  
42  
43 672 linear effect. To test whether the mean effect size differed significantly between moderator  
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45 673 categories, we used the  $Q_M$  statistic, with a significant value indicating that the moderator  
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47 674 accounts for a significant proportion of the between-study heterogeneity (Koricheva *et al.*,  
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49 675 2013). We also ran these models with the intercept term dropped to obtain estimates of the  
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51 676 mean effect size for each categorical moderator level (in effect running a separate meta-  
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3 677 analysis for each factor level). All meta-regressions were tested including directionless effect  
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5 678 sizes. To improve our ability to detect biologically relevant differences, we excluded any trait  
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8 679 categories with fewer than five effect sizes when performing meta-regressions.  
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10 680 For the testes size data set, we also explicitly tested whether the use of the GSI could inflate  
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12 681 the differences between male tactics in fish, in two ways. First, we estimated the mean effect  
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14 682 size for the subset of fish studies that did not use the GSI. Second, we searched for raw testes  
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16 683 allometry data, in order to compare directly effect size estimates from the same males derived  
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18 684 from ANCOVA and GSI approaches. We found raw data for testes allometry for 18 out of 51  
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20 685 studies. We tested whether these two approaches resulted in significantly different effect size  
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22 686 estimates using a paired *t*-test comparing the Hedges' *d* values ( $N = 35$  comparisons and 30  
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24 687 species).  
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28 688 We searched for two signs of publication bias. First, we tested for evidence of publication  
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30 689 bias against non-significant results. One outcome of this type of publication bias is a  
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32 690 significant relationship between effect size and study variance, driven by 'missing' effect  
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34 691 sizes of small effect and with small sample sizes (a 'small study effect': Koricheva *et al.*,  
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36 692 2013). We tested for this relationship using a meta-regression with the inverse standard error  
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38 693 (also known as study precision) as a fixed factor, and phylogeny, species, study ID, and  
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40 694 observation ID as random factors. Second, we tested for a change in the average effect size  
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42 695 over time, which could reflect a change in the speed with which certain types of studies are  
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44 696 published (Jennions & Møller, 2002). This could arise if studies with non-significant results  
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46 697 are less likely to be published when a research field is young. We tested for a temporal trend  
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48 698 in effect sizes using a meta-regression with publication year as a fixed factor, and phylogeny,  
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50 699 species, study ID, and observation ID as random factors.  
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### 701 III. RESULTS

#### 702 (1) Testes size

703 The testes size data set consisted of 74 effect sizes from 51 studies and 53 species. Over half  
704 of the effect sizes came from fish (44 effect sizes, 28 species). We obtained sneaker  
705 frequency data for 45 species in this data set. Overall, there was no significant difference in  
706 investment in testes size between male ARTs (mean  $d = 0.87$ , 95% CI =  $-0.16$  to  $1.90$ ,  $k =$   
707  $74$ ; Fig. 1A). This remained the case after removing the one directionless effect size (mean  $d$   
708 =  $0.90$ , 95% CI =  $-0.15$  to  $1.95$ ,  $k = 73$ ), and after removing the two *Onthophagus* species  
709 with uncertain phylogenetic placement (mean  $d = 0.87$ , 95% CI =  $-0.15$  to  $1.89$ ,  $k = 72$ ). The  
710 data set was characterised by high total heterogeneity (total  $I^2 = 95.93$ ), with 32.45%  
711 attributable to phylogenetic history, 29.82% to species-level differences, 20.54% to study-  
712 level differences, and the remaining 13.11% to observation-level differences.

713 Meta-regression showed that sneakers have significantly larger testes than non-sneakers in  
714 fish, but there was no difference in invertebrates or other vertebrates (Fig. 1B; Table 2).

715 There was also a significant effect of measurement: sneakers were found to have significantly  
716 larger testes than non-sneakers when using the GSI, but not when using relative testes size  
717 (Fig. 1B; Table 2). Importantly, in 29 out of 44 fish studies testes size was measured using  
718 the GSI. To test whether the significant difference between tactics in fish could be driven by  
719 the inappropriate use of this metric, we used two approaches. First, we estimated the average  
720 effect size for fish studies that did not use this metric. After removing GSI effect sizes from  
721 the data set, there was no significant difference between sneaker and non-sneaker males in  
722 relative testes size for fish (mean  $d = 1.25$ , 95% CI =  $-0.06$  to  $2.56$ ,  $k = 15$ ; Fig. S6), and no  
723 significant difference in mean effect size between the three taxonomic groups ( $Q_M = 1.74$ ,  $P$   
724 =  $0.42$ ,  $k = 44$ , marginal  $R^2 = 0.18$ ; Fig. S6). Second, we directly compared effect sizes  
725 estimated from the same raw testes allometry data, using both the GSI approach and the

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3 726 recommended ANCOVA approach. For the subset of studies for which raw testes allometry  
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5 727 data were available (35 comparisons from 30 species), we found that using the average GSI  
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7 728 resulted in a significantly larger difference between male tactics than when using an  
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10 729 ANCOVA (paired  $t$ -test,  $t_{34} = 6.05$ ,  $P < 0.001$ ; Fig. 2). Importantly, this significant effect  
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12 730 remained when only comparing fish species (14 comparisons of 12 species; paired  $t_{13} = 3.95$ ,  
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14 731  $P = 0.002$ ). Further, across all 35 comparisons, using GSI was more likely to result in a  
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16 732 statistically significant result (25 of 35 cases, filled circles in Fig. 2) than when using  
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18 733 ANCOVA (11 of 35 cases, open circles in Fig. 2).  
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21 734 Meta-regression showed that sneakers have significantly larger testes than non-sneakers in  
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23 735 species with external fertilisation, but not those with internal fertilisation (Table 2). However,  
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25 736 there is an almost total overlap between taxonomic group and fertilisation type in the data set  
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27 737 (42 out of 44 effect sizes for fish were from species with external fertilisation), so we cannot  
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29 738 separate these two effects (although both factors explain around 30% of the sample variance:  
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31 739 Table 2). The difference in testes investment between male ARTs was not influenced  
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33 740 significantly by whether tactics were fixed or state-dependent (Table 2: tactic type). There  
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35 741 was no significant linear (slope  $\beta = -0.25$ , 95% CI =  $-2.09$  to  $1.59$ ) or quadratic relationship  
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37 742 between the difference in testes investment between male ARTs and the frequency of  
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39 743 sneakers in the population (Table 2). There was a trend for the mean effect size to decrease  
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41 744 with study publication year, but not significantly so ( $\beta = -0.05$ , 95% CI =  $-0.10$  to  $0.003$ ;  
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43 745 Table 2). The relationship between effect size and study precision was significantly  
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45 746 asymmetric ( $\beta = -0.28$ , 95% CI =  $-0.48$  to  $-0.08$ ; Fig. 1A; Table 2), with a positively-skewed  
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47 747 distribution. Sample sizes, meta-analytic means and 95% confidence intervals for each factor  
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49 748 level are presented in Table S3.  
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## 750 (2) Sperm quantity

751 The sperm quantity data set consisted of 49 effect sizes from 43 studies and 32 species. The  
752 majority of data came from fish (36 effect sizes, 21 species). We obtained sneaker frequency  
753 data for 22 species in this data set. Overall, there was no significant difference in investment  
754 in sperm quantity between male ARTs (mean  $d = -0.16$ , 95% CI =  $-2.14$  to  $1.81$ ,  $k = 49$ ; Fig.  
755 3A). This result was the same after removing the four directionless effect sizes (mean  $d = -$   
756  $0.13$ , 95% CI =  $-2.19$  to  $1.94$ ,  $k = 45$ ). The data set was characterised by high total  
757 heterogeneity (total  $I^2 = 97.04$ ), with 73.68% attributable to phylogenetic history, 10.87% to  
758 species-level differences, 7.03% to study-level differences, and the remaining 5.45% to  
759 observation-level differences.

760 The difference in sperm quantity between male ARTs depended on how sperm quantity was  
761 measured; sperm density and volume were higher for sneakers, whereas sperm number was  
762 higher for non-sneakers (Fig. 3B; Table 2). However, in no case did the mean estimate differ  
763 significantly from zero. The difference in sperm quantity between ARTs was positively  
764 related to the proportion of sneakers in the population ( $\beta = 2.40$ , 95% CI =  $0.44$  to  $4.36$ ;  
765 Table 2; Fig. 4). Adding a quadratic term to the model increased the amount of heterogeneity  
766 explained by sneaker frequency (Table 2), but the quadratic term itself did not differ  
767 significantly from zero ( $z = -1.76$ ,  $P = 0.08$ ). The difference in sperm quantity between male  
768 ARTs was not significantly influenced by taxonomic group (Fig. 3B), fertilisation mode,  
769 whether tactics were fixed or state-dependent (tactic type), or whether sperm expenditure or  
770 allocation was examined (Table 2). There was also no effect of study precision ( $\beta = 0.08$ ,  
771 95% CI =  $-0.28$  to  $0.45$ ; Table 2). However, there was a significant negative relationship  
772 between effect size and the year in which a study was published ( $\beta = -0.06$ , 95% CI =  $-0.13$   
773 to  $-0.002$ ; Fig. S7; Table 2); this trend appears to be driven by a higher proportion of studies

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3 774 showing negative effects in the last five years. Sample sizes, meta-analytic means and 95%  
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5 775 confidence intervals for each factor level are presented in Table S4.  
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### 10 777 **(3) Sperm traits**

12 778 The sperm traits data set consisted of 128 effect sizes from 55 studies and 33 species. The  
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14 779 majority of data came from fish (102 effect sizes, 22 species). We obtained sneaker frequency  
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16 780 data for 23 species in this data set. Overall, there was no significant difference in sperm traits  
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18 781 between male ARTs (mean  $d = 0.14$ , 95% CI =  $-0.05$  to  $0.33$ ,  $k = 128$ ; Fig. 5A). This result  
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20 782 was the same after removing the ten directionless effect sizes (mean  $d = 0.15$ , 95% CI =  $-$   
21  
22 783  $0.04$  to  $0.35$ ,  $k = 118$ ), and after incorporating a variance matrix to account for potential non-  
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24 784 independence of sperm traits measured on the same males (Table S5). The data set was  
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26 785 characterised by high total heterogeneity (total  $I^2 = 74.8\%$ ), with 0.9% attributable to  
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28 786 phylogenetic history, 17.1% to species-level differences, 8.72% to study-level differences,  
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30 787 and the remaining 48.1% to observation-level differences.  
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33 788 The difference in sperm traits between male ARTs differed according to which sperm trait  
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35 789 was measured (Table 2). However, only ATP content had an estimate that differed  
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37 790 significantly from zero (Fig. 5B). The difference in sperm traits between male ARTs was not  
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39 791 significantly influenced by taxonomic group (Fig. 5B), mode of fertilisation, or tactic type  
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41 792 (Table 2). There was no significant linear ( $\beta = -0.32$ , 95% CI =  $-0.86$  to  $0.21$ ) or quadratic  
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43 793 relationship between the difference in sperm traits between male ARTs and the frequency of  
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45 794 sneakers in the population (Table 2). There was also no significant relationship between the  
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47 795 difference in sperm traits between male ARTs and study precision ( $\beta = -0.004$ , 95% CI =  $-$   
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49 796  $0.20$  to  $0.19$ ; Table 2). There was a marginally non-significant trend for the mean effect size  
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51 797 to decrease with study publication year ( $\beta = -0.02$ , 95% CI =  $-0.05$  to  $0.0006$ ; Table 2).  
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3 798 Sample sizes, meta-analytic means and 95% confidence intervals for each factor level are  
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5 799 presented in Table S5.  
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10 801 **IV. DISCUSSION**

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12 802 We systematically searched the literature for studies comparing ejaculate investment and  
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14 803 sperm traits between males using different types of ARTs. We found data from 92 studies and  
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16 804 67 species; more than double the 29 species surveyed by Kustra & Alonzo (2020). Despite  
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18 805 this larger data set, our quantitative results broadly matched their qualitative results. We  
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20 806 found that, after controlling for body size, male fish (but not any other taxonomic groups)  
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22 807 using tactics that elevate sperm competition risk, or that had a reduced investment in traits  
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24 808 that increase mating success, had significantly larger testes than males using other alternative  
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26 809 tactics. However, this pattern disappears when we restrict the analysis to those studies that do  
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28 810 not use the GSI as a measure of testes investment. Males exhibiting different ARTs did not  
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30 811 differ significantly in sperm number (either sperm allocation or expenditure), nor in other  
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32 812 sperm traits, with the exception of sperm ATP content in fish. We failed to detect the  
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34 813 predicted quadratic relationship between sneaker frequency and the difference in post-mating  
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36 814 investment between ARTs in any of the three data sets. However, we did detect a significant  
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38 815 positive linear relationship between sneaker frequency and the difference in sperm quantity  
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40 816 between ARTs, thus showing that the abundance of sneakers does influence the average  
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42 817 ejaculate investment of males exhibiting ARTs to some extent. Finally, contrary to our  
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44 818 predictions, differences in testes size, sperm number or sperm traits between male ARTs were  
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46 819 unaffected by the extent to which tactics were flexible.  
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3 **821 (1) Appraising the evidence**  
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6 822 In fishes, males exhibiting tactics associated with an increased risk of sperm competition, or a  
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8 823 reduced investment in traits that increase mating success, had relatively larger testes than  
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10 824 males exhibiting alternative tactics. This result supports predictions based on sperm  
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12 825 competition theory (Parker, 1990*a,b*; Gage *et al.*, 1995; Ball & Parker, 2003; Parker &  
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14 826 Pizzari, 2010). Why is this relationship present in fish but not in any other taxonomic group?  
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17 827 We suggest three potential explanations. First, more data were available for fish than for  
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19 828 other taxonomic groups, increasing our statistical power (Kustra & Alonzo, 2020). Second,  
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21 829 almost all (26 of 28) of the fish species in the testes size data set exhibit external fertilisation,  
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23 830 whereas the vast majority (24 of 25) of the remaining species exhibit internal fertilisation.  
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26 831 This pattern might therefore be explained by differences in fertilisation mode, given that: (a)  
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28 832 sperm limitation is likely to be more important in external fertilisers; and (b) strong sperm  
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30 833 precedence or cryptic female choice in internal fertilisers is expected to weaken the  
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32 834 relationship between sperm number and fertilisation (Fitzpatrick, 2020). However, we found  
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34 835 no effect of fertilisation mode in the sperm quantity or sperm traits data sets. Further testing  
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36 836 of this relationship is difficult without more data on internally fertilising fish species showing  
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38 837 ARTs.  
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42 838 Third, and most importantly, the use of the GSI as a measure of testes size is widespread in  
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44 839 studies of fish, but rare in other taxa. In the testes size data set, 29 of 44 fish effect sizes used  
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46 840 the GSI approach, whereas only 1 of 30 of the non-fish effect sizes did. As discussed in  
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48 841 Section I.2, the GSI is an unsuitable metric to use when comparing male tactics, because it  
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50 842 only controls properly for body size when the relationship between testes size and body size  
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52 843 is isometric (Tomkins & Simmons, 2002). When the slope of the relationship between testes  
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54 844 size and body size is less than 1, the difference in testes investment between large and small  
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56 845 male morphs is overestimated. Instead, the use of an ANCOVA is recommended, which  
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3 846 directly accounts for positive or negative allometry, as well as differences in allometry  
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5 847 between male morphs (Tomkins & Simmons, 2002). We provide two forms of evidence that  
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7 848 the significant difference in testes investment seen for fish is driven by the use of this  
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10 849 inappropriate metric. First, the effect disappears when studies using the GSI to measure testes  
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12 850 investment are excluded. Second, re-analysis of raw testes allometry data (35 comparisons,  
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14 851 30 species) showed that the GSI approach resulted in a significantly larger effect size than the  
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16 852 ANCOVA approach, both for the full data set and when only considering fish. We believe  
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18 853 this is the strongest evidence yet that GSI is an inappropriate method to compare testes  
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20 854 investment between male ARTs.  
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24 855 We found no evidence for differences in sperm quantity or sperm traits between male ARTs  
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26 856 that differ in sperm competition risk. The only exception was sperm ATP content in fish.  
27  
28 857 Across five species of fish, sneaker male sperm contained more ATP per cell than non-  
29  
30 858 sneaker male sperm. Intraspecific studies have shown a positive relationship between ATP  
31  
32 859 content and sperm motility (e.g. Christen, Gatti & Billard, 1987; Perchec *et al.*, 1995;  
33  
34 860 Burness *et al.*, 2004). However, the ATP content of a sperm cell depends on the balance  
35  
36 861 between production before and after ejaculation (either through respiration or glucose or lipid  
37  
38 862 catabolism; Werner & Simmons, 2008), and consumption during cellular maintenance and  
39  
40 863 motility (Tourmente *et al.*, 2019). This means that high cell ATP content could potentially  
41  
42 864 reflect high initial stores, high production after ejaculation, low consumption, or a  
43  
44 865 combination of all three (e.g. Christen *et al.*, 1987). All of the effect sizes in our data set  
45  
46 866 reflect stored ATP levels, as ATP content was measured in stripped (not ejaculated) sperm,  
47  
48 867 immediately after sampling, and before activation by contact with fresh water or sea water. It  
49  
50 868 is therefore unclear whether this difference between male tactics also exists for ATP  
51  
52 869 production or consumption. Nevertheless, we suggest this result should be interpreted with  
53  
54 870 caution, for two reasons. First, it is derived from only seven effect sizes, from six studies  
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3 871 (Table S5). Second, sperm ATP content is assumed to improve fertilisation success by  
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5 872 increasing sperm swimming speed, motility or longevity (or all three). However, none of  
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7 873 these three traits differed between male tactics in our data set, even though we obtained larger  
8  
9 874 sample sizes than those for ATP content. Our ability to detect a significant difference in  
10  
11 875 sperm traits between ARTs could have been reduced because we combined estimates from  
12  
13 876 multiple sperm traits which may exhibit functional or resource-allocation trade-offs (Snook,  
14  
15 877 2005). However, widespread trade-offs in the same direction would be revealed in our  
16  
17 878 analysis *via* differences in the average sign of the effect size for different sperm traits. For  
18  
19 879 example, a speed–longevity trade-off could result in a positive effect size for sperm  
20  
21 880 swimming speed and a negative effect size for sperm longevity. However, when considering  
22  
23 881 each sperm trait separately, only sperm ATP content differed significantly between male  
24  
25 882 tactics (Table S5), which suggests that such trade-offs do not act in the same direction across  
26  
27 883 species, at least in relation to differences in ART. Indeed, such trade-offs are also not  
28  
29 884 apparent when comparing multiple sperm traits between ARTs within the same species  
30  
31 885 (Kustra & Alonzo, 2020). This suggests either that such trade-offs do not typically constrain  
32  
33 886 the evolution of sperm traits across the animal kingdom, or that species can solve any trade-  
34  
35 887 offs in multiple ways.

36  
37 888 Theory predicts that the difference in post-mating investment between guarders and sneakers  
38  
39 889 should be greatest when sneakers are at an intermediate frequency in the population (Parker,  
40  
41 890 1990*b*; Gage *et al.*, 1995). We failed to confirm this prediction: there was no significant  
42  
43 891 quadratic relationship between sneaker frequency and the difference in post-mating  
44  
45 892 investment between ARTs in any of the three data sets. However, there was a significantly  
46  
47 893 positive linear relationship between sneaker frequency and effect size for the sperm quantity  
48  
49 894 data set, even though the average difference between ARTs was close to zero. Such a linear  
50  
51 895 relationship could arise due to a lack of data at high sneaker frequencies, which reduces our  
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3 896 power to detect the predicted decrease in the disparity between ARTs in this region. We thus  
4  
5 897 consider this to be tentative evidence showing that the abundance of sneakers does indeed  
6  
7 898 influence the average ejaculate investment of males exhibiting alternative tactics to some  
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10 899 extent. We may have failed to find a relationship between sneaker frequency and the  
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12 900 difference in post-mating investment between ARTs for the testes size and sperm traits data  
13  
14 901 sets because of data limitations. For example, we were typically only able to obtain an  
15  
16 902 estimate of sneaker frequency from a single population for each species, even though for  
17  
18 903 some species we had post-mating trait data from more than one population. Therefore, there  
19  
20 904 may be important among-population variation in sneaker frequency that we could not account  
21  
22 905 for. It is also important to note that the average frequency of sneakers in the population is  
23  
24 906 related to, but not identical to, the average frequency of *sneaking* per mating event. The  
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26 907 difference between sneaker and sneaking frequency can often be large. For example, in the  
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28 908 cichlid *Lamprologus callipterus* dwarf (sneaker) males may comprise around half of the  
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30 909 population, but were found to participate in only 5% of observed spawning events (Wirtz  
31  
32 910 Ocana *et al.*, 2014). Additionally, the frequency of sneaking is likely to be very variable  
33  
34 911 across the breeding season and depending on the immediate social and abiotic environment.  
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36 912 Such variability may be relevant for sperm traits which can be varied rapidly in response to  
37  
38 913 immediate social cues, but less relevant for traits such as testes size which change over  
39  
40 914 evolutionary time. However, this does not mean that sneaker frequency is an irrelevant metric  
41  
42 915 when considering post-mating traits. This is because sneaker frequency tells us what the  
43  
44 916 evolutionarily stable frequency of each male tactic is, which influences the *average* sperm  
45  
46 917 competition risk across all contexts and individuals. If this average risk differs between  
47  
48 918 ARTs, then it will influence the optimal investment into sperm and ejaculate traits  
49  
50 919 irrespective of spatial or temporal variation in sneaking frequency. Importantly, such a stable  
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52 920 frequency exists for both fixed tactics [in which the relative reproductive success of male  
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3 921 ARTs is stabilised at equilibrium by negative frequency-dependent selection (Gross, 1991;  
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5 922 Shuster & Wade, 1991)] and state-dependent tactics; in the latter case, the frequency of high-  
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8 923 quality ‘dominant’ males in the population will influence the threshold at which poor-  
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10 924 condition individuals switch to an alternative tactic (e.g. Tomkins & Brown, 2004).  
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12 925 All three data sets were characterised by very high heterogeneity. While high heterogeneity is  
13  
14 926 commonly seen in ecological meta-analyses (Senior *et al.*, 2016), it does reduce the power of  
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16 927 the analysis to detect small effects due to putative moderators, if other sources of variation  
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18 928 cannot readily be identified and accounted for. Partitioning of heterogeneity suggested that  
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20 929 the proportion of variation explained by species-level and phylogenetic differences combined  
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22 930 was high for both the testes size data set (62%) and the sperm quantity data set (85%). This  
23  
24 931 suggests that both of these traits evolve slowly, possibly because of constraints on testes  
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26 932 function. By contrast, for the sperm traits data set only 18% of heterogeneity could be  
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28 933 attributed to phylogenetic or species-level differences, suggesting fewer constraints on their  
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30 934 evolution. Notably, the proportion of variance explained by any of the nine tested moderator  
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32 935 variables was small for all three data sets (with the exception of taxonomic group and mode  
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34 936 of fertilisation for the testes size data set). Therefore, much of the effect size heterogeneity  
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36 937 remains unexplained, especially for the sperm traits data set. Several factors could explain  
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38 938 this heterogeneity, including complex changes in the immediate social environment (e.g.  
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40 939 local variation in the number and types of rival males present during spawning), other  
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42 940 species-specific selection pressures on male post-mating traits [e.g. *Lamprologus callipterus*  
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44 941 sneaker males face a higher sperm competition risk but occupy a favoured role during  
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46 942 spawning (Schutz *et al.*, 2010; Taborsky *et al.*, 2018); see Section IV.3], and functional trade-  
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48 943 offs between sperm traits [e.g. a trade-off between swimming speed and sperm longevity  
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50 944 (Levitan, 2000); see Section IV.3].  
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## 946 (2) Publication bias

947 We detected some evidence for publication bias in the three data sets. All three data sets  
948 showed a decrease in the mean effect size over time, although only significantly so for sperm  
949 quantity. Hence, studies showing no difference in post-mating traits between male ARTs, or a  
950 difference in the opposite direction to that typically predicted, are now published more often  
951 than in the 1990s. This could be for a variety of reasons, including an increase in sample size  
952 or improved methodological rigour over time, changes in editorial policy or in the types of  
953 study systems being investigated, or the fact that early theoretical investigations (e.g. Parker,  
954 1990*a,b*; Gage *et al.*, 1995) were influential and led to a genuine publication bias against non-  
955 confirmatory results. The funnel plot for testes sizes was significantly asymmetric, with a  
956 positively-skewed distribution. This pattern could arise if studies reporting a negative effect  
957 size are less likely to be published. However, our analysis indicated that the testes size data  
958 set was significantly heterogeneous in relation to taxonomic group, fertilisation mode and  
959 measurement type. We therefore suggest that the asymmetry is driven by true heterogeneity  
960 in the data set, rather than biased publication practices (Nakagawa & Santos, 2012).

961

## 962 (3) Explaining the incongruence between theory and data

963 Taken together, these results suggest that the current empirical evidence that male ARTs  
964 differ consistently in their investment into sperm and ejaculates is very weak. This is  
965 surprising, given that almost all theoretical models predict that sneaker males should invest  
966 more than non-sneaker males into post-mating traits (Parker, 1990*a,b*; Gage *et al.*, 1995; Ball  
967 & Parker, 2003). We have several potential explanations for the incongruence between theory  
968 and empirical data. First, males exhibiting ARTs may not differ significantly in sperm  
969 competition risk. One reason for this would be if sneakers typically make up a high  
970 proportion of males in the population (Parker, 1990*b*; Gage *et al.*, 1995; Simmons *et al.*,

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3 971 2007). We obtained these data for 53 species across all three data sets. Across these 53  
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5 972 species, sneaker frequency ranged from 2% of males in the cichlid *Amatliana siquia*  
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7 973 (Clotfelter *et al.*, 2017), to 87% of males in the dusky frillgoby *Bathygobius fuscus*  
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9 974 (Takegaki, Kaneko & Matsumoto, 2012), with an average of 39% (Fig. S5). Importantly,  
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11 975 non-sneakers outnumber sneakers by 2:1 or more in only 23 of the 53 species, and in fact  
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13 976 sneakers outnumber non-sneakers in 18 of the remaining 30 species. Therefore, sneaker  
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15 977 males are certainly not rare for the majority of species in our sample, so that non-sneakers  
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17 978 may typically face a similar sperm competition risk to sneakers (assuming sneaker frequency  
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19 979 is a reasonable proxy for the frequency of breeding events that involve sperm competition;  
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21 980 but see Wirtz Ocana *et al.*, 2014). Second, males often face multiple selection pressures in  
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23 981 relation to sperm and ejaculate investment. For example, in the cichlid fish *Lamprologus*  
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25 982 *callipterus*, dwarf (sneaker) males attempt to steal fertilisations from larger, nesting males  
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27 983 (Schutz *et al.*, 2010; Taborsky *et al.*, 2018). However, females spawn in empty shells  
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29 984 collected by nesting males, and their small size means that sneaker males can enter these  
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31 985 shells during spawning and ejaculate much closer to the eggs than can nesting males (Schutz  
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33 986 *et al.*, 2010; Taborsky *et al.*, 2018). Thus, while nesting males generally face lower sperm  
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35 987 competition than sneaking males, they also occupy a disfavoured role, and could benefit from  
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37 988 investing more into sperm and ejaculate traits to compensate. This example illustrates how  
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39 989 multiple factors may act simultaneously to influence sperm investment of different ARTs in  
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41 990 complex ways.

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43 991 Sperm competition models are also simplistic in three key ways. First, models assume that  
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45 992 fertilisation is the result of a 'fair raffle', whereby a male's chance of fertilising a female's  
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47 993 eggs is directly proportional to how many sperm he produces (Parker, 1990a,b; Gage *et al.*,  
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49 994 1995; Ball & Parker, 2003). This assumption may be met in broadcast-spawning external  
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51 995 fertilisers, but such species rarely show ARTs (and no examples are present in this analysis).  
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3 996 By contrast, in many other external fertilisers, a male's proximity to a female during gamete  
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5 997 release may be much more important than how many sperm he produces (Taborsky *et al.*,  
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7 998 2018), and in internal fertilisers first- or last-male sperm precedence or cryptic female choice  
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9 999 (a 'loaded raffle') will act to obscure the relationship between sperm number and fertilisation  
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11 1000 success (Simmons, 2001). Second, models do not consider functional trade-offs between  
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13 1001 post-mating traits (Kustra & Alonzo, 2020) which could limit the ability of ejaculate or sperm  
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15 1002 traits to evolve independently of each other (Snook, 2005; Simmons & Fitzpatrick, 2012).  
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17 1003 Third, models typically assume that males exhibiting different ARTs have the same overall  
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19 1004 energy budget, which they divide differentially between pre- and post-mating traits (Kustra &  
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21 1005 Alonzo, 2020). However, in species with state-dependent ARTs sneaker males will be in  
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23 1006 poorer condition than non-sneaker males, and hence less able to afford to increase their  
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25 1007 absolute investment into sperm or ejaculate traits. The fact that ejaculate and sperm traits may  
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27 1008 also be influenced by individual condition or diet (Macartney *et al.*, 2019) suggests that  
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29 1009 sneaker males may often be unable to produce larger ejaculates or higher-quality sperm  
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31 1010 because of energetic limitations. Males exhibiting ARTs may also differ in resource  
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33 1011 allocation even when the choice of tactic is not condition dependent. For example, at certain  
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35 1012 points in the breeding season guarding males may have few resources to invest into ejaculates  
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37 1013 because of the conflicting demands of territory defence, female courtship and brood care  
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39 1014 (Taborsky, 2008).  
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41 1015 It has also been questioned whether the traits commonly measured in empirical studies are  
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43 1016 appropriate proxies for post-mating investment. For example, as discussed above, GSI has  
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45 1017 been criticised as an inappropriate measure of size-corrected investment in testes tissue  
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47 1018 (Tomkins & Simmons, 2002). Sperm competition risk is not the only factor that influences  
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49 1019 ejaculate size or sperm production; large testes may also be important for males with high  
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51 1020 mating rates independent of levels of sperm competition (Vahed & Parker, 2012) or in  
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3 1021 species in which females lay large clutches (Emerson, 1997). Additionally, the relationship  
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5 1022 between sperm traits and fertilisation ability is complex (Snook, 2005; Simmons &  
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7 1023 Fitzpatrick, 2012), and predictions are often based on verbal arguments with dubious  
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9 1024 assumptions. For example, the general assumption that longer sperm are better swimmers is  
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11 1025 likely to be unfounded, especially for internal fertilisers (Humphries, Evans & Simmons,  
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13 1026 2008). It may be more appropriate in future to focus on sperm traits that have stronger causal  
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15 1027 links to sperm performance, such as the ratio of flagellum length to head length (Humphries  
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17 1028 *et al.*, 2008), or sperm ATP content (Tourmente *et al.*, 2019).  
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19 1029 There are also other ejaculate components that we did not consider here but which may play  
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21 1030 an important role in mediating male fertilisation success (Kustra & Alonzo, 2020). For  
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23 1031 example, studies of fish with male ARTs have shown that both the amount (Poli *et al.*, 2018)  
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25 1032 and composition of the male seminal fluid differs between tactics (Gombar *et al.*, 2017).  
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27 1033 Further, seminal fluid may improve the competitiveness of sperm from the same males  
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29 1034 (Locatello *et al.*, 2013; Bartlett *et al.*, 2017; Poli *et al.*, 2018; Gasparini, Pilastro & Evans,  
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31 1035 2020), or even reduce the competitiveness of sperm from males exhibiting the alternative  
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33 1036 tactic (Locatello *et al.*, 2013; Lewis & Pitcher, 2017*b*). This latter observation raises the  
34  
35 1037 possibility that sneaker and guarder males could be engaged in a molecular ‘arms race’, with  
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37 1038 sneaker males evolving seminal fluid components that impair guarder sperm competitiveness,  
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39 1039 and guarders evolving traits that resist the effect of these components. Nevertheless,  
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41 1040 differences in seminal fluid between male ARTs have been investigated in only three fish  
42  
43 1041 species. Until we have more data, we cannot rule out the possibility that, when compared to  
44  
45 1042 non-sneaker males, sneaker males consistently produce more seminal fluid per mating, or  
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47 1043 produce non-sperm components of the ejaculate that are more competitive. Another factor  
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49 1044 which has been mostly ignored is cryptic female choice, which occurs in both internal and  
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51 1045 external fertilisers and has the potential to alter the relative competitiveness of sperm from  
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3 1046 different tactics (Simmons, 2001; Fitzpatrick, 2020). For example, in the ocellated wrasse  
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5 1047 *Symphodus ocellatus* female ovarian fluid increases sperm swimming speed, and this likely  
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7 1048 enhances the competitiveness of dominant males, who produce fewer, faster sperm than  
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9 1049 sneaker males (Alonzo, Stiver & Marsh-Rollo, 2016).  
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#### 15 1051 **(4) Future directions**

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17 1052 In summary, our meta-analyses show that the current evidence for consistent differential  
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19 1053 investment into post-mating traits by males exhibiting different ARTs is weak, especially in  
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21 1054 relation to sperm quantity and individual sperm traits. However, all three data sets were  
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23 1055 characterised by high heterogeneity, well beyond that attributable to sampling error alone,  
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25 1056 which remains mostly unexplained. It remains unclear if the incongruence between data and  
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27 1057 theory is due to theory not taking real-world complexity into account, empirical studies that  
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29 1058 focus on the wrong post-mating traits, or both. However, there is clearly a need to reassess  
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31 1059 the validity of the assumptions underlying mathematical models of sperm competition. For  
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33 1060 example, the assumption that fertilisation follows a fair raffle is likely to be unrealistic for  
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35 1061 most species (Simmons, 2001). If such assumptions do not apply widely, it does not mean  
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37 1062 that a model is incorrect; rather that only species that match these assumptions are  
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39 1063 appropriate test subjects. Further, it may be naïve to expect to see the same general patterns  
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41 1064 across divergent taxa given how much species vary, even within the same genus. While the  
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43 1065 disparate species represented in our meta-analysis do indeed exhibit similar ARTs, there are  
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45 1066 many important biological and ecological differences among species (for example in their  
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47 1067 intra- and inter-sexual interactions, the importance of different sperm traits for determining  
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49 1068 male fertilisation success, or the mechanisms of sperm utilisation by females) which could  
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51 1069 obscure any general patterns. In light of these points, we have several clear recommendations  
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53 1070 for researchers. First, the GSI should not be used to compare gonadal investment between  
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3 1071 male tactics. This is not a new recommendation, but we hope that by expanding the original  
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5 1072 comparison by Tomkins & Simmons (2002) from 5 to 30 species, we provide very strong  
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7 1073 evidence in support of abandoning the GSI. Second, we need more empirical data linking  
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9 1074 sperm traits to fertilisation success in target species. As it is, we are in danger of measuring  
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11 1075 sperm quality using traits that do not directly influence sperm competitiveness (Snook, 2005;  
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13 1076 Simmons & Fitzpatrick, 2012). We should also not assume that the post-mating traits that  
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15 1077 partially determine male fertilisation success in one or a few species will do so in all species  
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17 1078 or different types of ART. Finally, we need new theory which takes into account complexities  
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19 1079 driven by the social environment, energetic constraints and male physiology, sperm function,  
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21 1080 and functional trade-offs between post-mating traits (Kustra & Alonzo, 2020).  
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## 28 1082 **V. CONCLUSIONS**

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31 1083 (1) We performed three meta-analyses examining how testes size, sperm number and sperm  
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33 1084 traits differ between males exhibiting ARTs that face either a high or a low sperm  
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35 1085 competition risk, or have high or low investment in traits that increase mating success.  
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37 1086 (2) Male fish exhibiting ARTs facing a high sperm competition risk had significantly larger  
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39 1087 testes after controlling for body size than those exhibiting tactics facing a low sperm  
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41 1088 competition risk. However, we suggest this difference is driven by the widespread use of GSI  
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43 1089 as a measure of testes investment in fish, which overestimates the difference in testes  
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45 1090 investment between male tactics when the relationship between testes size and body size is  
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47 1091 not isometric.  
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51 1092 (3) There was no significant difference in sperm quantity between males exhibiting different  
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53 1093 ARTs, regardless of whether it was measured in the testes or following ejaculation.  
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56 1094 (4) There was no significant difference in sperm traits between males exhibiting different  
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58 1095 ARTs, except for sperm ATP content in fish.  
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3 1096 (5) The difference in post-mating investment between male ARTs was not influenced by  
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5 1097 taxonomic group or by the extent to which tactics were flexible. However, the difference in  
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7 1098 sperm quantity between ARTs increased as sneakers became more common in the  
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9 1099 population. The difference in testes size between male ARTs was greater for external than  
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11 1100 internal fertilisers.

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14 1101 (6) Overall, there is little evidence that male ARTs differ substantially in investment into  
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16 1102 sperm and ejaculates. The incongruence between theoretical and empirical results could be  
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18 1103 explained if (a) theoretical models fail to account for differences in overall resource levels  
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20 1104 between males exhibiting different ARTs or fundamental trade-offs between investment into  
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22 1105 different ejaculate and sperm traits, and (b) studies often use sperm or ejaculate traits that do  
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24 1106 not reflect overall post-mating investment or relate to fertilisation success.

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27 1107 (7) We recommend that future studies: (a) cease using the GSI to quantify gonadal  
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29 1108 investment; (b) seek empirical data linking specific sperm traits to fertilisation success in a  
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31 1109 range of species; (c) compare non-sperm components of the ejaculate between male ARTs;  
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33 1110 and (d) develop theoretical models that take into account the presence of multiple selection  
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35 1111 pressures acting on male post-mating investment, variable patterns of sperm precedence,  
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37 1112 differences in energy budgets between males exhibiting ARTs, and functional trade-offs  
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39 1113 between sperm traits.

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## 46 **VI. ACKNOWLEDGEMENTS, AUTHOR CONTRIBUTIONS AND DATA**

### 47 **ACCESSIBILITY**

48  
49  
50  
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4  
5 performed meta-analysis, and wrote the first draft of the paper. M.J.A.S. developed the  
6  
7 methods and screened studies. M.D.J. and L.W.S. conceived the study, developed the  
8  
9 methods, and contributed to writing.  
10  
11

12 *Data accessibility:* All data and code used in the analysis are available from Figshare  
13 (10.6084/m9.figshare.19174604).  
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## 18 **VII. REFERENCES**

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20 References included in the meta-analysis are highlighted with an asterisk.

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22 Additional references used in the supporting information are indicated with a † symbol.

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## 45 **VIII. SUPPORTING INFORMATION**

46 Additional supporting information may be found online in the Supporting Information section  
47  
48 at the end of the article.

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51 **Appendix S1.** PRISMA-EcoEvo checklist.

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53 **Fig. S1.** PRISMA diagram summarising the literature search and study screening processes.

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56 **Table S1.** Methods for calculating the standardised mean difference (Hedges' *d*) and the  
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58 location of the data collected.  
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3 **Fig. S2.** Phylogenetic tree for the 53 species in the testis size data set.  
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5 **Fig. S3.** Phylogenetic tree for the 32 species in the sperm quantity data set.  
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7 **Fig. S4.** Phylogenetic tree for the 33 species in the sperm traits data set.  
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9 **Table S2.** Sources used for the sneaker frequency data.  
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11 **Fig. S5.** Histogram showing the distribution of sneaker frequency across 54 species.  
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13 **Fig. S6.** Differences in testes size (Hedges'  $d$ ) between male alternative reproductive tactics  
14 (ARTs) in relation to taxonomic group, after removing studies using the gonadosomatic  
15 index.  
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18 **Table S3.** Mean effect size estimates (Hedges'  $d$ ), 95% confidence intervals, and sample  
19 sizes for the testis size data set.  
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22 **Fig. S7.** Relationship between effect size (Hedges'  $d$ ) and publication year for the sperm  
23 quantity data set.  
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26 **Table S4.** Mean effect size estimates (Hedges'  $d$ ), 95% confidence intervals, and sample  
27 sizes for the sperm quantity data set.  
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30 **Table S5.** Mean effect size estimates (Hedges'  $d$ ), 95% confidence intervals, and sample  
31 sizes for the sperm traits data set.  
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### 34 **Figure legends**

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44 **Fig. 1.** Difference in testes size (Hedges'  $d$ ) between male alternative reproductive tactics  
45 (ARTs) in relation to (A) study variance, and (B) taxonomic group (top panel) and size  
46 measure (bottom panel). In A, the dashed vertical line represents the meta-analytic mean, and  
47 the dotted lines are the 95% pseudo-confidence interval. In B, points are scaled according to  
48 study variance (precision). In all panels, black points represent the meta-analytic mean, and  
49 black bars show the 95% confidence interval.  $k$  = number of effect sizes for each category.  
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6 **Fig. 2.** Comparison of two methods for comparing the difference in relative testes size  
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8 (Hedges'  $d$ ) between male alternative reproductive tactics (ARTs): the gonadosomatic index  
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10 (blue points) or ANCOVA (red points). Horizontal lines connect effect size estimates derived  
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12 from the same raw data. Filled and open circles represent cases in which a statistical test  
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14 (either a  $t$ -test or ANCOVA) detected a significant or non-significant difference, respectively  
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16 in relative testes size between ARTs.  
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21 **Fig. 3.** Difference in sperm quantity (Hedges'  $d$ ) between male alternative reproductive  
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23 tactics (ARTs) in relation to (A) study variance (precision), and (B) taxonomic group (top  
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25 panel) and quantity measure (bottom panel). In A, the dashed vertical line represents the  
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27 meta-analytic mean, and the dotted lines are the 95% pseudo-confidence interval. In B, points  
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29 are scaled according to study variance (precision). In all panels, black points represent the  
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31 meta-analytic mean, and black bars show the 95% confidence interval.  $k$  = number of effect  
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33 sizes for each category.  
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40 **Fig. 4.** The relationship between the proportion of sneakers in the population and the  
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42 difference in sperm quantity between male alternative reproductive tactics (ARTs). Each  
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44 bubble represents an effect size ( $N = 53$ ), with bubble size scaled to effect size precision  
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46 (inverse standard error; larger bubbles reflect studies with larger sample sizes). The dashed  
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48 line shows the predicted line from a meta-regression including sneaker frequency as a  
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50 covariate. Dotted lines show the 95% confidence intervals for the predicted line.  
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56 **Fig. 5.** Difference in sperm traits (Hedges'  $d$ ) between male alternative reproductive tactics  
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58 (ARTs) in relation to (A) study variance (precision), and (B) taxonomic group (top panel) and  
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3 sperm trait (bottom panel). In A, the dashed vertical line represents the meta-analytic mean,  
4 and the dotted lines are the 95% pseudo-confidence interval. In B, points are scaled according  
5 to study variance (precision). In all panels, black points represent the meta-analytic mean, and  
6 black bars show the 95% confidence interval.  $k$  = number of effect sizes for each category.  
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Table 1. Overview of the 18 alternative reproductive tactics (ARTs) included in the data set, with a description of how each is predicted to influence investment into sperm or ejaculate traits. SC, sperm competition.

Species	ARTs	Reason for inclusion	Positive effect size
<b>Roach</b> <i>Rutilus rutilus</i>	Attractive vs unattractive	Unattractive males face greater SC risk	Unattractive > attractive
<b>Golden julie</b> <i>Julidochromis ornatus</i>	Breeder vs cooperative breeder	Cooperative breeders face greater SC risk	Cooperative breeder > breeder
<b>Masked julie</b> <i>Julidochromis transcriptus</i>			
<b>Golden julie</b> <i>Julidochromis ornatus</i>	Breeder vs helper	Helpers face greater SC risk	Helper > breeder
<b>Masked julie</b> <i>Julidochromis transcriptus</i>			
<b>Red-backed fairy wren</b> <i>Malurus melanocephalus</i>			
<b>Chinook salmon</b> <i>Oncorhynchus tshawytscha</i>	Dominant vs subordinate	Subordinates face greater SC risk	Subordinate > dominant
<b>Dunnock</b> <i>Prunella modularis</i>			
<b>Arctic char</b> <i>Salvelinus alpinus</i>			
<b>Ant</b> <i>Cardiocondyla obscurior</i>	Fighter vs disperser	Dispersers face greater SC risk	Disperser > fighter
<b>Atlantic horseshoe crab</b> <i>Limulus polyphemus</i>	Guarder vs satellite	Satellites face greater SC risk	Satellite > guarder
<b>Quacking frog</b> <i>Crinia georgiana</i>	Guarder vs sneaker	Sneakers face greater SC risk	Sneaker > guarder
<b>Slender inshore squid</b> <i>Doryteuthis plei</i>			
<b>European earwig</b> <i>Forficula auricularia</i>			
<b>Black goby</b> <i>Gobius niger</i>			
<b>Wellington tree weta</b> <i>Hemideina crassidens</i>			
<b>Spear squid</b> <i>Heterololigo bleekeri</i>			
<b>Longear sunfish</b> <i>Lepomis megalotis</i>			
<b>Masu salmon</b> <i>Oncorhynchus masou</i>			
<b>Chinook salmon</b> <i>Oncorhynchus tshawytscha</i>			
<b>Plainfin midshipman</b> <i>Porichthys notatus</i>			
<b>European bitterling</b> <i>Rhodeus amarus</i>			
<b>Peacock blenny</b> <i>Salaria pavo</i>			
<b>Atlantic salmon</b> <i>Salmo salar</i>			
<b>Brown trout</b> <i>Salmo trutta</i>			
<b>Harvestman</b> <i>Serracutisoma proximum</i>			
<b>Bluehead wrasse</b> <i>Thalassoma bifasciatum</i>			
<b>Grass goby</b> <i>Zosterisessor ophiocephalus</i>			
<b>Dung beetle</b> <i>Lethrus apterus</i>	Major vs minor	Minors face greater SC risk	Minor > major
<b>Dung beetle</b> <i>Onthophagus aeruginosis</i>			

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3	<b>Dung beetle</b> <i>Onthophagus alcyonides</i>			
4	<b>Dung beetle</b> <i>Onthophagus australis</i>			
5	<b>Dung beetle</b> <i>Onthophagus binodis</i>			
6	<b>Dung beetle</b> <i>Onthophagus cribripennis</i>			
7	<b>Dung beetle</b> <i>Onthophagus fuliginosus</i>			
8	<b>Dung beetle</b> <i>Onthophagus gazella</i>			
9	<b>Dung beetle</b> <i>Onthophagus haagi</i>			
10	<b>Dung beetle</b> <i>Onthophagus hecate</i>			
11	<b>Dung beetle</b> <i>Onthophagus nigriventris</i>			
12	<b>Dung beetle</b> <i>Onthophagus nodulifer</i>			
13	<b>Dung beetle</b> <i>Onthophagus rupicapra</i>			
14	<b>Dung beetle</b> <i>Onthophagus sloanei</i>			
15	<b>Dung beetle</b> <i>Onthophagus taurus</i>			
16	<b>Dung beetle</b> <i>Onthophagus vermiculatus</i>			
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18	<b>Seba's short-tailed bat</b> <i>Carollia perspicillata</i>	Harem vs sneaker	Sneakers face greater SC risk	Sneaker > harem
19	<b>Cichlid</b> <i>Neolamprologus mondabu</i>			
20	<b>Dunnock</b> <i>Prunella modularis</i>	Monogamous vs polyandrous	Polyandrous males face greater SC risk	Polyandrous > monogamous
21	<b>Dusky frillgoby</b> <i>Bathygobius fuscus</i>	Nesting vs sneaker	Sneakers face greater SC risk	Sneaker > nesting
22	<b>Cichlid</b> <i>Lamprologus callipterus</i>			
23	<b>Cichlid</b> <i>Lamprologus lemairii</i>			
24	<b>Sand goby</b> <i>Pomatoschistus minutus</i>			
25	<b>Molly Miller</b> <i>Scartella cristata</i>			
26	<b>Corkwing wrasse</b> <i>Symphodus melops</i>			
27	<b>Ocellated wrasse</b> <i>Symphodus ocellatus</i>			
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29	<b>Tree swallow</b> <i>Tachycineta bicolor</i>	Paired vs extra-pair	Extra-pair males face greater SC risk	Extra-pair > paired
30	<b>Cichlid</b> <i>Amatitlania siquia</i>	Parental vs sneaker	Sneakers face greater SC risk	Sneaker > parental
31	<b>Three-spined stickleback</b> <i>Gasterosteus aculeatus</i>			
32	<b>Pumpkinseed</b> <i>Lepomis gibbosus</i>			
33	<b>Bluegill</b> <i>Lepomis macrochirus</i>			
34	<b>Round goby</b> <i>Neogobius melanostomus</i>			
35	<b>Cichlid</b> <i>Telmatochromis temporalis</i>			
36	<b>Cichlid</b> <i>Telmatochromis vittatus</i>			
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38	<b>Common shrew</b> <i>Sorex araneus</i>	Resident vs searcher	Searchers face greater SC risk	Searcher > resident
39	<b>Bluenose shiner</b> <i>Pteronotopsis welaka</i>	Territorial vs non-territorial	Non-territorial males face greater SC risk	Non-territorial > territorial
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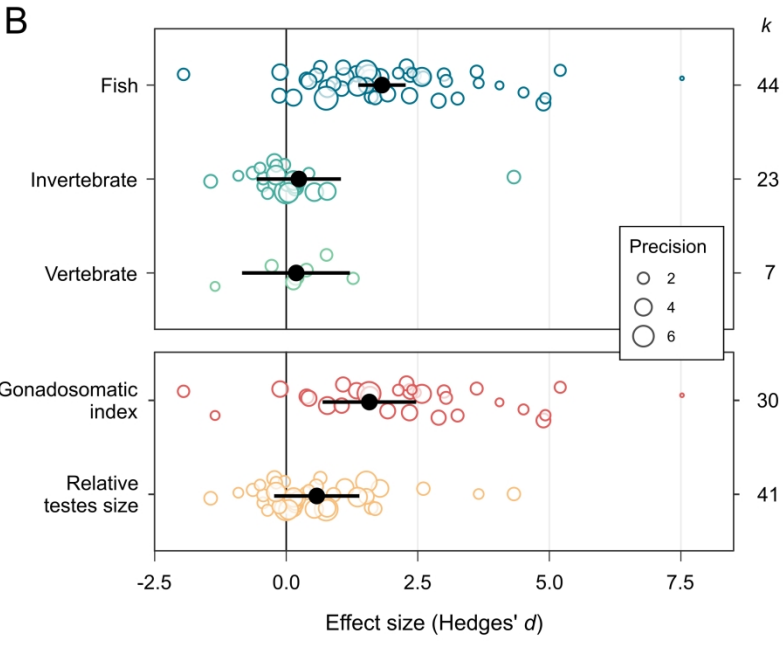
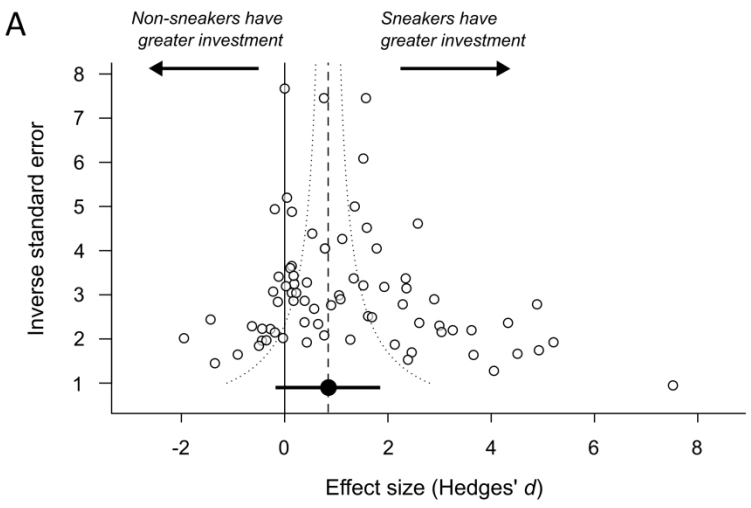
<b>Cortez triplefin</b> <i>Axoclinus nigricaudus</i>	Territorial vs sneaker	Sneakers face greater SC risk	Sneaker > territorial
<b>Painted dragon</b> <i>Ctenophorus pictus</i>			
<b>Carmine triplefin</b> <i>Enneanectes carminalis</i>			
<b>Ruff</b> <i>Calidris pugnax</i>	Territorial vs female mimic	Female-mimics invest less in courtship	Female mimic > territorial
<b>Melanzona guppy</b> <i>Poecilia parae</i>	Consensual vs coercive matings	Coercive males invest less in courtship	Coercive > consensual
<b>Guppy</b> <i>Poecilia reticulata</i>			
<b>Panuco swordtail</b> <i>Xiphophorus nigrensis</i>			

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Table 2. Meta-regression results for all three data sets. Each moderator variable was tested using a separate meta-regression model.  $k$  is the number of effect sizes included in each test. The  $Q_M$  statistic tests whether the moderator variable significantly influences the mean effect size. Marginal  $R^2$  is the amount of variance explained by each moderator. Significant effects are highlighted in grey.

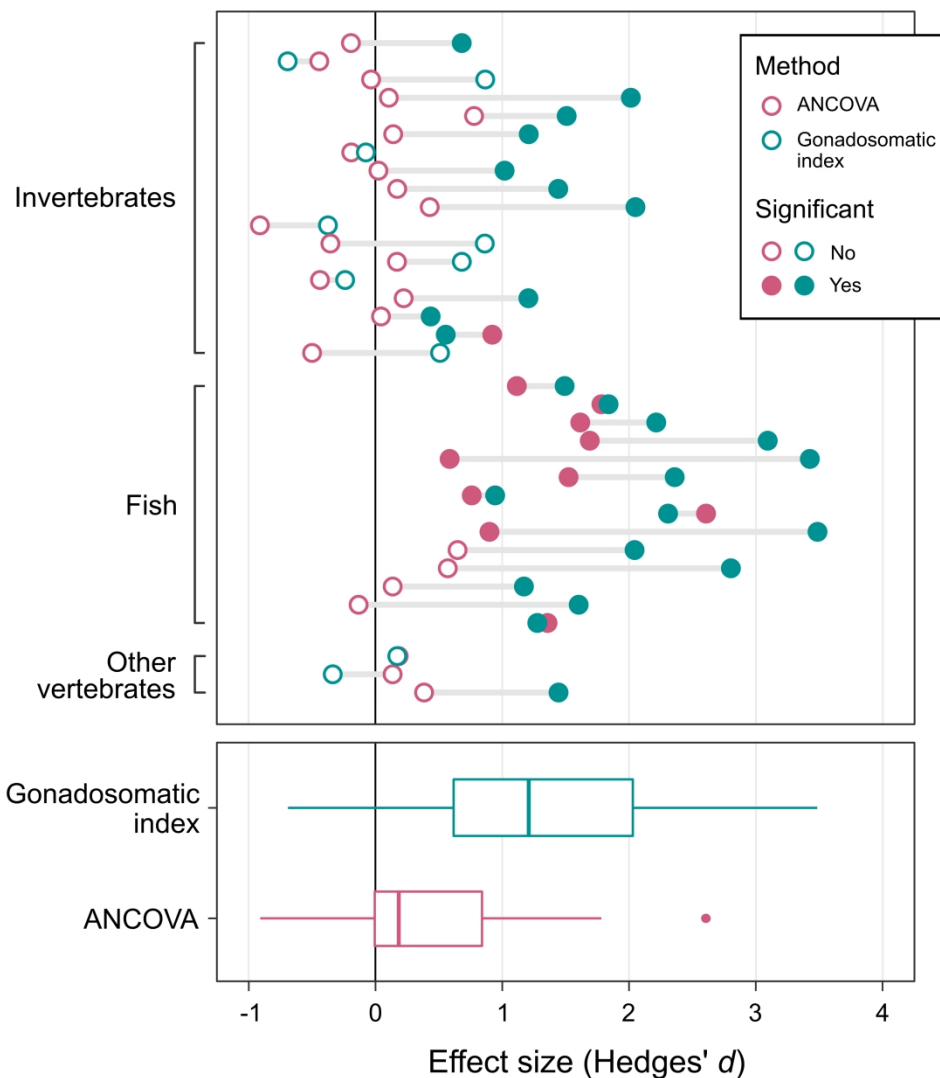
Factor	Testes size				Sperm quantity				Sperm traits			
	$k$	$Q_M$	$P$	Marginal $R^2$	$k$	$Q_M$	$P$	Marginal $R^2$	$k$	$Q_M$	$P$	Marginal $R^2$
Taxonomic group	74	16.37	<0.001	0.29	49	0.84	0.66	0.11	128	0.05	0.97	0.002
Mode of fertilisation	74	17.34	<0.001	0.30	49	0.17	0.68	0.001	128	0.23	0.63	0.004
Tactic type	73	0.24	0.63	0.01	45	1.03	0.31	0.02	121	1.38	0.50	0.03
Measurement	71	8.42	0.004	0.12	48	10.23	0.006	0.12	-	-	-	-
Sperm trait	-	-	-	-	-	-	-	-	128	18.52	0.001	0.18
Sperm allocation vs expenditure	-	-	-	-	49	1.43	0.23	0.02	-	-	-	-
Sneaker frequency (linear)	62	0.07	0.79	0.001	31	5.77	0.02	0.08	89	1.39	0.24	0.04
Sneaker frequency (quadratic)	62	0.07	0.96	0.001	31	9.1	0.01	0.13	89	1.37	0.5	0.04
Study precision	74	7.54	0.01	0.07	49	0.20	0.66	0.002	128	0.002	0.97	<0.001
Publication year	74	3.38	0.07	0.04	49	4.03	0.04	0.05	128	3.67	0.06	0.06

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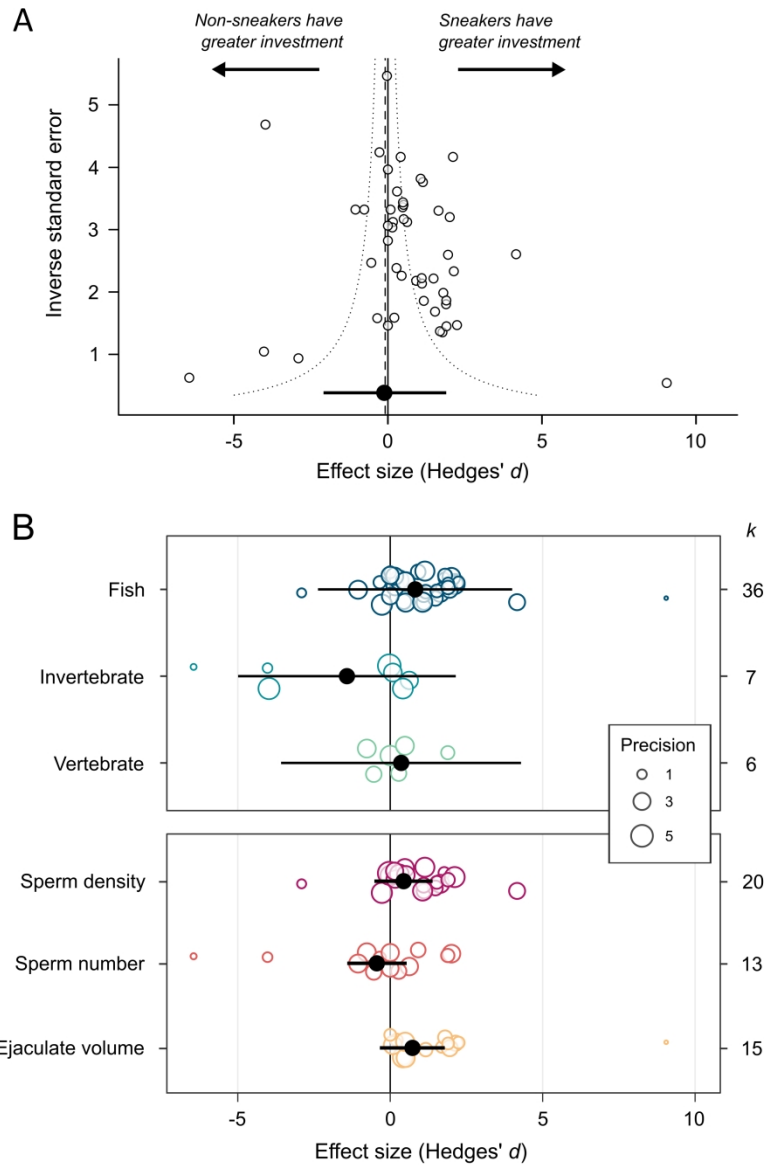
Difference in testes size (Hedges'  $d$ ) between male alternative reproductive tactics (ARTs) in relation to (A) study variance, and (B) taxonomic group (top panel) and size measure (bottom panel). In A, the dashed vertical line represents the meta-analytic mean, and the dotted lines are the 95% pseudo-confidence interval. In B, points are scaled according to study variance (precision). In all panels, black points represent the meta-analytic mean, and black bars show the 95% confidence interval.  $k$  = number of effect sizes for each category.

281x398mm (236 x 236 DPI)



Comparison of two methods for comparing the difference in relative testes size (Hedges' *d*) between male alternative reproductive tactics (ARTs): the gonadosomatic index (blue points) or ANCOVA (red points). Horizontal lines connect effect size estimates derived from the same raw data. Filled and open circles represent cases in which a statistical test (either a t-test or ANCOVA) detected a significant or non-significant difference, respectively in relative testes size between ARTs.

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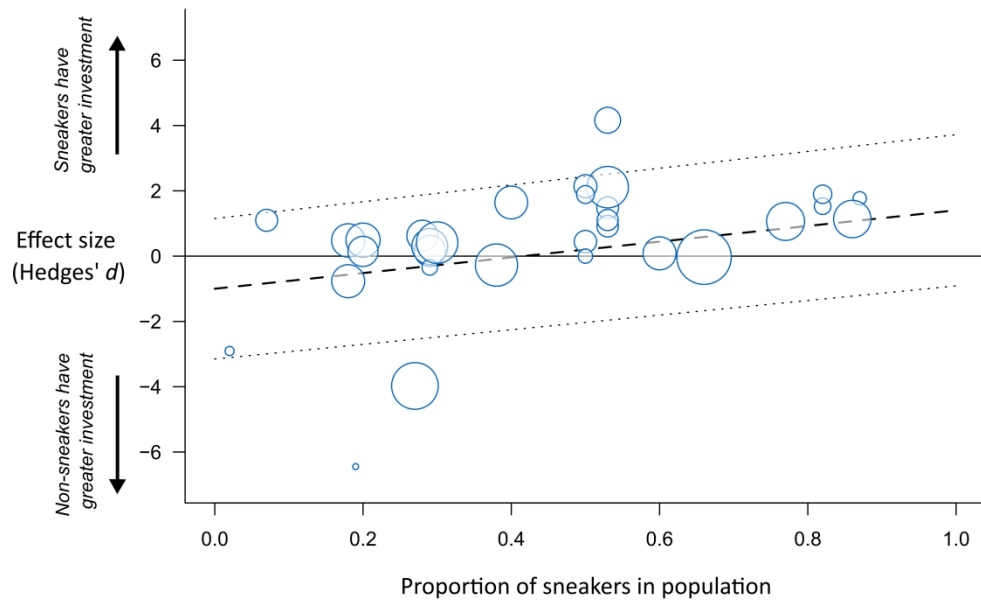


45 Difference in sperm quantity (Hedges'  $d$ ) between male alternative reproductive tactics (ARTs) in relation to  
 46 (A) study variance (precision), and (B) taxonomic group (top panel) and quantity measure (bottom panel).  
 47 In A, the dashed vertical line represents the meta-analytic mean, and the dotted lines are the 95% pseudo-  
 48 confidence interval. In B, points are scaled according to study variance (precision). In all panels, black  
 49 points represent the meta-analytic mean, and black bars show the 95% confidence interval.  $k$  = number of  
 50 effect sizes for each category.

51 304x440mm (236 x 236 DPI)

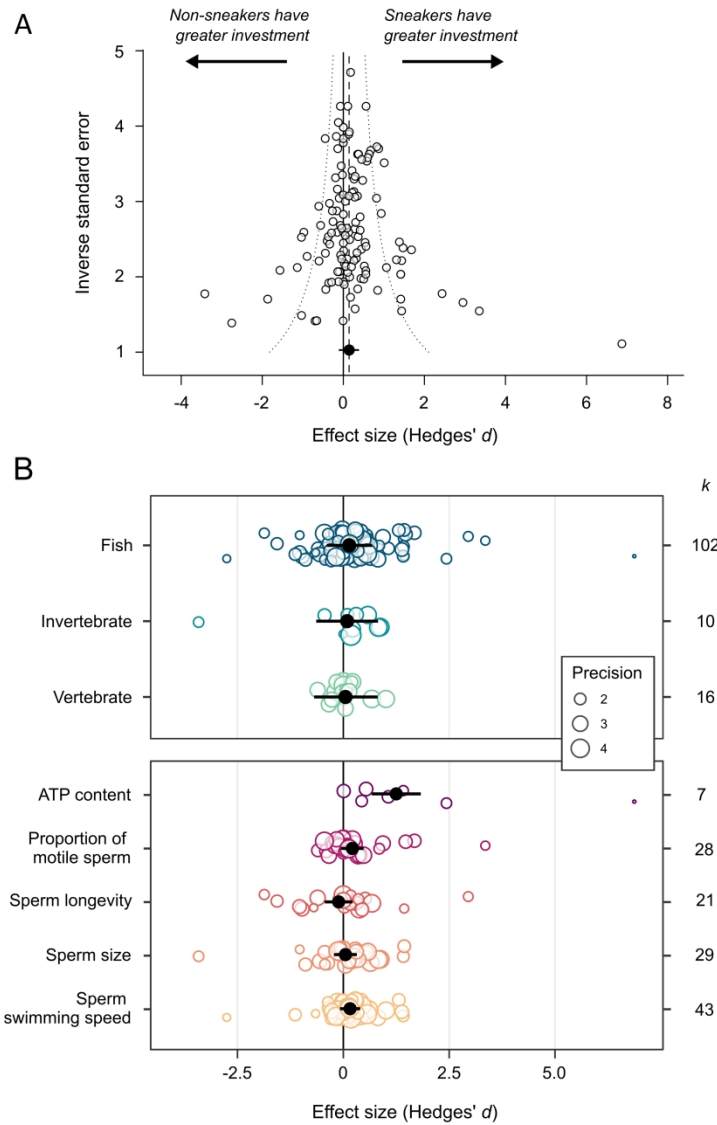
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The relationship between the proportion of sneakers in the population and the difference in sperm quantity between male alternative reproductive tactics (ARTs). Each bubble represents an effect size ( $N = 53$ ), with bubble size scaled to effect size precision (inverse standard error; larger bubbles reflect studies with larger sample sizes). The dashed line shows the predicted line from a meta-regression including sneaker frequency as a covariate. Dotted lines show the 95% confidence intervals for the predicted line.

501x314mm (236 x 236 DPI)



45 Difference in sperm traits (Hedges'  $d$ ) between male alternative reproductive tactics (ARTs) in relation to (A)

46 study variance (precision), and (B) taxonomic group (top panel) and sperm trait (bottom panel). In A, the

47 dashed vertical line represents the meta-analytic mean, and the dotted lines are the 95% pseudo-confidence

48 interval. In B, points are scaled according to study variance (precision). In all panels, black points represent

49 the meta-analytic mean, and black bars show the 95% confidence interval.  $k$  = number of effect sizes for

50 each category.

51 278x431mm (236 x 236 DPI)

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Dougherty et al., 2022 PRISMA checklist

## Appendix S1: PRISMA-EcoEvo checklist

Male alternative reproductive tactics and sperm competition: a meta-analysis

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## Dougherty et al., 2022 PRISMA checklist

The PRISMA-EcoEvo extension was published in 2021 (O’Dea *et al.*, 2021). It consists of a 27-item checklist and guidance for reporting systematic reviews and meta-analyses of primary research in ecology and evolutionary biology. Within each item, sub-items are given a percentage score (calculated using the Shiny app: <https://prisma-ecoevo.shinyapps.io/checklist/>). Higher item scores thus indicate that a higher proportion of sub-items are reported in the manuscript.

Checklist item	Item score	Sub-item number	Sub-item	Reported by authors?	Notes
<b>Title and abstract</b>	<b>100%</b>	1.1	Identify the review as a systematic review, meta-analysis, or both	Yes	Page 2
		1.2	Summarise the aims and scope of the review	Yes	Page 2
		1.3	Describe the data set	Yes	Page 2
		1.4	State the results of the primary outcome	Yes	Page 2-3
		1.5	State conclusions	Yes	Page 3
		1.6	State limitations	Yes	Page 2-3
<b>Aims and questions</b>	<b>80%</b>	2.1	Provide a rationale for the review	Yes	Page 9
		2.2	Reference any previous reviews or meta-analyses on the topic	Yes	Page 9
		2.3	State the aims and scope of the review (including its generality)	Yes	Page 14
		2.4	State the primary questions the review addresses (e.g. which moderators were tested)	Yes	Pages 14-15
		2.5	Describe whether effect sizes were derived from experimental and/or observational comparisons	No	n/a

## Dougherty et al., 2022 PRISMA checklist

Checklist item	Item score	Sub-item number	Sub-item	Reported by authors?	Notes
<b>Review registration</b>	<b>0%</b>	3.1	Register review aims, hypotheses (if applicable), and methods in a time-stamped and publicly accessible archive and provide a link to the registration in the methods section of the manuscript. Ideally registration occurs before the search, but it can be done at any stage before data analysis.	No	n/a
		3.2	Describe deviations from the registered aims and methods	No	n/a
		3.3	Justify deviations from the registered aims and methods	No	n/a
<b>Eligibility criteria</b>	<b>100%</b>	4.1	Report the specific criteria used for including or excluding studies when screening titles and/or abstracts, and full texts, according to the aims of the systematic review (e.g. study design, taxa, data availability)	Yes	Pages 17-21
		4.2	Justify criteria, if necessary (i.e. not obvious from aims and scope)	Yes	Pages 17-21
<b>Finding studies</b>	<b>100%</b>	5.1	Define the type of search (e.g. comprehensive search, representative sample)	Yes	Pages 15-17
		5.2	State what sources of information were sought (e.g. published and unpublished studies, personal communications)	Yes	Page 15
		5.3	Include, for each database searched, the exact search strings used, with keyword combinations and Boolean operators	Yes	Pages 15-16

## Dougherty et al., 2022 PRISMA checklist

Checklist item	Item score	Sub-item number	Sub-item	Reported by authors?	Notes
		5.4	Provide enough information to repeat the equivalent search (if possible), including the timespan covered (start and end dates)	Yes	Page 15
<b>Study selection</b>	<b>100%</b>	6.1	Describe how studies were selected for inclusion at each stage of the screening process (e.g. use of decision trees, screening software)	Yes	Page 17
		6.2	Report the number of people involved and how they contributed (e.g. independent parallel screening)	Yes	Page 17
<b>Data collection process</b>	<b>67%</b>	7.1	Describe where in the reports data were collected from (e.g. text or figures)	Yes	Page 21-22
		7.2	Describe how data were collected (e.g. software used to digitize figures, external data sources)	Yes	Page 22
		7.3	Describe moderator variables that were constructed from collected data (e.g. number of generations calculated from years and average generation time)	No	n/a
		7.4	Report how missing or ambiguous information was dealt with during data collection (e.g. authors of original studies were contacted for missing descriptive statistics, and/or effect sizes were calculated from test statistics)	Yes	Page 22
		7.5	Report who collected data	Yes	Page 22

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## Dougherty et al., 2022 PRISMA checklist

Checklist item	Item score	Sub-item number	Sub-item	Reported by authors?	Notes
		7.6	State the number of extractions that were checked for accuracy by co-authors	No	n/a
<b>Data items</b>	<b>100%</b>	8.1	Describe the key data sought from each study	Yes	Page 21-22
		8.2	Describe items that do not appear in the main results, or which could not be extracted due to insufficient information	Yes	Page 21-22
		8.3	Describe main assumptions or simplifications that were made (e.g. categorising both 'length' and 'mass' as 'morphology')	Yes	Pages 17-21
		8.4	Describe the type of replication unit (e.g. individuals, broods, study sites)	Yes	Page 27
<b>Assessment of individual study quality</b>	<b>0%</b>	9.1	Describe whether the quality of studies included in the systematic review or meta-analysis was assessed (e.g. blinded data collection, reporting quality, experimental versus observational)	No	n/a
		9.2	Describe how information about study quality was incorporated into analyses (e.g. meta-regression and/or sensitivity analysis)	No	n/a
<b>Effect size measures</b>	<b>100%</b>	10.1	Describe effect size(s) used	Yes	Page 21
		10.2	Provide a reference to the equation of each calculated effect size (e.g. standardised mean difference, log response ratio) and (if applicable) its sampling variance	Yes	Pages 21-22

## Dougherty et al., 2022 PRISMA checklist

Checklist item	Item score	Sub-item number	Sub-item	Reported by authors?	Notes
		10.3	If no reference exists, derive the equations for each effect size and state the assumed sampling distribution(s)	No	n/a
<b>Missing data</b>	<b>0%</b>	11.1	Describe any steps taken to deal with missing data during analysis (e.g. imputation, complete case, subset analysis)	No	n/a
		11.2	Justify the decisions made to deal with missing data	No	n/a
<b>Meta-analytic model description</b>	<b>100%</b>	12.1	Describe the models used for synthesis of effect sizes	Yes	Pages 27-28
		12.2	The most common approach in ecology and evolution will be a random-effects model, often with a hierarchical/multilevel structure. If other types of models are chosen (e.g. common/fixed effects model, unweighted model), provide justification for this choice	No	n/a
<b>Software</b>	<b>100%</b>	13.1	Describe the statistical platform used for inference (e.g. R)	Yes	Page 27
		13.2	Describe the packages used to run models	Yes	Page 27
		13.3	Describe the functions used to run models	Yes	Page 27
		13.4	Describe any arguments that differed from the default settings	No	n/a
		13.5	Describe the version numbers of all software used	Yes	Page 27



## Dougherty et al., 2022 PRISMA checklist

Checklist item	Item score	Sub-item number	Sub-item	Reported by authors?	Notes
<b>Non-independence</b>	<b>100%</b>	14.1	Describe the types of non-independence encountered (e.g. phylogenetic, spatial, multiple measurements over time)	Yes	Pages 27-28
		14.2	Describe how non-independence has been handled	Yes	Pages 27-28
		14.3	Justify decisions made	Yes	Pages 27-28
<b>Meta-regression and model selection</b>	<b>50%</b>	15.1	Provide a rationale for the inclusion of moderators (covariates) that were evaluated in meta-regression models	Yes	Page 28
		15.2	Justify the number of parameters estimated in models, in relation to the number of effect sizes and studies (e.g. interaction terms were not included due to insufficient sample sizes)	No	n/a
		15.3	Describe any process of model selection	No	n/a
<b>Publication bias and sensitivity analysis</b>	<b>100%</b>	16.1	Describe assessments of the risk of bias due to missing results (e.g. publication, time-lag, and taxonomic biases)	Yes	Page 29
		16.2	Describe any steps taken to investigate the effects of such biases (if present)	Yes	Page 29

## Dougherty et al., 2022 PRISMA checklist

Checklist item	Item score	Sub-item number	Sub-item	Reported by authors?	Notes
		16.3	Describe any other analyses of robustness of the results, e.g. due to effect size choice, weighting or analytical model assumptions, inclusion or exclusion of subsets of the data, or the inclusion of alternative moderator variables in meta-regressions	Yes	Page 28
<b>Clarification of post hoc analyses</b>	<b>0%</b>	17.1	When hypotheses were formulated after data analysis, this should be acknowledged.	No	n/a
<b>Metadata, data, and code</b>	<b>100%</b>	18.1	Share metadata (i.e. data descriptions)	Yes	10.6084/m9.figshare.19174604
		18.2	Share data required to reproduce the results presented in the manuscript	Yes	10.6084/m9.figshare.19174604
		18.3	Share additional data, including information that was not presented in the manuscript (e.g. raw data used to calculate effect sizes, descriptions of where data were located in papers)	Yes	10.6084/m9.figshare.19174604
		18.4	Share analysis scripts (or, if a software package with graphical user interface (GUI) was used, then describe full model specification and fully specify choices)	Yes	10.6084/m9.figshare.19174604
<b>Results of study selection process</b>	<b>100%</b>	19.1	Report the number of studies screened	Yes	Figure 1
		19.2	Report the number of studies excluded at each stage of screening	Yes	Figure 1

## Dougherty et al., 2022 PRISMA checklist

Checklist item	Item score	Sub-item number	Sub-item	Reported by authors?	Notes
		19.3	Report brief reasons for exclusion from the full text stage	Yes	Figure 1
		19.4	Present a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-like flowchart ( <a href="http://www.prisma-statement.org">www.prisma-statement.org</a> ).	Yes	Figure 1
<b>Sample sizes and study characteristics</b>	<b>80%</b>	20.1	Report the number of studies and effect sizes for data included in meta-analyses	Yes	Pages 29-33
		20.2	Report the number of studies and effect sizes for subsets of data included in meta-regressions	Yes	Pages 29-33
		20.3	Provide a summary of key characteristics for reported outcomes (either in text or figures; e.g. one quarter of effect sizes reported for vertebrates and the rest invertebrates)	Yes	Pages 29-33
		20.4	Provide a summary of limitations of included moderators (e.g. collinearity and overlap between moderators)	Yes	Pages 29-33
		20.5	Provide a summary of characteristics related to individual study quality (risk of bias)	No	n/a
<b>Meta-analysis</b>	<b>100%</b>	21.1	Provide a quantitative synthesis of results across studies, including estimates for the mean effect size, with confidence/credible intervals	Yes	Pages 29-33

## Dougherty et al., 2022 PRISMA checklist

Checklist item	Item score	Sub-item number	Sub-item	Reported by authors?	Notes
<b>Heterogeneity</b>	<b>100%</b>	22.1	Report indicators of heterogeneity in the estimated effect (e.g. $I^2$ , tau <sup>2</sup> and other variance components)	Yes	Pages 29-33
<b>Meta-regression</b>	<b>50%</b>	23.1	Provide estimates of meta-regression slopes (i.e. regression coefficients) and confidence/credible intervals	Yes	Pages 30-34
		23.2	Include estimates and confidence/credible intervals for all moderator variables that were assessed (i.e. complete reporting)	Yes	Tables S2-S4
		23.3	Report interactions, if they were included	No	n/a
		23.4	Describe outcomes from model selection, if done (e.g. $R^2$ and AIC)	No	n/a
<b>Outcomes of publication bias and sensitivity analysis</b>	<b>100%</b>	24.1	Provide results for the assessments of the risks of bias (e.g. Egger's regression, funnel plots)	Yes	Pages 29-33
		24.2	Provide results for the robustness of the review's results (e.g. subgroup analyses, meta-regression of study quality, results from alternative methods of analysis, and temporal trends)	Yes	Pages 30-33
<b>Discussion</b>	<b>100%</b>	25.1	Summarise the main findings in terms of the magnitude of effect	Yes	Pages 33-34
		25.2	Summarise the main findings in terms of the precision of effects (e.g. size of confidence intervals, statistical significance)	Yes	Pages 33-34

## Dougherty et al., 2022 PRISMA checklist

Checklist item	Item score	Sub-item number	Sub-item	Reported by authors?	Notes
		25.3	Summarise the main findings in terms of their heterogeneity	Yes	Page 42
		25.4	Summarise the main findings in terms of their biological/practical relevance	Yes	Pages 33-36
		25.5	Compare results with previous reviews on the topic, if available	Yes	Page 33
		25.6	Consider limitations and their influence on the generality of conclusions, such as gaps in the available evidence (e.g. taxonomic and geographical research biases)	Yes	Pages 33-40
<b>Contributions and funding</b>	<b>100%</b>	26.1	Provide names, affiliations, and funding sources of all co-authors	Yes	Pages 1, 41
		26.2	List the contributions of each co-author	Yes	Page 42
		26.3	Provide contact details for the corresponding author	Yes	Page 1
		26.4	Disclose any conflicts of interest	No	n/a
<b>References</b>	<b>100%</b>	27.1	Provide a reference list of all studies included in the systematic review or meta-analysis	Yes	Pages 42-51
		27.2	List included studies as referenced sources (e.g. rather than listing them in a table or supplement)	Yes	Pages 42-51

Dougherty et al., 2022 supplementary tables and figures

## Supplementary tables and figures:

Male alternative reproductive tactics and sperm competition: a meta-analysis

Liam R. Dougherty<sup>1\*</sup>, Michael J. A. Skirrow<sup>2</sup>, Michael D. Jennions<sup>2</sup>, and Leigh W. Simmons<sup>3</sup>

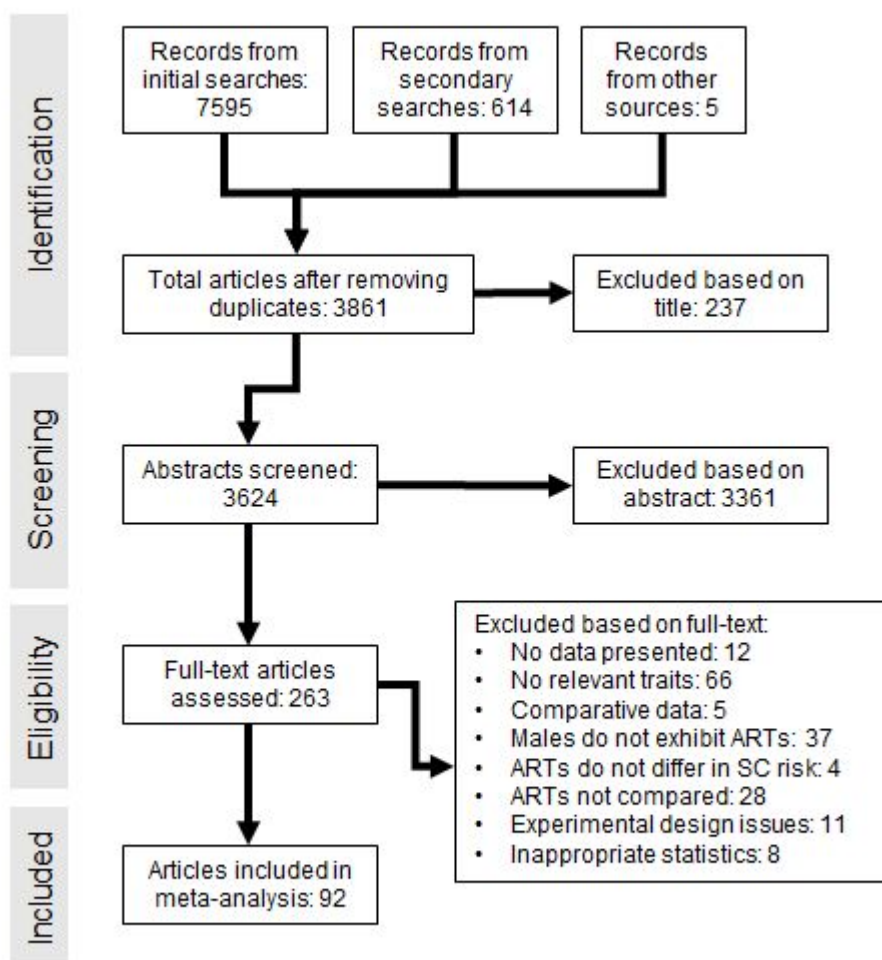
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<sup>2</sup>*Evolution, Ecology and Genetics, Research School of Biology, The Australian National University, Canberra, ACT, 0200, Australia*

<sup>3</sup>*Centre for Evolutionary Biology, School of Biological Sciences, The University of Western Australia, Crawley, WA, 6009, Australia*

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Dougherty et al., 2022 supplementary tables and figures



**Fig. S1.** PRISMA diagram summarising the literature search and study screening processes.

## Dougherty et al., 2022 supplementary tables and figures

**Table S1.** Methods for calculating the standardised mean difference (Hedges'  $d$ ), and the location of the data collected. For the remaining three papers [Hettiey & Roberts (2005), Munguia-Steyer et al. (2012), and Smith (2012)] no directional effect size data was obtained.

Study	Method	Data location
Almeida <i>et al.</i> (2012)	Means and variances	Table 1
Alonzo <i>et al.</i> (2016)	Means and variances	Figure 2
Apostolico & Marian (2017)	Mann-Whitney $U$ test	Table 1
Apostolico & Marian (2018)	Means and variances	Text
Awata <i>et al.</i> (2006)	ANCOVA	Figure 1
Awata <i>et al.</i> (2008)	ANCOVA	Figure 2
	Means and variances	Table 2
Bartlett <i>et al.</i> (2017)	$t$ -test	Supplementary data
Bleeker <i>et al.</i> (2017)	Mann-Whitney $U$ test	Figure 3
Burness <i>et al.</i> (2004)	$t$ -test	Text
	Means and variances	Figure 1
Burness <i>et al.</i> (2005)	Means and variances	Figure 1
	$t$ -test	Text
Butts <i>et al.</i> (2012)	$t$ -test	Text
	Means and variances	Text
Butts <i>et al.</i> (2017)	Means and variances	Figure 3
Byrne (2004)	ANCOVA	Figure 1
	Means and variances	Figure 2
Clotfelter <i>et al.</i> (2017)	Means and variances	Figures 4 & 5
Cote <i>et al.</i> (2009)	Means and variances	Figure 1 & Table 1
Fasel <i>et al.</i> (2017)	Means and variances	Figures 1 & 2
Fitzpatrick <i>et al.</i> (2007)	Means and variances	Figure 1 & Table 2
Fitzpatrick <i>et al.</i> (2016)	ANCOVA	Figure 2
	Means and variances	Figures 3 & 4
Flannery <i>et al.</i> (2013)	Means and variances	Figure 2, text
	ANCOVA	Text
Fletcher (1999)	Means and variances	Table 1
Gage <i>et al.</i> (1995)	Mann-Whitney $U$ test	Text
	ANCOVA	Figure 2
	Means and variances	Text
Goncalves <i>et al.</i> (2008)	Mann-Whitney $U$ test	Figure 2
Green <i>et al.</i> (2020)	Mann-Whitney $U$ test	Figure 2
Hettiey & Roberts (2007)	Means and variances	Table 1
	ANCOVA	Figure 1a
Hurtado-Gonzales & Uy (2009)	ANCOVA	Figure 5
	Means and variances	Table 3 & Text
Iwata <i>et al.</i> (2011)	Means and variances	Text
Jennings & Philipp (1992)	$t$ -test	Text
Katoh <i>et al.</i> (2005)	Means and variances	Figure 2b
Kelly (2008)	ANCOVA	Figure 2
	Means and variances	Text
Kortet <i>et al.</i> (2004)	Means and variances	Figure 1
Koseki & Maekawa (2002)	Means and variances	Text
Kvarnemo <i>et al.</i> (2010)	ANCOVA	Figure 2c
Lara <i>et al.</i> (2020)	Means and variances	Figure 1
Leach & Montgomerie (2000)	$t$ -test	Text
Lenhert <i>et al.</i> (2017)	Means and variances	Text
Lewis & Pitcher (2017b)	Means and variances	Figure 2
Lewis & Pitcher (2017a)	Means and variances	Figure 1 & Figure 2



## Dougherty et al., 2022 supplementary tables and figures

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3	Locatello <i>et al.</i> (2007)	<i>t</i> -test	Text
4		Means and variances	Figure 1 & Figure 2
5	Locatello <i>et al.</i> (2013)	Means and variances	Figure 1
6	Loveland <i>et al.</i> (2021)	ANCOVA	Raw data
7	Makiguchi <i>et al.</i> (2016)	Means and variances	Figure 2
8		ANCOVA	Raw data
9	Marentette <i>et al.</i> (2009)	Means and variances	Figure 4 & Table 1
10	Mazzoldi <i>et al.</i> (2000)	Means and variances	Table 3
11	Meniri <i>et al.</i> (2019)	Means and variances	Table 2
12	Miller <i>et al.</i> (2019)	Mann-Whitney <i>U</i> test	Figure 1b
13	Nakanishi & Takegaki (2019)	Mann-Whitney <i>U</i> test	Figure 2
14		<i>t</i> -test	Text
15		Means and variances	Figure 3
16	Neat (2001)	Means and variances	Figure 5
17		<i>t</i> -test	Text
18	Neat <i>et al.</i> (2003)	Means and variances	Table 1
19	Neff <i>et al.</i> (2003)	Means and variances	Figure 3
20	Olsson <i>et al.</i> (2009)	Means and variances	Text
21	Ota & Kohda (2006)	Means and variances	Figure 3
22	Ota <i>et al.</i> (2010)	Means and variances	Figure 1
23	Ota <i>et al.</i> (2011)	ANCOVA	Figure 2
24	Ota <i>et al.</i> (2014a)	ANCOVA	Figure 5
25	Ota <i>et al.</i> (2014b)	Means and variances	Supplementary data
26	Peer <i>et al.</i> (2000)	Means and variances	Table 1
27	Pilastro & Bisazza (1999)	Mann-Whitney <i>U</i> test	Text
28	Poli <i>et al.</i> (2018)	Means and variances	Figure 1
29	Pujolar <i>et al.</i> (2012)	ANCOVA	Figure 1
30	Rasotto & Mazzoldi (2002)	Means and variances	Figure 2, Table 2
31	Rosa <i>et al.</i> (2019)	Means and variances	Figures 3 & 4
32	Rowe <i>et al.</i> (2010)	Means and variances	Table 1
33	Rudolfson <i>et al.</i> (2006)	Means and variances	Figures 1, 3 & 4
34	Saraiva <i>et al.</i> (2010)	Means and variances	Table 1
35	Sasson <i>et al.</i> (2015)	Means and variances	Text
36	Sato <i>et al.</i> (2004)	Means and variances	Table 1
37	Scharer & Robertson (1999)	Means and variances	Text
38	Schrempf <i>et al.</i> (2016)	Means and variances	Text
39	Schutz <i>et al.</i> (2010)	Means and variances	Text
40	Simmons & Buzatto (2014)	Means and variances	Figure 2
41	Simmons <i>et al.</i> (1999)	Means and variances	Text
42	Simmons <i>et al.</i> (2007)	ANCOVA	Raw data
43	Smith & Reichard (2013)	Paired <i>t</i> -test	Text
44	Smith & Ryan (2010)	Means and variances	Table 1
45		ANCOVA	Figure 1
46	Stockley <i>et al.</i> (1994)	Means and variances	Table 1
47	Stoltz & Neff (2006)	Mann-Whitney <i>U</i> test	Raw data
48		Means and variances	Text
49	Taborsky <i>et al.</i> (2018)	Mann-Whitney <i>U</i> test	Figure 3
50		Means and variances	Figure 2, text
51	Tomkins & Simmons (2002)	ANCOVA	Figure 1d
52	Uglem <i>et al.</i> (2000)	Means and variances	Figure 4
53	Uglem <i>et al.</i> (2001)	<i>t</i> -test	Text
54		Mann-Whitney <i>U</i> test	Text
55	Uglem <i>et al.</i> (2002)	Means and variances	Table 1
56		<i>t</i> -test	Text
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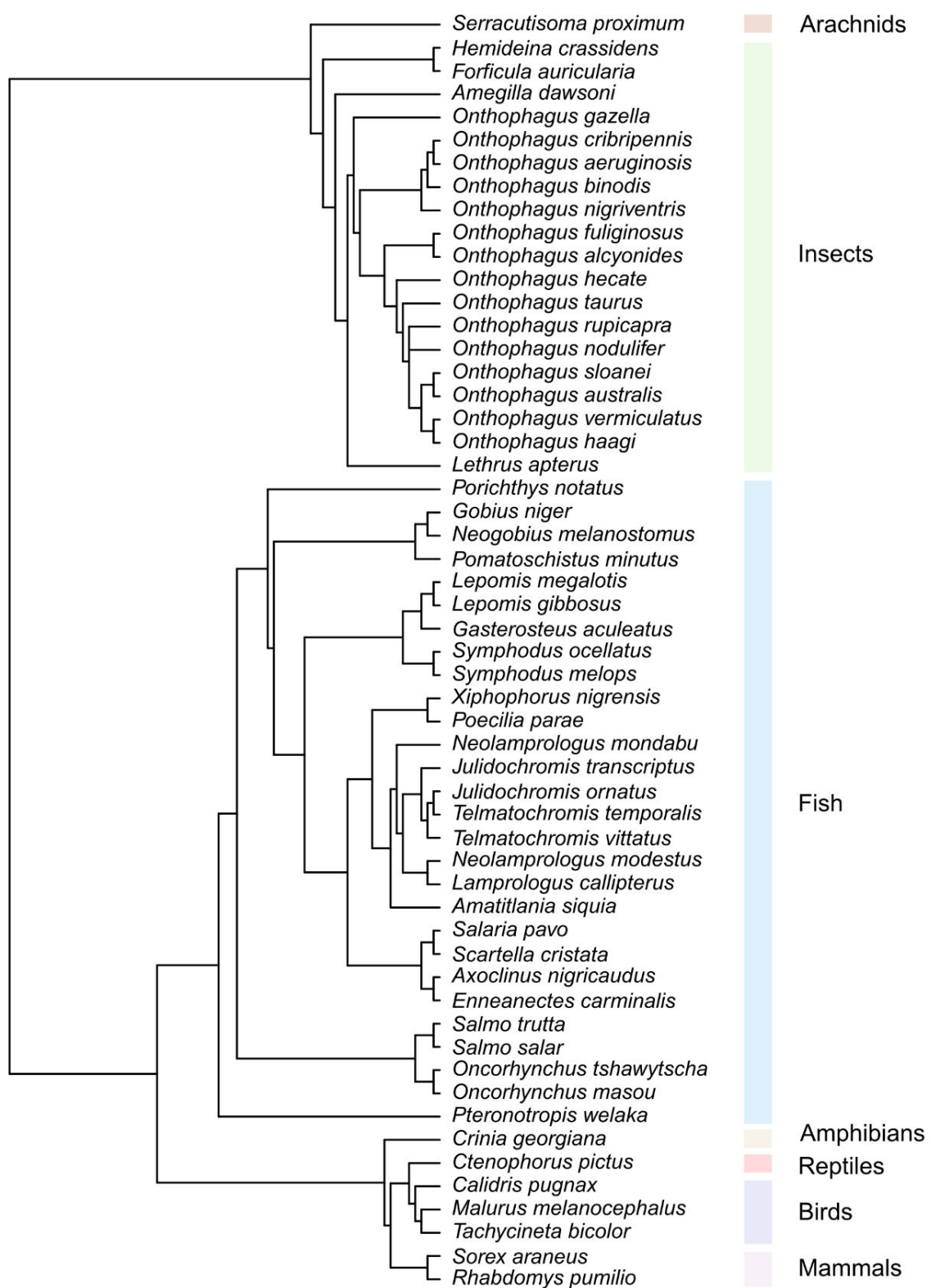
## Dougherty et al., 2022 supplementary tables and figures

		Means and variances	Text
	Vladic (2000)	Mann-Whitney $U$ test	Figure 2a
		Means and variances	Figure 1
	Vladic (2006)	Means and variances	Table 2
	Vladic & Jarvi (2001)	Means and variances	Table 1
	Vladic <i>et al.</i> (2002)	Means and variances	Table 1, text
	Vladic <i>et al.</i> (2010)	Means and variances	Table 1
	Warner & Lejeune (1985)	Means and variances	Table 2
	Yamamoto <i>et al.</i> (2015)	Mann-Whitney $U$ test	Figure 2
		Means and variances	Figure 2, Table 1
	Young <i>et al.</i> (2013)	Means and variances	Raw data

For Review Only

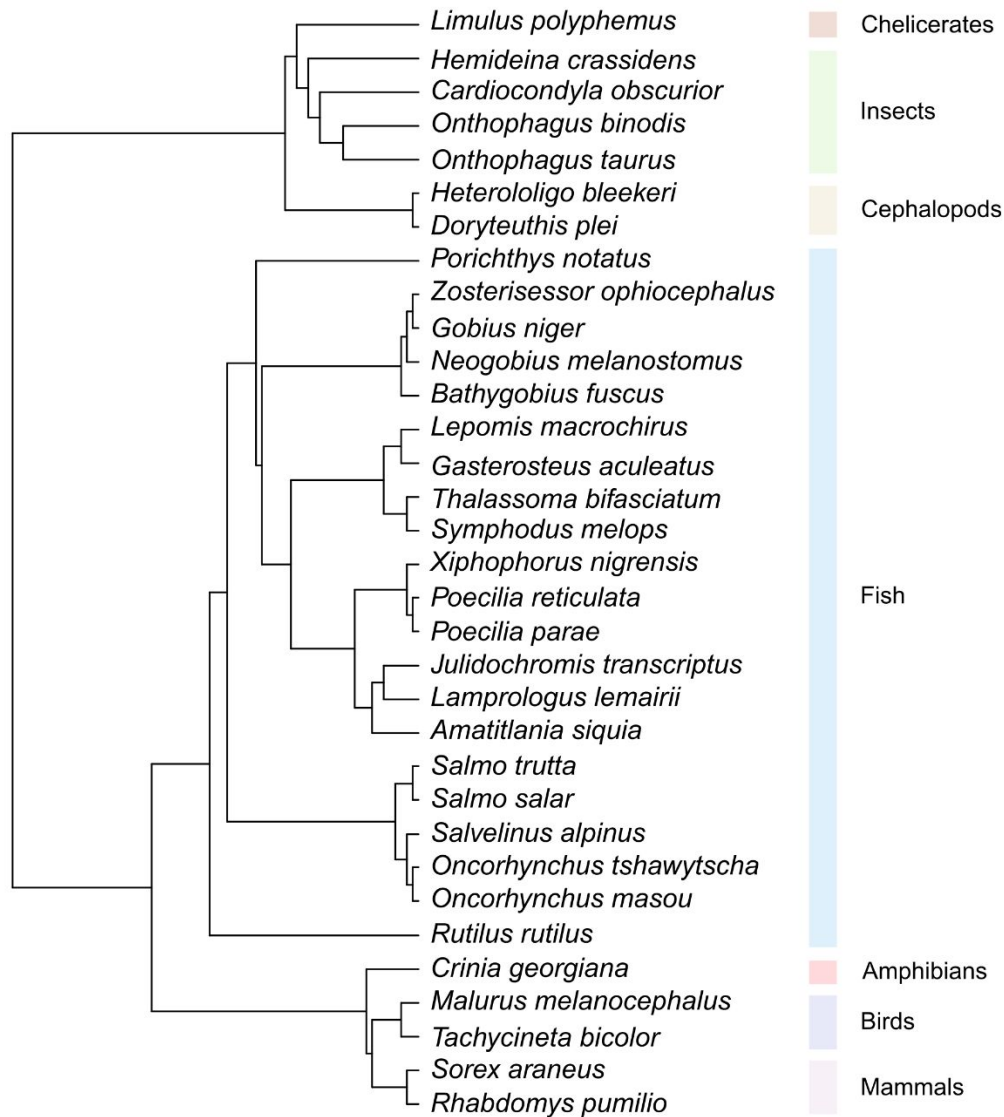
Dougherty et al., 2022 supplementary tables and figures

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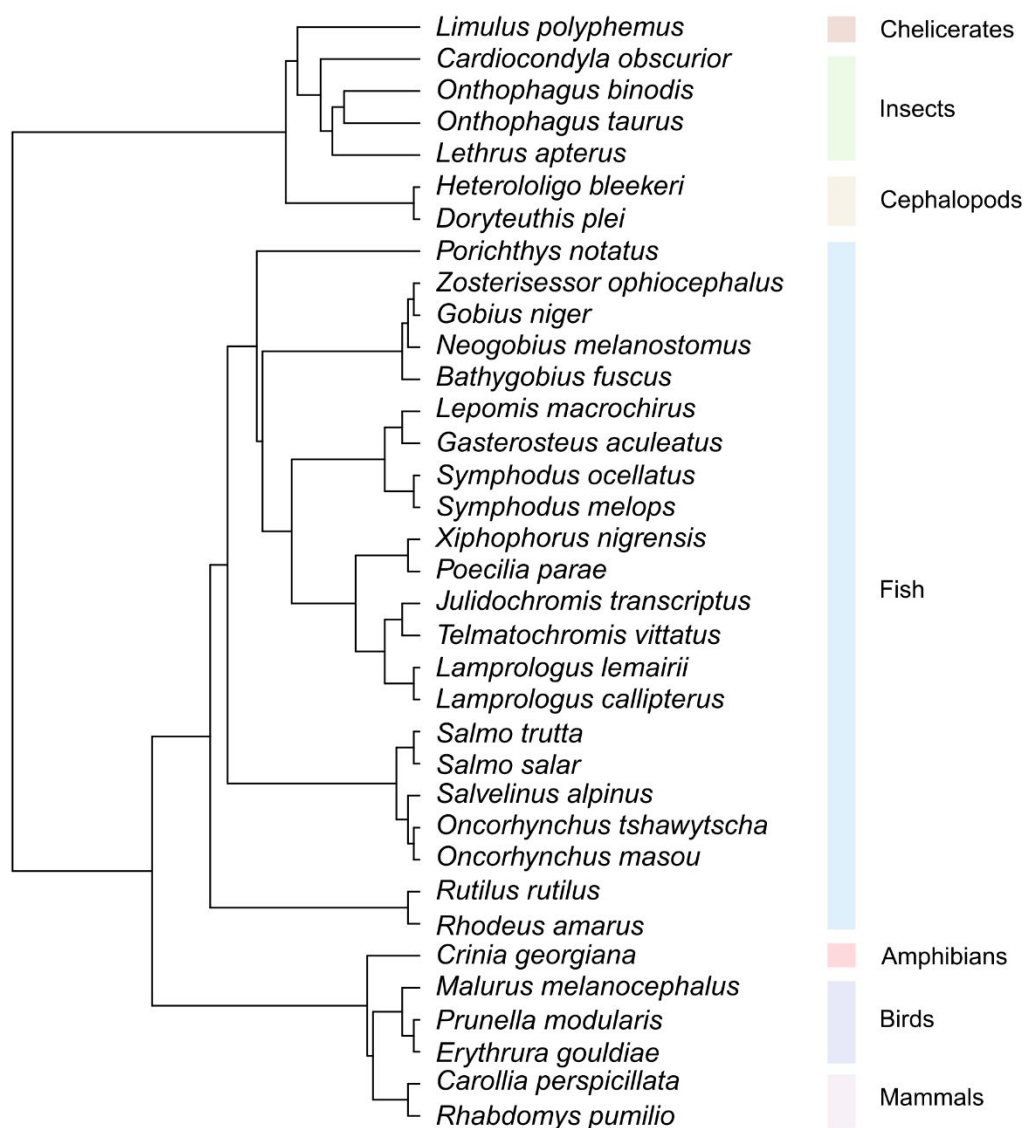
**Fig. S2.** Phylogenetic tree for the 53 species in the testis size data set. Note that the branch lengths are not time-calibrated.

## Dougherty et al., 2022 supplementary tables and figures



**Fig. S3.** Phylogenetic tree for the 32 species in the sperm quantity data set. Note that the branch lengths are not time-calibrated.

Dougherty et al., 2022 supplementary tables and figures



**Fig. S4.** Phylogenetic tree for the 33 species in the sperm traits data set. Note that the branch lengths are not time-calibrated.

Dougherty et al., 2022 supplementary tables and figures

**Table S2.** Sources used for the sneaker frequency data. See main text for references.

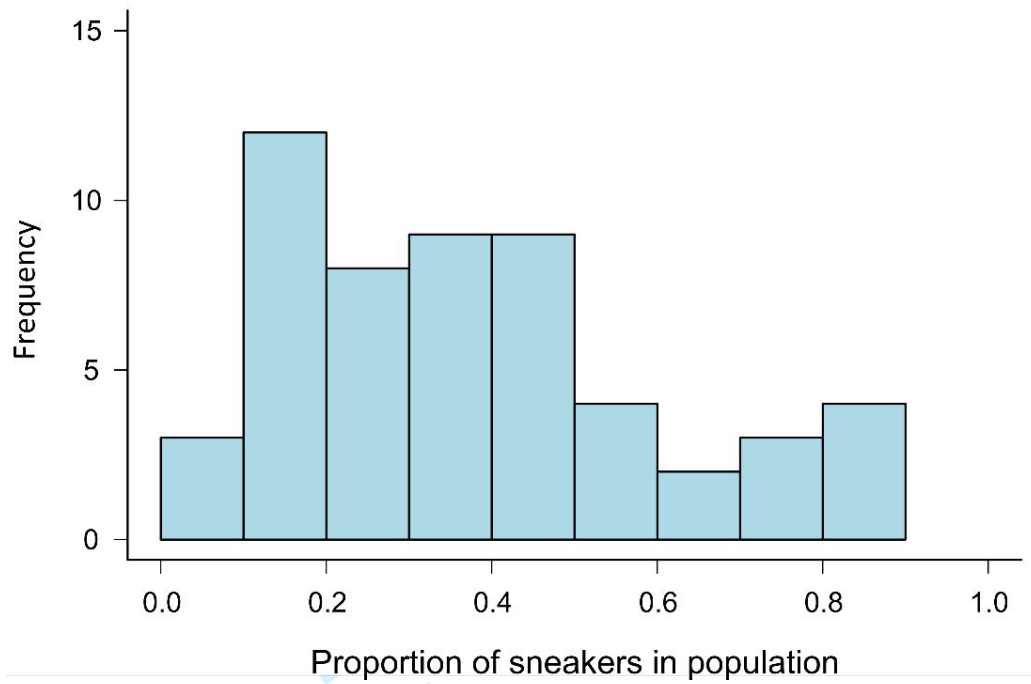
Species	Strategies	Sneaker frequency	Source
<i>Amatitlania siquia</i>	Parental vs sneaker	0.02	Clotfelter <i>et al.</i> (2017)
<i>Axoclinus nigricaudus</i>	Territorial vs sneaker	0.76	Neat (2001)
<i>Bathygobius fuscus</i>	Nesting vs sneaker	0.87	Takegaki <i>et al.</i> (2012)
<i>Carollia perspicillata</i>	Harem vs sneaker	0.8	Fasel <i>et al.</i> (2017)
<i>Ctenophorus pictus</i>	Territorial vs sneaker	0.39	Olsson <i>et al.</i> (2007)
<i>Doryteuthis plei</i>	Guarder vs sneaker	0.19	Iwata & Sakurai (2007)
<i>Enneanectes carminalis</i>	Territorial vs sneaker	0.39	Neat (2001)
<i>Forficula auricularia</i>	Guarder vs sneaker	0.4	Tomkins & Simmons (2002)
<i>Gasterosteus aculeatus</i>	Parental vs sneaker	0.38	Cote <i>et al.</i> (2009)
<i>Gobius niger</i>	Guarder vs sneaker	0.4	Rasotto & Mazzoldi (2002)
<i>Hemideina crassidens</i>	Guarder vs sneaker	0.28	Kelly (2005)
<i>Heterololigo bleekeri</i>	Guarder vs sneaker	0.27	Iwata <i>et al.</i> (2011)
<i>Julidochromis ornatus</i>	Breeder vs cooperative breeder	0.15	Awata <i>et al.</i> (2006)
<i>Lamprologus callipterus</i>	Nesting vs sneaker	0.51	Sato <i>et al.</i> (2004)
<i>Lamprologus lemairii</i>	Nesting vs sneaker	0.5	Ota <i>et al.</i> (2014b)
<i>Lepomis gibbosus</i>	Parental vs sneaker	0.54	Almeida <i>et al.</i> (2012)
<i>Lepomis macrochirus</i>	Parental vs sneaker	0.53	Gross (1982)
<i>Lepomis megalotis</i>	Guarder vs sneaker	0.26	Jennings & Philipp (1992)
<i>Lethrus apterus</i>	Guarder vs sneaker	0.14	Rosa <i>et al.</i> (2019)
<i>Limulus polyphemus</i>	Guarder vs satellite	0.66	Brockmann (1990)
<i>Malurus melanocephalus</i>	Breeder vs helper	0.18	Rowe <i>et al.</i> (2010)
<i>Neogobius melanostomus</i>	Parental vs sneaker	0.5	Marentette <i>et al.</i> (2009)
<i>Neolamprologus mondabu</i>	Harem vs sneaker	0.14	Ota <i>et al.</i> (2014a)
<i>Oncorhynchus masou</i>	Guarder vs sneaker	0.86	Koseki & Maekawa (2002)
<i>Oncorhynchus tshawytscha</i>	Guarder vs sneaker	0.29	Flannery <i>et al.</i> (2013)
<i>Onthophagus aeruginosis</i>	Guarder vs sneaker	0.2	Simmons <i>et al.</i> (2007)
<i>Onthophagus alcyonides</i>	Guarder vs sneaker	0.17	Simmons <i>et al.</i> (2007)
<i>Onthophagus australis</i>	Guarder vs sneaker	0.47	Simmons <i>et al.</i> (2007)
<i>Onthophagus binodis</i>	Guarder vs sneaker	0.3	Simmons <i>et al.</i> (2007)
<i>Onthophagus cribripennis</i>	Guarder vs sneaker	0.45	Simmons <i>et al.</i> (2007)
<i>Onthophagus fuliginosus</i>	Guarder vs sneaker	0.41	Simmons <i>et al.</i> (2007)
<i>Onthophagus gazella</i>	Guarder vs sneaker	0.34	Simmons <i>et al.</i> (2007)
<i>Onthophagus haagi</i>	Guarder vs sneaker	0.32	Simmons <i>et al.</i> (2007)
<i>Onthophagus hecate</i>	Guarder vs sneaker	0.29	Simmons <i>et al.</i> (2007)
<i>Onthophagus nigriventris</i>	Guarder vs sneaker	0.48	Simmons <i>et al.</i> (2007)
<i>Onthophagus nodulifer</i>	Guarder vs sneaker	0.21	Simmons <i>et al.</i> (2007)
<i>Onthophagus rupicapra</i>	Guarder vs sneaker	0.61	Simmons <i>et al.</i> (2007)
<i>Onthophagus sloanei</i>	Guarder vs sneaker	0.17	Simmons <i>et al.</i> (2007)
<i>Onthophagus taurus</i>	Guarder vs sneaker	0.6	Simmons <i>et al.</i> (2007)
<i>Onthophagus vermiculatus</i>	Guarder vs sneaker	0.21	Simmons <i>et al.</i> (2007)
<i>Pomatoschistus minutus</i>	Nesting vs sneaker	0.175	Kvarnemo <i>et al.</i> (2010)
<i>Porichthys notatus</i>	Guarder vs sneaker	0.07	Fitzpatrick <i>et al.</i> (2016)

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3	<i>Pteronotropis welaka</i>	Territorial vs non-territorial	0.5	Fletcher (1999)
4	<i>Rutilus rutilus</i>	Attractive vs unattractive	0.5	Kortet <i>et al.</i> (2004)
5	<i>Salaria pavo</i>	Guarder vs sneaker	0.36	Almada <i>et al.</i> (1994)
6	<i>Scartella cristata</i>	Nesting vs sneaker	0.15	Neat <i>et al.</i> (2003)
7	<i>Serracutisoma proximum</i>	Guarder vs sneaker	0.1	Munguia-Steyer <i>et al.</i> (2012)
8	<i>Sorex araneus</i>	Resident vs searcher	0.5	Stockley <i>et al.</i> (1994)
9	<i>Symphodus melops</i>	Nesting vs sneaker	0.2	Uglem <i>et al.</i> (2000)
10	<i>Symphodus ocellatus</i>	Nesting vs sneaker	0.85	Warner & Lejeune (1985)
11	<i>Telmatochromis temporalis</i>	Parental vs sneaker	0.4	Katoh <i>et al.</i> (2005)
12	<i>Telmatochromis vittatus</i>	Parental vs sneaker	0.14	Ota & Kohda (2006)
13	<i>Thalassoma bifasciatum</i>	Guarder vs sneaker	0.77	Warner & Robertson (1978)
14	<i>Zosterisessor ophiocephalus</i>	Guarder vs sneaker	0.82	Scaggiante <i>et al.</i> (1999)
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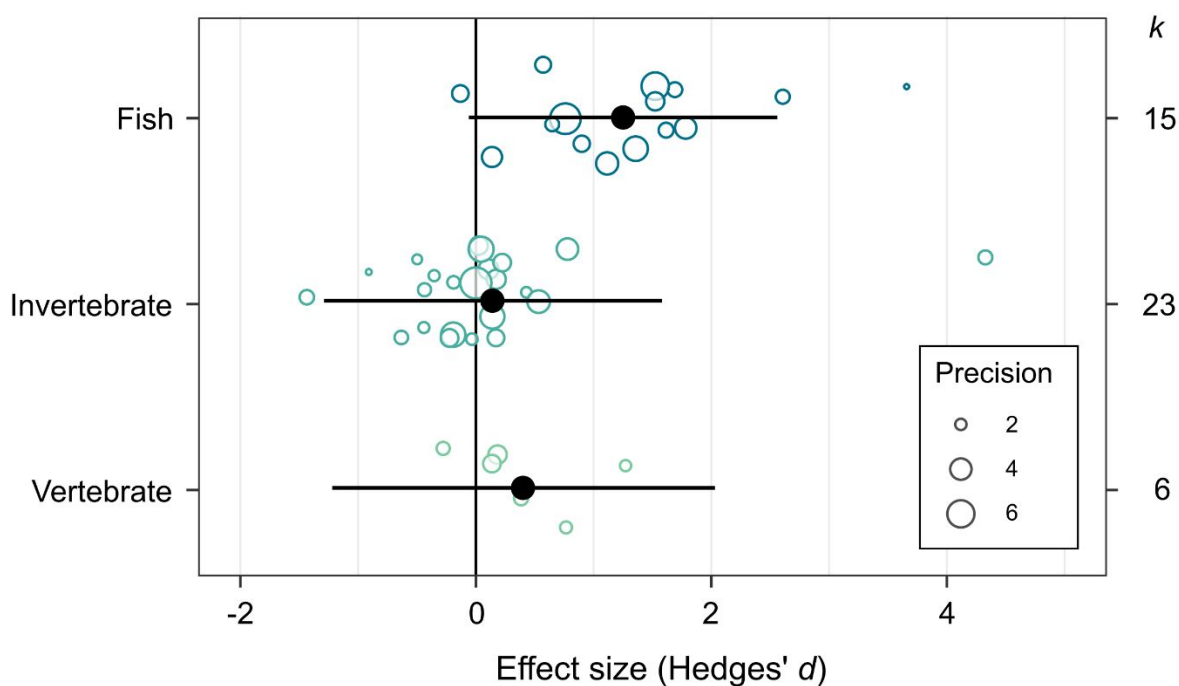
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**Fig. S5.** Histogram showing the distribution of sneaker frequency across 54 species.



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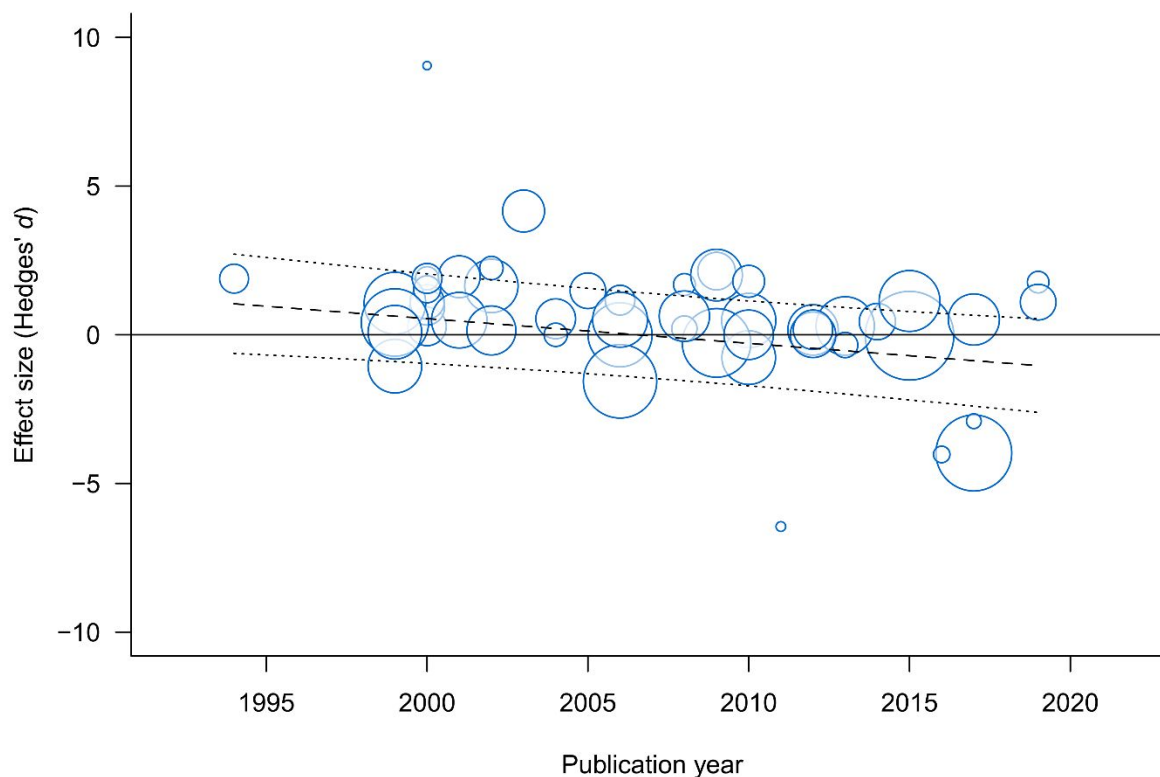
**Fig. S6.** Difference in testes size (Hedges'  $d$ ) between male alternative reproductive tactics (ARTs) in relation to taxonomic group, after removing studies using the gonadosomatic index. Points are scaled according to study variance (precision). Black points represent the meta-analytic mean, and black bars show the 95% confidence interval.  $k$  = number of effect sizes for each category.

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**Table S3.** Mean effect size estimates (Hedges'  $d$ ), 95% confidence intervals, and sample sizes for the testes size data set. Means for the categorical moderator variables were obtained using a minus-intercept meta-regression, performed separately for each moderator. GSI, gonadosomatic index.

Factor	Level	Effect sizes	Studies	Species	Mean $d$	95% CI lower	95% CI upper
All data		74	51	53	0.87	-0.16	1.90
Directionless effect sizes removed		73	50	52	0.90	-0.15	1.95
Polytomy removed		72	51	51	0.87	-0.15	1.89
Taxonomic group	Fish	44	37	28	1.82	1.37	2.27
	Invertebrate	23	7	19	0.24	-0.56	1.04
	Vertebrate	7	7	6	0.19	-0.84	1.21
Mode of fertilisation	External	44	37	27	1.84	1.39	2.29
	Internal	30	14	26	0.24	-0.36	0.84
Tactic type	Fixed	47	28	32	0.96	-0.18	2.11
	Plastic	1	1	1	0.00	-2.87	2.87
	State-dependent	26	22	20	0.73	-0.53	1.99
Measurement	GSI	30	25	20	1.57	0.67	2.46
	Relative testes size	41	23	34	0.58	-0.24	1.39
	Absolute testes size	3	3	3	0.80	-0.96	2.56

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**Fig. S7.** Relationship between effect size (Hedges'  $d$ ) and publication year for the sperm quantity data set ( $k = 49$ ). Each bubble represents an effect size, and bubble size is scaled to effect size precision (inverse standard error; larger bubbles reflect larger sample sizes). The dashed line shows the predicted line from a meta-regression including study year as a covariate. Dotted lines show the 95% confidence intervals for the predicted line.

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**Table S4.** Mean effect size estimates (Hedges'  $d$ ), 95% confidence intervals, and sample sizes for the sperm quantity data set. Means for the categorical moderator variables were obtained using a minus-intercept meta-regression, performed separately for each moderator.

Factor	Level	Effect sizes	Studies	Species	Mean $d$	95% CI lower	95% CI upper
All data		49	43	32	-0.16	-2.14	1.81
Directionless effect sizes removed		45	39	30	-0.13	-2.19	1.94
Taxonomic group	Fish	36	32	21	0.82	-2.34	3.98
	Invertebrate	7	6	7	-1.42	-4.98	2.14
	Vertebrate	6	5	4	0.35	-3.62	4.31
Mode of fertilisation	External	35	31	20	-0.03	-2.10	2.04
	Internal	14	12	12	-0.30	-2.39	1.79
Tactic type	Fixed	27	24	15	0.12	-2.17	2.41
	Plastic	4	4	4	-0.52	-2.99	1.95
	State-dependent	18	15	14	-0.46	-2.77	1.86
Measurement	Sperm density	20	19	14	0.44	-0.52	1.39
	Sperm number	13	13	12	-0.44	-1.41	0.54
	Ejaculate volume	15	12	10	0.73	-0.34	1.79
	Spermatophore size	1	1	1	-3.30	-5.73	-0.88
Sperm allocation vs expenditure	Allocation	7	6	7	-0.69	-2.79	1.41
	Expenditure	42	37	26	0.09	-1.86	2.04

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**Table S5.** Mean effect size estimates (Hedges'  $d$ ), 95% confidence intervals, and sample sizes for the sperm traits data set. Means for the categorical moderator variables were obtained using a minus-intercept meta-regression, performed separately for each moderator.

Factor	Level	Effect sizes	Studies	Species	Mean $d$	95% CI lower	95% CI upper
All data		128	55	33	0.14	-0.05	0.33
Directionless effect sizes removed		118	52	33	0.15	-0.04	0.35
Variance matrix ( $r = 0.25$ )		128	55	33	0.14	-0.07	0.34
Variance matrix ( $r = 0.5$ )		128	55	33	0.12	-0.12	0.37
Variance matrix ( $r = 0.75$ )		128	55	33	0.12	-0.16	0.38
Taxonomic group	Fish	102	43	22	0.14	-0.40	0.68
	Invertebrate	10	6	7	0.09	-0.64	0.82
	Vertebrate	16	6	4	0.05	-0.70	0.80
Mode of fertilisation	External	103	43	22	0.08	-0.26	0.43
	Internal	25	12	11	0.19	-0.22	0.60
Tactic type	Fixed	65	30	15	0.25	0.00	0.51
	Plastic	7	4	4	0.08	-0.52	0.67
	State-dependent	56	21	15	0.05	-0.21	0.30
Trait	ATP content	7	6	5	1.25	0.67	1.83
	Proportion of motile sperm	28	25	17	0.21	-0.06	0.48
	Sperm longevity	21	19	14	-0.11	-0.44	0.22
	Sperm size	29	25	22	0.05	-0.22	0.32
	Sperm swimming speed	43	36	22	0.16	-0.08	0.40