

The causal relationship between sexual selection and sexual size dimorphism in marine gastropods

Terence P. T. Ng^{1,a}, Emilio Rolán-Alvarez^{2,3,a}, Sara Saltin Dahlén⁴, Mark S. Davies⁵, Daniel Estévez², Richard Stafford⁶, Gray A. Williams^{1*}

¹ The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China ² Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, 36310 Vigo Spain

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[Centro de Investigación Mariña da Universidade de Vigo](#)

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Department of Marine Sciences - Tjärnö, University of Gothenburg, SE-452 96 Strömstad, Sweden ⁵ Faculty of Applied Sciences, University of Sunderland, Sunderland, U.K. ⁶

Faculty of Science and Technology, Bournemouth University, U.K.

^a

Contributed equally to this work

*Correspondence: Gray A. Williams, The Swire Institute of Marine Science, The University of Hong Kong, Pokfulam Road, Hong Kong

E-mail: hrsbwga@hku.hk

Telephone: (852) 2809 2551

Fax: (852) 2809 2197

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1 **The causal relationship between sexual selection and sexual**
2 **size dimorphism in marine gastropods**

3

4 **Abstract**

5 Sexual size dimorphism is widespread among dioecious species but its underlying
6 driving forces are often complex. A review of sexual size dimorphism in marine
7 gastropods revealed two common patterns: firstly, sexual size dimorphism, with
8 females being larger than males, and secondly females being larger than males in
9 mating pairs; both of which suggest sexual selection as being causally related with
10 sexual size dimorphism. To test this hypothesis, we initially investigated mechanisms
11 driving sexual selection on size in three congeneric marine gastropods with different
12 degrees of sexual size dimorphism, and, secondly, the correlation between
13 male/female sexual selection and sexual size dimorphism across several marine
14 gastropod species. Male mate choice via mucus trail following (as evidence of sexual
15 selection) was found during the mating process in all three congeneric species,
16 despite the fact that not all species showed sexual size dimorphism. There was also a
17 significant and strong negative correlation between female sexual selection and
18 sexual size dimorphism across 16 cases from seven marine gastropod species. These
19 results suggest that sexual selection does not drive sexual size dimorphism. There

20 was, however, evidence of males utilizing a similar mechanism to choose mates (i.e.
21 selecting a female slightly larger than own size) which may be widespread among
22 gastropods, and in tandem with present variability in sexual size dimorphism among
23 species, provide a plausible explanation of the observed mating patterns in marine
24 gastropods.

25

26 **Keywords:** assortative mating, mate choice, male-male competition, snail, trail
27 following

28

29 **Declarations of interest: none**

30

31

32 **Introduction**

33 Most taxonomic groups of gonochoric animals exhibit sexual size dimorphism, where
34 body size differs between sexes, a pattern which has intrigued evolutionary biologists
35 since Darwin (Andersson, 2007). In most cases, the male is larger than the
36 female, but there are many exceptions (reviewed in Andersson, 1994). The occurrence
37 of such dimorphism begs the questions of why the sexes should differ in a trait that
38 should be, *a priori*, strongly correlated between sexes (as every individual has half of

39 the genome from both parents) and this has provoked a variety of alternative
40 evolutionary explanations (reviewed in Andersson, 1994; Blanckenhorn, 2005;
41 Fairbairn et al., 2007; Shine, 1989). The most common trend, males being larger than
42 females, has often been explained in terms of sexual selection favouring larger males
43 in relation to the female optimum (Blanckenhorn, 2005). The opposite trend, females
44 being larger than males, can be explained as a result of fecundity selection favouring
45 larger sizes in females in relation to the male optimum (Andersson, 1994;
46 Blanckenhorn, 2005). To date, the mutual contribution from multiple selective forces
47 is the most widely accepted explanation for sexual size dimorphism (Andersson 1994;
48 vs. Rodrigues O'Hara & Westoby 2016; but see Blanckenhorn, 2005, for alternative
49 explanations). Nevertheless, it is generally difficult to test these multiple selective
50 forces which may involve evolutionary and ecological/behavioural mechanisms
51 (Blanckenhorn, 2005).

52 Marine gastropods offer several advantages for the study of evolutionary causes
53 of sexual size dimorphism, as in most gastropods females are larger than males
54 (opposite to the general trend in many other animals); and potential behavioural
55 mechanisms for driving sexual selection can be directly measured in the wild. In fact,
56 compared to our current knowledge about reproductive behaviour in vertebrates and

57 insects, sexual selection and sexual conflict theory have only recently been
58 investigated in marine gastropods (Angeloni, 2003; Evanno, Madec, &
59 Arnaud, 2005; Johannesson, Saltin, Duranovic, Havenhand, & Jonsson, 2010; Leonard,
60 1991, 2005). Most marine gastropods are gonochoric and the majority of sexual
61 selection studies have been carried out on species in the family Littorinidae
62 (Erlandsson & Johannesson, 1994; Erlandsson & Rolán-Alvarez, 1998; Johannesson et
63 al., 2016; Ng & Williams, 2014; Rolán-Alvarez & Ekendahl, 1996; Saur, 1990; Zahradnik,
64 Lemay, & Boulding, 2008); probably as a result of their wide distribution, high
65 abundance (Reid, 1989; Rolán-Alvarez, Austin, & Boulding, 2015) and the fact that
66 sexes can be readily identified (Reid, 1986, 1989). There have, however, also been
67 studies on *Neptunea arthritica* (Lombardo & Goshima, 2010, 2011; Lombardo,
68 Takeshita, Abe, & Goshima, 2012) and *Rapana venosa* (Xue, Zhang, & Liu, 2016) as well
69 as studies on sexual selection on size in several other species (Table 1).

70 The goal of the present paper is to use marine gastropods as model organisms for
71 understanding the causes of sexual size dimorphism, using direct measurement of
72 mating pairs in the wild to allow natural, *in-situ*, estimation of sexual selection (and its
73 behavioural mechanism). First, we provide an overview of these findings to integrate
74 and interpret the patterns found in marine gastropods and, second, we propose a

75 general strategy that can be invoked to understand the causal drivers of the observed
76 patterns.

77

78 **What is the current state of knowledge?**

79 In gonochoric marine gastropods the mating process is often initiated by a male
80 following the mucus trail of a female, and this is the first stage at which selection for
81 size may occur (Ng et al., 2013). Size-related mate choice during trail following has, for
82 example, been demonstrated in *Littorina saxatilis* (Johannesson et al., 2008) with
83 males preferring to follow females larger than themselves. This appears to be a general
84 phenomenon in littorinids, resulting in size-dependent male mate preference (e.g.
85 *Littorina fabalis* and *Littoraria ardouini*; Ng & Williams, 2014; Saltin, Schade, &
86 Johannesson, 2013).

87 In general, males (in gonochoric species) or sperm donors (in hermaphroditic
88 species) tend to mate with females or sperm recipients larger than themselves (Table
89 1). Males also, in general, copulate with larger females for longer durations than with
90 smaller females (Table 1; Erlandsson & Johannesson, 1994; Hollander, Lindegarth, &
91 Johannesson, 2005; Saur, 1990). Most species also show sexual size dimorphism, with
92 females being larger than males, but the coincidence between the mating pattern and

93 sexual size dimorphism does not hold for *Echinolittorina vidua* and *Littorina littorea*,
94 where sexes are typically of similar size (Table 1). Interestingly, in one species,
95 *Assiminea japonica*, the direction of sexual size dimorphism and also the size
96 differences between mated males and females are reversed as compared to other
97 Gastropoda (males being larger than females), suggesting a causal relationship
98 between these patterns (Blanckenhorn, 2005).

99 During copulation, selection occurs via inter-individual interactions. Male-male
100 competition can, for example, occur when a rival male physically challenges a mating
101 male (Gibson, 1965; Ng, Davies, Stafford, & Williams, 2016; Zahradnik et al., 2008). In
102 a few species, females may reject males, through mechanisms such as pushing away
103 or even biting the penis (e.g. *Littorina littorea*, Saur, 1990; *Neptunea arthritica*,
104 Lombardo & Goshima, 2010); *Littoraria melanostoma*, Ng & Williams, 2015), indicating
105 some degree of female influence over choice and male reproductive success. A recent
106 study has also shown that, despite being polyandrous, paternity in *Littorina saxatilis* is
107 biased towards certain fathers, suggesting the possibility of postcopulatory (perhaps
108 due to sperm competition) sexual selection for male size (Johannesson et al., 2016).

109

110 **Sexual selection and size dimorphism**

111 While most studies have been confined to investigate a single mechanism at a single
112 mating stage, usually under laboratory conditions, taken together these studies
113 indicate that sexual selection on size in marine gastropods can occur at a number of
114 different times during the mating process (before, during and after copulation)
115 through a number of different mechanisms (Ng, 2013; Ng & Williams,
116 2014). The close coincidence between mating pattern and sexual size dimorphism
117 (Table 1) suggests that the mechanism that is driving sexual selection is also
118 contributing to sexual size dimorphism. A similar mechanism has been proposed in
119 black scavenger flies (*Sepsis* species), where sexual selection acting differentially on
120 males, plus increased fecundity favouring large size in females, contributed to drive
121 sexual size dimorphism (but see alternative explanations reviewed in Blanckenhorn,
122 2005).

123 To investigate why previous studies have shown an association between sexual
124 selection and sexual size dimorphism, we evaluated the mechanisms that may cause
125 male and female size sexual selection across several marine gastropod species.

126 Firstly, we assessed the various behavioural mechanisms of sexual selection
127 throughout the mating process (from trail following to copulation) in three

128 *Echinolittorina* species from Hong Kong that differ in their degree of sexual size
129 dimorphism. Secondly, the strength of sexual selection (using standardized selection
130 estimates) on male and female size was investigated in seven littorinid species from
131 two genera (*Echinolittorina* and *Littorina*). The methodology used was identical to
132 those employed in previous studies (Erlandsson & Johannesson, 1994; Erlandsson &
133 Rolán-Alvarez, 1998; Johannesson, Rolán-Alvarez, & Ekendahl, 1995; Rolán-Alvarez,
134 Carvajal-Rodríguez, et al., 2015; Rolán-Alvarez, Erlandsson, Johannesson, & Cruz,
135 1999) to allow a rigorous interspecific comparison of patterns of sexual selection and,
136 importantly, to identify any general patterns among marine gastropods.

137

138 **Material and Methods**

139 **Definitions of sexual selection**

140 Sexual selection has been considered a controversial concept since Darwin's definition
141 (Andersson, 1994; Futuyma & Kirkpatrick, 2016). In this paper, we adhere to the
142 population genetic definition where sexual selection is viewed as a component of
143 natural selection typically being caused, as proposed by Darwin, by two biological
144 mechanisms; mate competition and mate choice (Arnold & Wade, 1984; Endler, 1986;
145 Lewontin, Kirk, & Crow, 1968; Merrell, 1950; Rolán-Alvarez, Carvajal-Rodríguez, et al.,

146 2015; Rolán-Alvarez & Caballero, 2000). Mate competition applies its selective effects
147 on the sex that the competition occurs within (i.e. intrasexual selection), while in
148 contrast, mate choice exerts its selective effects on the opposite sex (i.e. inter-sexual
149 selection). The consequences of sexual selection have, therefore, often been
150 considered at different stages of the reproductive cycle, depending on the study
151 species (reviewed in Andersson, 1994), but typically are subdivided into the pre-
152 copulatory and post-copulatory stages (Eberhard, 1991). In this study, we focus
153 exclusively on pre-copulatory sexual selection (termed sexual selection from now) for
154 practical reasons, although the potential for post-copulatory sexual selection has been
155 established in several gastropod species (Johannesson et al., 2016; Rolán-Alvarez,
156 Austin, et al., 2015).

157

158 **Mechanisms of sexual selection in three *Echinolittorina* species**

159 In this study, field measurements of the whole mating process (i.e. from trail following
160 to copulation, see detailed text and video descriptions in Ng & Williams, 2014) were
161 obtained for *Echinolittorina malaccana*, *E. radiata* and *E. vidua* in JuneJuly (the hot and
162 wet season in Hong Kong, see Kaehler & Williams, 1996, when sea surface
163 temperatures varied between 27.3-28.4°C, EPD 2012), 2012 at C p d' Aguilar Marine

164 Reserve, Hong Kong (22° 12' 27" N 114° 15' 33" E). Trail following was evident when
165 snails were awash by the rising tide during the mating season (Ng et al., 2016).
166 Specifically, this behaviour occurs when an individual (referred to as a tracker) travels
167 along the mucus path of another individual (i.e. the marker, Davies & Beckwith, 1999)
168 for more than five seconds (see Supplementary Material S1). Trail following individuals
169 were visually identified on the shore. If the male subsequently mounted an individual
170 that he followed, copulation duration was measured from the moment the male had
171 positioned himself in the copulation position until he left (see Gibson, 1965, and Saur,
172 1990). Although it is extremely difficult to see the insertion of the penis into the
173 female's cavity *in situ*, the period during which a male remained in the copulation
174 position is considered a reliable estimate of copulation duration (Saur, 1990). Females
175 appeared to have no strategies to reject males during these stages; either through
176 preventing males from following their trails or from copulating with them (e.g. such as
177 the rejection behaviour displayed by *Littoraria melanostoma*, Ng & Williams, 2015)
178 and, in most cases, the females continued to move and feed on the rock surface. Given
179 this lack of response by the females, we assume any variation in frequency of
180 mounting and/or copulation duration among females of different sizes was solely a
181 result of male mate choice. Finally, all pairs

182 (*Echinolittorina malaccana*: $n = 53$; *E. radiata*: $n = 56$; *E. vidua*: $n = 43$) were collected
183 after copulation, sexed and their shell lengths (± 0.1 mm) measured using vernier
184 callipers in the laboratory.

185 To determine if there was mate choice based on snail size during trail following
186 and consequent mounting, we tested if pairs with female size $>$ male size were more
187 frequent than pairs with male size $>$ female size using a Chi-square test. In addition, as
188 an indication of male mate choice during copulation, two t-tests were conducted
189 to compare the copulation duration of snails in these two categories, and multiple
190 regression was used to investigate the relative contribution of male and female size to
191 the observed variation in copulation duration.

192

193 **Strength of sexual selection in seven littorinid species**

194 To test for generality in the patterns of sexual selection on size, we used published
195 material from *Littoraria flava*, *Littorina saxatilis* (Cardoso, Costa, & Loureiro, 2007;
196 Erlandsson & Rolán-Alvarez, 1998), and *Echinolittorina malaccana* and *E. radiata* (Ng
197 et al., 2016). In this study, we also incorporated unpublished data from

198 *Echinolittorina malaccana*, *E. radiata* and *E. vidua* from Cape d' Agu Marine Reserve,
199 Hong Kong (22° 12' 27" N 114° 15' 33" E, in June-July 2012); *Littorina fabalis* from
200 Abelleira, NW Spain (42° 47' 46.91" N, 9° 1' 20.44" W, in July 2014 and
201 July 2016); and *L. littorea* and *L. saxatilis* (crab ecotype) from Långholmen, Sweden
202 (58°53'05.72" N, 11°07'00.67" E, in May 2014). The experimental design varied slightly
203 between locations and species, but basically consisted of the collection of copulating
204 pairs and unmated neighbouring snails (hereafter 'reference' snails, 4-10 individuals).
205 The distance of these reference snails to the mating pair depended on snail density
206 and was within a 25-cm radius for *Echinolittorina malaccana*, *E. radiata*, *E. vidua*,
207 *Littorina littorea* and *L. saxatilis*, and within 10-cm for *L. fabalis*. The mating pairs and
208 reference snails were returned to the laboratory where species, sex and size (as
209 described above) were recorded.

210 Sexual size dimorphism was investigated using two-tailed *t*'s *t*-tests (using all
211 mating and reference individuals), and deviation from a 1:1 sex ratio was examined
212 using binomial tests (again using all the reference snails). The sexual selection intensity
213 index (standardized selection differential; *SS*), was used to compare the strength of
214 sexual selection between different populations (see Arnold & Wade, 1984; Falconer &
215 Mackay, 1996). *SS* on male and female size was measured as the mean size of the

216 mating males or females minus the mean size of reference males or females, divided
217 by the standard deviation of the size of reference males or females (SS_m or SS_f ; see
218 Cardoso et al., 2007; Erlandsson & Rolán-Alvarez, 1998).

219 Sexual selection on size was tested by one-way ANOVA using the fixed factor mating
220 (mated or reference individuals) for each sex separately, with juvenile snails (either
221 with immature sexual organs or smaller than adult size (following Erlandsson & Rolán-
222 Alvarez, 1998; Mak, 1996) excluded from the analyses.

223

224 **Dimorphism and sexual selection, how are they related?**

225 To investigate the possible causal relationship between male/female sexual selection
226 and sexual size dimorphism in marine gastropods we propose two alternative
227 evolutionary scenarios with subsequent predictions that can be empirically tested as
228 follows:

229 1) The first scenario is that sexual size dimorphism is just a consequence of male
230 sexual selection [see Blackernhorn 2005]. This would occur if fecundity selection
231 would always favour larger females, but sexual selection would favour larger males
232 only in certain cases (resulting in a low level of sexual size dimorphism). Under this
233 scenario a high level of sexual size dimorphism would occur exclusively when sexual

234 selection does not favour larger males (see Fig. 1). This mechanism, if it occurs in most
235 gastropod species, would predict a negative correlation between male sexual
236 selection (SS_m) and sexual size dimorphism. A variation of this explanation would be
237 that sexual selection in both sexes is the main driver of sexual size dimorphism (see
238 Blanckenhorn, 2005). In that case, differential sexual selection between sexes (i.e. SS_f
239 $> SS_m$), would result in female size being systematically larger than male size (Fig. 1).
240 We would, therefore, expect a positive correlation between differential sexual
241 selection ($SS_{f-m} = SS_{female} - SS_{male}$; or SS_f) and sexual size dimorphism across populations
242 and species.

243 2) A second evolutionary scenario is that sexual size dimorphism is pre-existing
244 and responsible for present-day levels of sexual selection, but we do not propose any
245 specific explanation for the sexual size dimorphism (as it could be caused by other
246 components of natural selection). A possible example of such a situation is when
247 differences in survivorship between sexes for size exist, causing different optima in
248 male and female size (see Blanckenhorn, 2005). Under this scenario, we propose that
249 the species-specific level of sexual selection is a consequence of certain mate choices
250 in tandem with pre-existing species-specific sexual size dimorphism. In gastropods and
251 most other species, there is positive assortative mating for size (Jiang, Bolnick, &

252 Kirkpatrick, 2013), which suggests mate choice may be based on a ‘similarity-like’
253 mechanism (Fernández-Meirama et al., 2017). If such similarity would be displaced
254 from the male optimum, for example if a male prefers to mate with a female of similar
255 size to himself (plus a certain constant value; as females are typically larger than males
256 in mating pairs, Table 1), then such a mechanism would result in a negative correlation
257 between SS_f (and SS_{f-m}) and sexual size dimorphism
258 (see explanation in Fig. 1). Interestingly, this prediction would never affect the
259 relationship between SS_m and sexual size dimorphism, as male mate choice will affect
260 SS_f but not SS_m .

261 The above two scenarios can only be tested when the same mechanism is prevalent
262 for most species, and if this is not the case, we would expect no correlation between
263 sexual selection and sexual size dimorphism. Using data from the seven studied
264 species (and several populations within each species), we tested these alternative
265 hypotheses for sexual selection (i.e. SS) and sexual size dimorphism (Table 2). Both
266 standardized and raw sexual size dimorphism value data were investigated, but as the
267 results were statistically very similar, we only present the standardized sexual size
268 dimorphism values. Spearman’s correlation coefficient (ρ) and corresponding

269 significance tests were used to estimate the strength of the sexual selection and sexual
270 size dimorphism relationship using SPSS 23.0 (SPSS Inc., Chicago, IL, U.S.A).

271

272 **Ethical note**

273 All individuals used were captured from non-endangered populations with high
274 densities and with corresponding permission of local authorities (Xunta de Galicia and
275 the Agriculture, Fisheries and Conservation Department, Hong Kong SAR
276 Government). In addition, due to the proximity of the sampling sites to the Swire
277 Institute of Marine Science, individuals of *Echinolittorina* spp. were captured,
278 measured in the laboratory and returned alive to the sampling sites; while the
279 remaining species which were collected from distant sites, were transported to the
280 laboratory and then anesthetized (by cold temperature) before submersion in
281 alcohol.

282

283 **Results**

284 **Interspecific sexual selection mechanisms with varying size dimorphism**

285 All cases (152) of trail following, except one, consisted of a male following a female
286 trail (i.e. females rarely followed trails to mate). Instances of males following a trail of

287 a different species were also rare (*Echinolittorina malaccana*, 6 out of 53 cases; *E.*
288 *radiata*, 6 out of 56 cases, representing ~11% of cases for both species; *E. vidua*, 0 out
289 of 43 cases), and in only half of these false trail-followings did the male subsequently
290 mount and take up the copulation position. This suggests that males can recognize and
291 differentiate the species laying the mucus trail, as well as between male and female
292 mucus trails as they trail-followed and mounted many more females than expected by
293 chance (Table 3). Most conspecific mountings (> 93%) resulted in copulations, but in a
294 few cases (*E. malaccana*, one case; *E. radiata* and *E. vidua*, two cases each) a male
295 mounted a conspecific female without copulation, and in four of these five cases (80%)
296 the female was much smaller (2.3-3.3 mm or 32-37% smaller) than the male. All
297 species showed a significantly higher frequency of males following a larger female
298 (than their own sizes) than expected by chance (Fig. 2a), suggesting a similar size-
299 dependent male mate preference during trail following (see Table 3). The same
300 mechanism, therefore, seems to be present in the three species despite their
301 differences in sexual size dimorphism.

302 Males did not, however, copulate for significantly longer with females larger than
303 themselves as compared to females smaller than themselves, with the exception of
304 *Echinolittorina vidua*. Differential copulation duration can, therefore, only be

305 explained by size-dependent mate preference in *E. vidua* (mean duration with larger
306 females \pm SD = 10.89 ± 0.72 min, and with smaller females = 7.36 ± 0.96 min; $t = 2.247$,
307 $df = 35$, $P < 0.05$, Fig. 2b), which is the only species which did not exhibit sexual size
308 dimorphism. Copulation duration, therefore, seems to be related to female rather
309 than male size (Table 4).

310

311 **Strength of sexual selection with varying size dimorphism**

312 Mated females were typically larger than unmated females (indicating positive sexual
313 selection on female size), and in 13 out of 16 (>80%) comparisons these differences
314 were significant (Table 2). In males the strength of sexual selection was generally
315 weaker, less clear, and species dependant (only 7 out of 16 (44%) comparisons were
316 significant, Table 2). There were similar, positive, sexual selection indices for both
317 sexes in *Echinolittorina malaccana*, *E. radiata*, *Littorina fabalis* and the sheltered
318 ecotype of *L. saxatilis* (Table 2). For the wave ecotype of *L. saxatilis* the sexual selection
319 indices were negative, indicating smaller females were selected by males, although
320 this was only significant in one population (Table 2). Apart from this one exception,
321 the overall trend in the family Littorinidae was for positive sexual selection on size in

322 both sexes of the seven species (including the sheltered ecotype of *L. saxatilis*, Table
323 2, overall standardized means \pm SD: males = 0.27 ± 0.153 ; females = 0.32 ± 0.083).

324

325 **The relationship between sexual selection and size dimorphism**

326 Overall, the relationship between SS_f (and SS_{f-m}) and sexual size dimorphism was
327 highly negative and significant across the whole data set (Table 2, $\rho_f = -0.77$, $df = 15$,
328 $P = 0.001$, Fig. 3; $\rho_{f-m} = -0.56$, $df = 15$, $P = 0.025$). The same trend was observed using
329 the mean values within species ($\rho_f = -0.89$, $df = 6$, $P = 0.007$, Fig. 3; $\rho_{f-m} = -0.79$, $df =$
330 6 , $P = 0.036$) or using the seven species but maintaining the two *L. saxatilis* ecotypes
331 separately ($\rho_f = -0.71$, $df = 7$, $P = 0.047$; $\rho_{f-m} = -0.74$, $df = 7$, $P = 0.037$). All these results
332 are in full agreement with expectations from scenario 2 (i.e. sexual size dimorphism
333 was pre-existing and not driven by sexual selection but other components of natural
334 selection). The relationship between SS_m and sexual size dimorphism, however,
335 showed a pattern contrary to scenario 1, but compatible with scenario 2 (see Fig. 1;
336 $\rho_{samples} = -0.14$, $df = 15$, $P = 0.613$, $\rho_{species} = 0.21$, $df = 6$, $P =$
337 0.645).

338

339 **Discussion**

340 Marine gastropods show sexual size dimorphism with, typically, the female being
341 larger than the male, which represents the opposite trend to many other gonochoric
342 species studied to date (Andersson, 1994; Blanckenhorn, 2005; Fairbairn et al., 2007).
343 Such a general, but unconventional, pattern should be particularly informative for our
344 understanding of the causes of sexual size dimorphism (see arguments in
345 Blanckenhorn, 2005). In marine gastropods, males also mate with females typically
346 larger than themselves and, even in hermaphroditic species, sperm donors generally
347 mate with larger sperm recipients (Table 1). We found no obvious link between any
348 life history traits and sexual size dimorphism, except that the relationship between
349 patterns of mating and sexual dimorphism may suggest a causal link between sexual
350 selection and size dimorphism as described in several studies (Blanckenhorn, 2005;
351 Rohner, Blanckenhorn, & Puniamoorthy, 2016, and references therein).

352 Our results showed clear support for the second proposed scenario, that the
353 observed sexual size dimorphism in many marine gastropods was pre-existing and not
354 necessarily driven by sexual selection. In addition, male and female sexual selection
355 was found in many marine gastropods and may be caused by the existence of a
356 common mate choice mechanism (males preferentially mate with females of the same
357 size plus a specific value, . . . 's m t - ' m sm Fernández-Meirama et al., 2017),

358 and such mechanism would produce a negative correlation between female (but not
359 male) sexual selection and sexual size dimorphism. This finding suggests that it is the
360 degree of sexual dimorphism which explains the observed patterns in female sexual
361 selection. The same relationship between these two variables is observed even when
362 there are populations and species that exhibit the opposite trends in sexual selection
363 or sexual size dimorphism, confirming the generality of the trend. Under this scenario,
364 species that have the largest size dimorphism, even when males prefer to mate with
365 larger females than themselves, could effectively still choose relatively small females
366 (i.e. still larger than the male) from the overall female population (see Fig. 1). When
367 we studied the mechanism of sexual selection in *Echinolittorina* species with different
368 levels of sexual size dimorphism in the wild, we observed the same mechanism of male
369 choice causing female sexual selection, confirming that sexual selection cannot explain
370 present levels of sexual size dimorphism.

371 In addition to sexual selection, other selection forces can also contribute to shape
372 size traits in these snails and different selection pressures may frequently counteract
373 each other (Blanckenhorn, 2005). Fecundity selection, for example, favours large size
374 in females (larger females carry more eggs or offspring, Hughes & Answer, 1982; Ng &
375 Williams, 2012; Ross & Berry, 1991; Zahradnik et al., 2008), but variability selection

376 driven by, for example, wave action could favour smaller male size (Johannesson et
377 al., 2008). Another scenario could be that male gastropods achieve a smaller size
378 compared to females just because of differential daily activities, as searching for mates
379 has been considered to impose a large daily energetic cost, while females focus
380 preferentially on foraging and feeding (Ng et al., 2013; Rolán-Alvarez, Austin, et al.,
381 2015; Zahradnik et al., 2008), causing differential growth rates between sexes (Riascos
382 & Guzman, 2010). Distinct natural selection components or life-history traits may,
383 therefore, act differentially on males and females to drive sexual size dimorphism in
384 marine gastropods, without the need to invoke any role of sexual
385 selection.

386 Another possibility would be that the observed sexual dimorphism does not have a
387 genetic basis. It is, for example, unknown whether differences in male and female
388 body sizes in gastropods are genetic in origin. Differential ecological strategies
389 between sexes could, therefore, affect the probability of survivorship at different sizes,
390 or affect the size at adult age or growth rate differences between sexes as recorded in
391 some pulmonates (Sutton, Zhao, & Carter, 2017). The niche hypothesis, which includes
392 the former possibility, has previously been proposed as a general explanation for
393 sexual size dimorphism (Shine, 1989), but it is rather difficult to test, as the ecological

394 conditions experienced may substantially vary from one organism to another.
395 However, this phenotypic version of the niche hypothesis assumes that body size
396 differences between sexes are not genetic in origin, and this prediction could be
397 experimentally tested.

398 On the other hand, both male and female sexual selection has been detected in
399 many marine gastropods. Male mate choice in littorinids appears to be initiated at the
400 trail following stage, where males generally follow mucus trails laid by females larger
401 than themselves (this study, Ng & Williams, 2014; Saltin et al., 2013). It can be argued
402 that this finding may be partially due to a higher probability of encountering larger
403 females, as females are generally larger than males (but see statistical test from Table
404 3). The same trend was, however, also found in *Echinolittorina vidua* which shows no
405 size sexual dimorphism. Further evidence of males having a preference for somewhat
406 larger females is provided from other littorinid species where a size-dependent male
407 mate preference was demonstrated in laboratory choice experiments (Erlandsson &
408 Kostylev, 1995; Johannesson et al., 2008; Ng & Williams, 2014), supporting the theory
409 that males have the ability to assess the size of females from their trails. This variety
410 of evidence, together with the correlation between sexual selection and size
411 dimorphism found in all studied species, suggests that there could be a conserved

412 mechanism in gastropods, where males typically show a fixed preference for females
413 slightly larger than themselves, causing the observed trend for sexual selection in
414 females across species.

415 A t g u g q u s t s ' w w u d m s s t f m s s d t w d s s d t s m p s t
416 t g s t f m ?' Selecting the largest available female may, in fact, not necessarily be
417 advantageous for a male because of the risk of sperm competition (Herdman, Kelly, &
418 Godin, 2004; Wedell, Gage, & Parker, 2002). Any fecundity-related benefits accruing
419 to a male that has mated with a large female may be offset by an associated fitness
420 cost of shared paternity if large females are more likely to be mated multiple times
421 (Herdman et al., 2004). A m 's s t t g f selecting females slightly larger than his own
422 size during trail following may, therefore, have an important implication for
423 maximizing reproductive success through investing in a range of larger females rather
424 than the largest female available (Widemo & Sæther, 1999). Another plausible reason
425 can be related to physical mating constraints, such that copulation becomes physically
426 more difficult for two individuals when their size difference exceeds a certain
427 threshold (Arnqvist, Rowe, Krupa, & Sih, 1996; Crespi, 1989). Size-dependent male
428 mate preference during trail following can, therefore, be a strategy driven by a balance
429 between a set of fitness costs and benefits (Herdman et al., 2004; Wedell et al., 2002).

430 Empirical and simulation studies will, however, be necessary to confirm this
431 interpretation.

432 The male sexual selection pattern (SS_m from Table 2), on the other hand, can be
433 caused by male-male competition. Aggressive physical male-male contests, for
434 example, have been reported in *E. malaccana* and *E. radiata* (Ng et al., 2016) as well
435 as in several other littorinids (Gibson, 1965; Ng & Williams, 2014; Zahradnik et al.,
436 2008) and other marine gastropods (e.g. *Strombus pugilis*, Bradshaw-Hawkins and
437 Sander 1981). In the littorinid species where such contests were observed, larger males
438 that fought with females were displaced
439 (Ng et al., 2016; Ng & Williams, 2014).

440 Smaller males copulated for longer than larger males in *Echinolittorina radiata*, which
441 may be interpreted as a form of 'prudent choice' (Fawcett & Johnstone, 2003), where
442 smaller males may be more judicious in investing their sperm due to the potentially
443 greater time and energy costs. Previous work has shown
444 that larger males were able to assess the size of their rivals and attack smaller rivals in
445 *E. radiata*, but not *E. malaccana* (Ng et al., 2016).

446 Copulating for longer could, therefore, be advantageous (in terms of fertilization
447 success) for smaller males when mating opportunities can be limited in comparison to

448 larger males. Further investigations into variation in male mate preference under
449 different levels of male-male competition are, however, needed to formally test this
450 hypothesis (see Franceschi, Lemaître, Cézilly, & Bollache, 2010).

451

452 **Conclusion**

453 Our study shows that there is a negative relationship between sexual selection
454 and sexual size dimorphism across many marine gastropod species, indicating that
455 such size dimorphism is unlikely to be produced by the mechanisms contributing to
456 sexual selection. Nevertheless, a common male mate choice (i.e. selecting a mate
457 slightly larger than their own body sizes) seems to explain the female sexual selection
458 observed in most studied marine gastropods. The level of size dimorphism along with
459 the size-dependent male mate preference may, therefore, explain the pattern of
460 sexual selection in marine gastropods. Such an apparently highly conserved
461 mechanism of mate choice in this diverse taxonomic group suggests that there may be
462 an important canalization of the mechanical/physiological traits used to search for
463 mates during reproduction, which may reflect the constraints imposed by the way the
464 snails move, and the multi-functional benefits of utilizing their mucus trails when
465 searching for a mating partner (Ng et al., 2013).

466 Blanckenhorn (2005) highlighted the difficulty in distinguishing between causal
467 *versus* consequential relationships, when trying to explain the origin of sexual size
468 dimorphism, particularly as most studies do not compare multiple species with the
469 same methodologies. Although the question of why females are larger than males in
470 gastropods remains unresolved, we have provided evidence to support a better
471 understanding of the causal and consequential relationships between sexual size
472 dimorphism and sexual selection in this large but under studied taxon.

473

474 **Data accessibility**

475 The data used for this study has been deposited in Dryad
476 (doi:10.5061/dryad.h214h8t; DATA NG et al 2018).

477

478

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717

718 **Figure and Table legends**

719

720 Figure 1. Explanation of the selection consequences of the same mating preference
721 mechanism in males (males of size S prefer to mate with females of size $S + X$, X being
722 any specific positive value) on differential *a priori* sexual size dimorphism scenarios
723 (scenarios A and B). The black normal distributions represent the male size distribution
724 in a hypothetical population, and two alternative female size distributions (scenarios
725 A and B). The red normal curves represent the hypothetical mating preference of
726 males in the population (notice that the preference distribution is displaced from the
727 male size distribution by a factor X). Scenario A assumes a low sexual size dimorphism,
728 and therefore the average male will choose (with the same mating preference; red
729 curve) the largest (within female size distribution) females, therefore causing a
730 positive SSf. Under scenario B, due to a large sexual size dimorphism, the same males
731 will choose females which are the smallest females within the female size distribution,
732 therefore causing negative SSf. Notice that in the two scenarios, the male mate choice
733 distribution has not changed (red distribution) but the resulting chosen female size
734 distribution changes depending on the particular level of size dimorphism in the
735 population.

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737 Figure 2. Frequency of male trail following (as percentage of cases observed; Figure

738 2a) and copulation duration between the two mating categories (white bars: females

739 smaller than males; black bar: females larger than males; Figure 2b) in the three

740 littorinids, *Echinolittorina malaccana*, *E. radiata* and *E. vidua*, at Cape d' Aguilar Marine

741 Reserve, Hong Kong. Significantly different results are indicated by asterisks (* $P < 0.05$,

742 ** $P < 0.01$, *** $P < 0.001$).

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744 Figure 3. Relationship between SS_f and sexual size dimorphism (both standardized) for

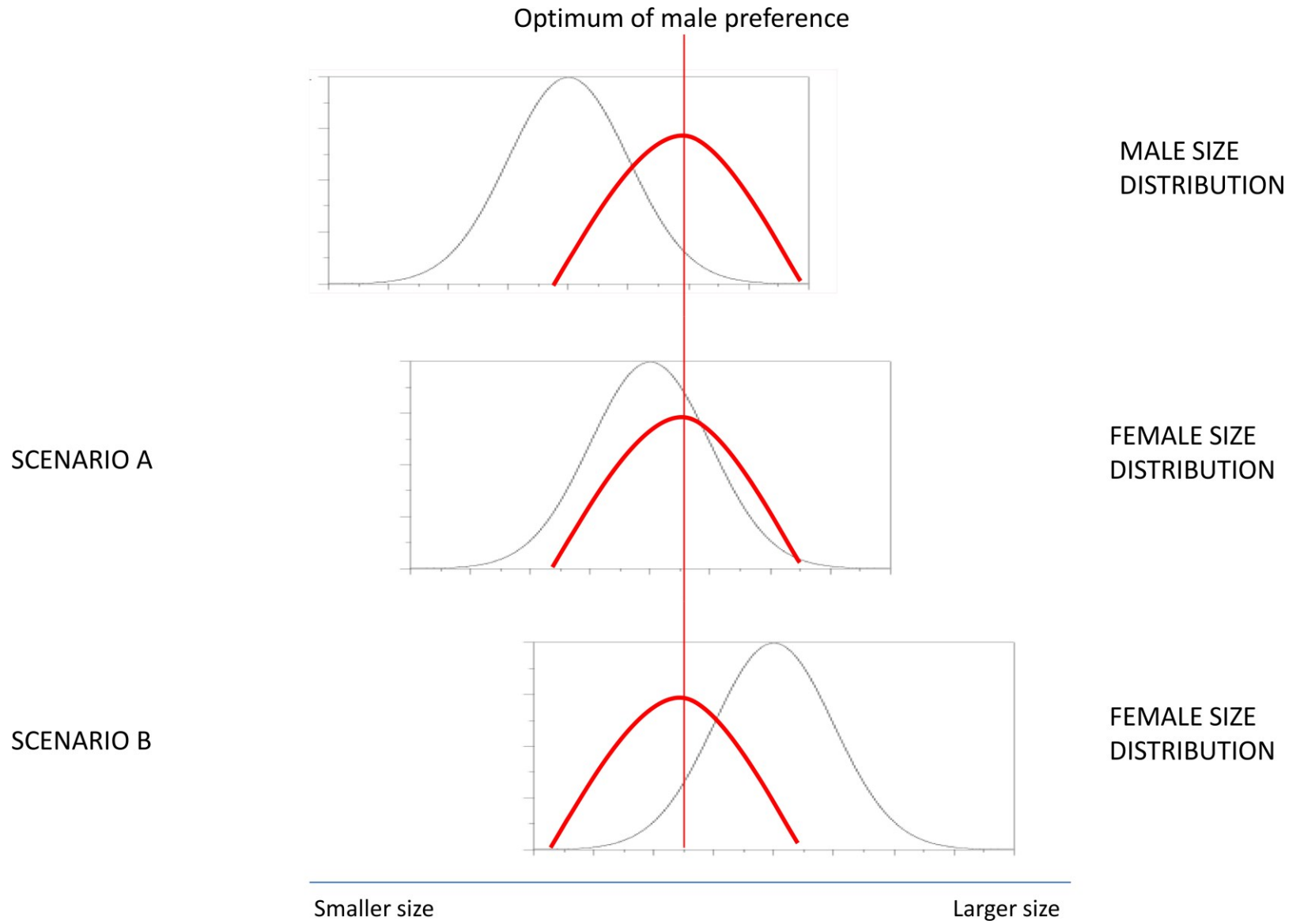
745 the whole data set (light squares) and for the means within the seven species (dark

746 circles). Correlation values and statistical significances are given in the text.

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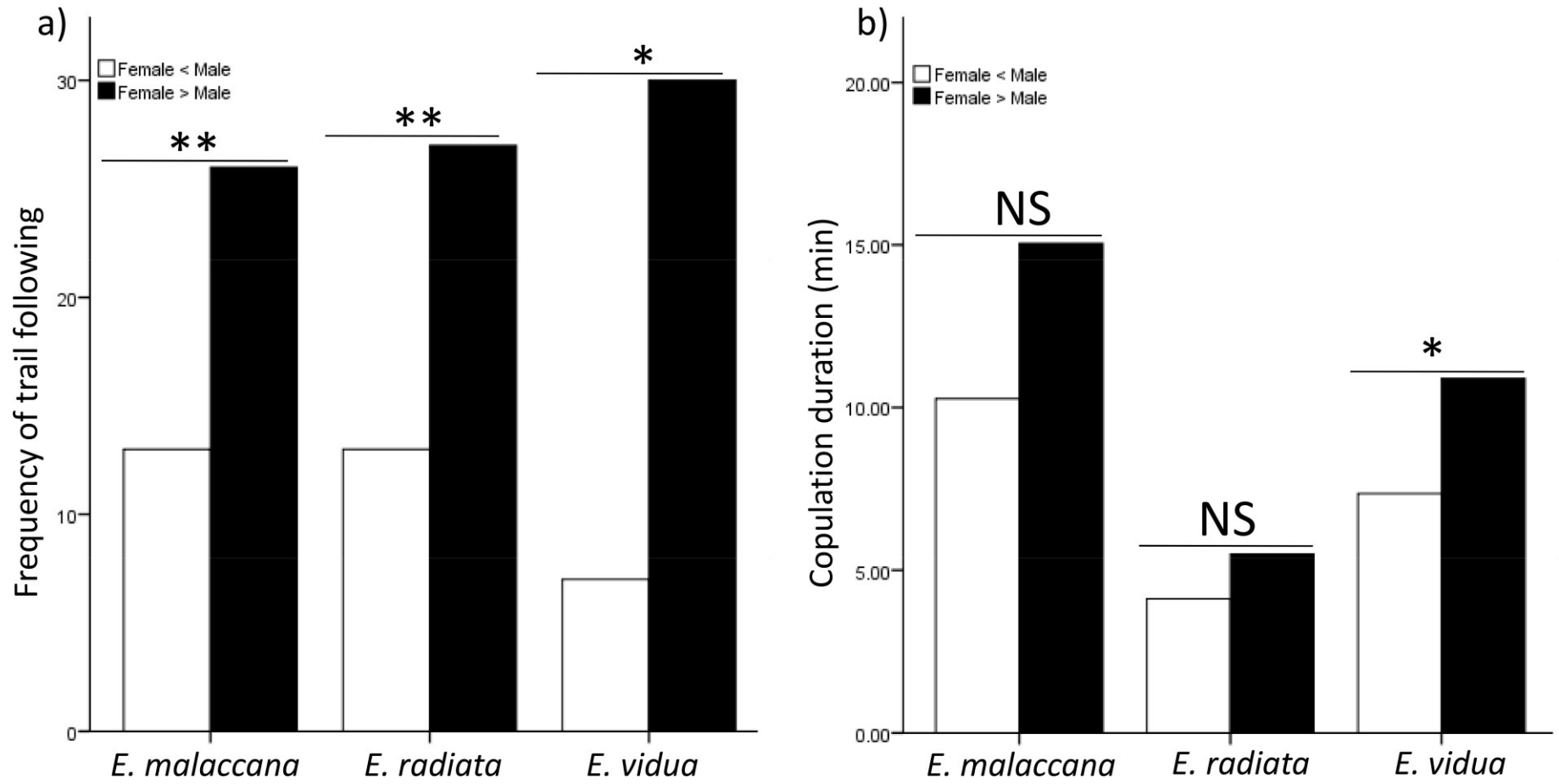
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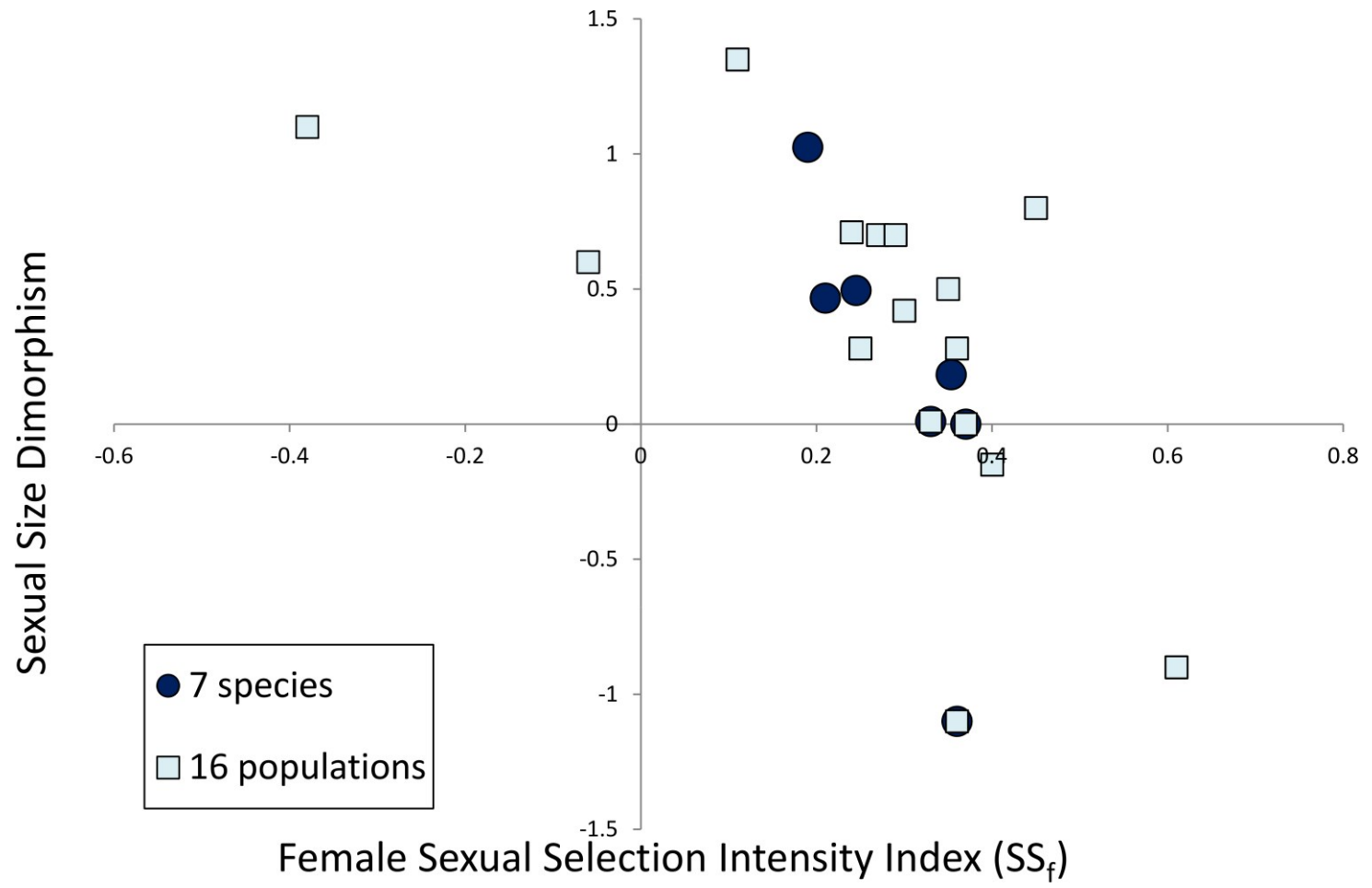
Figure 1



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Figure 2

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776 **Figure 3.** 777
778 **Table 1** Literature review.

Species	Reproductive mode	Developmental mode	Sex Ratio	Sexual Dimorphism in size	Mating pattern (size)	Reference
<i>Siphonaria capensis</i>	H	P	-	-	SR = SD	(Pal, Erlandsson, & Sköld, 2006)
<i>Aplysia vaccaria</i>	H	P	-	-	SR > SD	(Angeloni & Bradbury, 1999)
<i>Aplysia punctata</i>	H	P	-	-	SR > SD	(Otsuka, Yves, & Tobach, 1980)
<i>Aplysia kurodai</i>	H	P	-	-	SR > SD	(Yusa, 1996)
<i>Alderia modesta</i>	H	P	-	-	SR > SD	(Angeloni, 2003)
<i>Buccinanops globulosus</i>	Di	D	♀ bias	♀ > ♂	♀ > ♂	(Avaca, Narvarte, & Martín, 2012, 2013)
<i>Littoraria flava</i>	Di	P	♀ bias	♀ > ♂	♀ > ♂	(Cardoso et al., 2007)
<i>Angustassiminea castanea</i>	Di	P		♀ > ♂	♀ > ♂	(Kurata & Kikuchi, 2000)
<i>Assiminea japonica</i>	Di	P		♂ > ♀	♂ > ♀	(Kurata & Kikuchi, 2000)
<i>Littoraria ardouiniana</i>	Di	P	♂ bias	♀ > ♂	♀ > ♂	(Ng et al., 2013; Ng & Williams, 2014)
<i>Littoraria melanostoma</i>	Di	P	1:1	♀ > ♂	♀ > ♂	(Ng, 2013)
<i>Echinolittorina malaccana</i>	Di	P	1:1	♀ > ♂	♀ > ♂	This study
<i>Echinolittorina radiata</i>	Di	P	♀ bias	♀ > ♂	♀ > ♂	This study
<i>Echinolittorina radiata</i>	Di	P	♂ bias	♀ > ♂	♀ > ♂	(Ito & Wada, 2006)
<i>Echinolittorina vidua</i>	Di	P	♀ bias	♀ = ♂	♀ > ♂	This study
<i>Littorina saxatilis</i> _{crab}	Di	D	1:1	♀ > ♂	♀ > ♂	(Erlandsson & Rolán-Alvarez, 1998; Hollander et al., 2005; Hull, 1998; Johannesson et al., 1995; Rolán-Alvarez et al., 1999; Saur, 1990) this study
<i>Littorina saxatilis</i> _{wave}	Di	D	1:1	♀ > ♂	♀ > ♂	This study
<i>Littorina fabalis</i>	Di	D	1:1	♀ > ♂	♀ > ♂	This study
<i>Littorina littorea</i>	Di	P	1:1	♀ = ♂	♀ > ♂	(Erlandsson & Johannesson, 1994; Saur, 1990)

A review of reproductive traits and mating patterns in relation to individual size in marine gastropods. Remark: The above studies were identified by searching in ISI WO f pu t s ud gt w ds “s xu ” “s t ” d “s ” wt G st p d “G st p d” “G st p d” “M us”) f m t f d “T p ”) with further sorting for marine species in November 2017. Abbreviations: H = hermaphrodite, Di = dioecious, D = direct, P = planktonic, SR = sperm recipient and SD = sperm donor.

Table 2. Analysis of sexual selection on size (shell length).

Species	Locality	Reference	Nm	Nu	ALE SIZE (mm)			FEMALE SIZE (mm)		
					Mated (Mean ± SD)	Unmated (Mean ± SD)	SS _m	Mated (Mean ± SD)	Unmated (Mean ± SD)	SS _f
<i>E. malaccana</i>	ShekO ₂₀₁₅	Ng et al., 2016	80	155	6.04 ± 1.01	5.71 ± 1.30	0.19	6.49 ± 1.23	5.56 ± 1.47	0.40***
	C p d' Agu ₂₀₁₂	This study	102	266	8.65 ± 0.88	8.18 ± 0.99	0.34**	9.01 ± 0.98	8.46 ± 1.14	0.36**
	C p d' Agu ₂₀₁₅	Ng et al., 2016	456	905	8.44 ± 1.38	8.11 ± 1.44	0.15**	9.23 ± 1.45	8.53 ± 1.58	0.30***
	Mean ± SD						0.23 ± 0.185			0.35** ± 0.253
<i>E. radiata</i>	C p d' Agu ₂₀₁₂	This study Ng et al., 2016	108	247	6.36 ± 1.51	6.12 ± 1.68	0.09	7.73 ± 1.62	7.47 ± 1.86	0.11
	C p d' Agu ₂₀₁₅		102	198	7.27 ± 1.54	6.83 ± 1.62	0.16	8.22 ± 1.53	7.53 ± 1.80	0.27*
	Mean ± SD						0.12 ± 0.049			0.19 ± 0.113
<i>E. vidua</i>	C p d' Agu ₂₀₁₂	This study	82	126	6.97 ± 1.06	6.72 ± 1.14	0.13	7.41 ± 1.02	6.73 ± 1.36	0.33**
<i>Littoraria flava</i>	Flexeira ₂₀₀₁	Cardoso et al., 2007	480	243	10.8 ± 1.72	10.9 ± 2.28	-0.06	11.8 ± 1.80	10.9 ± 2.31	0.37***
		This study	190	375	6.98 ± 0.67	6.68 ± 0.95	0.22***	7.60 ± 0.78	6.96 ± 1.13	0.25***
<i>Littorina fabalis</i>	Abelleira ₂₀₁₄	This study	292	549	6.63 ± 0.81	6.34 ± 0.89	0.22**	7.42 ± 0.97	7.05 ± 0.96	0.24***
	Abelleira ₂₀₁₆						0.22** ±			0.24** ± 0.01
	Mean ± SD									
<i>L. littorea</i>	Långholmen ₂₀₁₄	This study	88	333	19.5 ± 2.05	19.1 ± 2.50	0.17	19.2 ± 2.87	18.0 ± 3.45	0.36*
	Saltö W ₁₉₉₄	E & R-A, 1998	44	74	11.5 ± 1.21	10.4 ± 2.17	0.34*	12.0 ± 1.17	11.1 ± 2.31	0.29
	Saltö S ₁₉₉₄	E & R-A, 1998	46	53	11.2 ± 1.39	10.4 ± 2.17	0.45**	12.1 ± 1.77	10.9 ± 1.75	0.35*
<i>L. saxatilis</i> _{crab}	Ängklavenbukten ₁₉₉	E & R-A, 1998	44	83	10.1 ± 1.04	8.8 ± 1.58	0.57***	10.7 ± 1.01	9.6 ± 1.65	0.45**

	Långholmen ₂₀₁₄	This study	96	365	10.6 ± 1.31	10.3 ± 1.47	0.21	10.8 ± 1.54	9.4 ± 2.34	0.61 ^{***}
							0.39 [*] ±			
		Mean ± SD								0.42 ^{**} ± 0.140
	Saltö1 ₁₉₉₄	E & R-A, 1998	76	167	5.5 ± 1.44	6.0 ± 1.65	-0.20	6.2 ± 1.06	7.1 ± 1.76	-0.38 [*]
<i>L. saxatilis</i> _{Swave}	Saltö2 ₁₉₉₄	E & R-A, 1998	76	167	4.7 ± 0.91	5.1 ± 0.77	-0.33	5.6 ± 1.05	5.7 ± 1.19	-0.06
		Mean ± SD					-0.27 ±			-0.22 ± 0.226

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782 Analysis of sexual selection on size (shell length) in males and females of seven littorinid species from three genera with locality and year of study. Nm= sample size of
783 mated individuals and Nu = sample size of unmated (reference) individuals. Sexual selection intensity (SSm and SSf) index is the difference between mated and unmated
784 males or females standardized by the SD of shell length of the population of males or females (see Erlandsson & Rolán-Alvarez, 1998). E & R-A 1998 is Erlandsson & Rolán-
785 Alvarez, 1998.

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786 **Table 3.** Evaluation of the mate choice mechanism

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Species	Expected malemale trail following	Expected malefemale trail following	Observed malemale trail following	Observed malefemale trail following	χ^2	<i>P</i>	<i>n</i>
<i>E. malaccana</i>	23 (48.1%)	24 (51.9%)	5 (10.6%)	42 (89.4%)	27.587	<0.001	47
<i>E. radiata</i>	21 (41.2%)	29 (58.8%)	6 (12.0 %)	44 (88.0%)	18.473	<0.001	50
<i>E. vidua</i>	17 (40.5%)	26 (59.5%)	3 (7.0%)	40 (93.0%)	19.068	<0.001	43

788 Chi-square tests to examine whether males followed females more than males than would be predicted by chance (taking into account the size distribution of females 789 in the sample). Expected (derived from sex ratios) and observed frequencies of males mounting conspecific males and females in the three littorinids: *Echinolittorina* 790 *malaccana*, *E. radiata* and *E. vidua*.

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Regression of size on copulation duration

793 **Table 4.** Male and female size contribution to copulation duration

Species	Copulation Duration (min)	N	Explained	Full Model		Explained	Step-Wise	
				Variables in Model	Partial r		Variable Chosen	Partial r
<i>E. malaccana</i>	13.8 ± 9.46	41	27.10%	Male	-0.02	27.10%	Female	0.52***
				Female	0.49***			
<i>E. radiata</i>	5.0 ± 2.87	42	13.80%	Male	-0.31*	n.s.	Male/Female	n.s.
				Female	0.32*			
<i>E. vidua</i>	10.3 ± 3.91	38	11.60%	Male	-0.09	10.90%	Female	0.33*
				Female	0.29 ^m			

794 Multiple regressions to evaluate the contribution of male and female size to the variation in copulation duration in three *Echinolittorina* species. Both the full model
 795 approach and the step-wise regressions gave similar results in relating male and female size to copulation duration in two of the three species, with the exception of
 796 *Echinolittorina radiata*. In *E. malaccana* female size was clearly the best predictor of copulation time, but this was less clear in *E. vidua*; while in *E. radiata* similar
 797 contributions of both male and female size (but in different directions) determined copulation duration. Copulation duration was generally longer in *E. malaccana* than
 798 in *E. vidua*, and longer in *E. vidua* than in *E. radiata*. Significant results are indicated by asterisks (n.s. = not significant, ^mp = 0.082, * p < 0.05, ** p < 0.01, *** p < 0.001).

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