

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

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Highlights

- Sexual size dimorphism is not driven by sexual selection in marine gastropods
- Female sexual selection [is driven by male choice and](#) sexual size dimorphism
- Males prefer to mate with females slightly larger than themselves
- This pattern of male choice may be widespread in marine gastropods

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
35 biologists since Darwin (Fairbairn, Blanckenhorn, & Székely, 2007). In most cases, the
36 male is larger than the female, but there are many exceptions (reviewed in
37 Andersson, 1994). The occurrence of such dimorphism begs the questions of why the
38 sexes should differ in a trait that should be, *a priori*, strongly correlated between
39 sexes (as every individual has half of the genome from both parents) and this has
40 provoked a variety of alternative evolutionary explanations (reviewed in Andersson,
41 1994; Blanckenhorn, 2005; Fairbairn et al., 2007; Shine, 1989). The most common
42 trend, males being larger than females, has often been explained in terms of sexual
43 selection favouring larger males in relation to the female optimum (Blanckenhorn,
44 2005). The opposite trend, females being larger than males, can be explained as a
45 result of fecundity selection favouring larger sizes in females in relation to the male
46 optimum (Andersson, 1994; Blanckenhorn, 2005). To date, the mutual contribution
47 from multiple selective forces is the most widely accepted explanation for sexual size
48 dimorphism (Andersson, 1994; Svenson, Brannoch, Rodrigues, O’Hanlon, & Wieland,
49 2016; but see Blanckenhorn, 2005, for alternative explanations). Nevertheless, it is
50 generally difficult to test these multiple selective forces which may involve

51 evolutionary and ecological/behavioural mechanisms (Blanckenhorn, 2005).

52 Marine gastropods offer several advantages for the study of evolutionary
53 causes of sexual size dimorphism, as in most gastropods females are larger than
54 males (opposite to the general trend in many other animals); and potential
55 behavioural mechanisms for driving sexual selection can be directly measured in the
56 wild. In fact, compared to our current knowledge about reproductive behaviour in
57 vertebrates and insects, sexual selection and sexual conflict theory have only
58 recently been 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;
64 Zahradnik, Lemay, & Boulding, 2008); probably as a result of their wide distribution,
65 high abundance (Reid, 1989; Rolán-Alvarez, Austin, & Boulding, 2015) and the fact
66 that sexes can be readily identified (Reid, 1986, 1989). There have, however, also
67 been studies on *Neptunea arthritica* (Lombardo & Goshima, 2010, 2011; Lombardo,
68 Takeshita, Abe, & Goshima, 2012) and *Rapana venosa* (Xue, Zhang, & Liu, 2016) as
69 well 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
71 for understanding the causes of sexual size dimorphism, using direct measurement
72 of mating pairs in the wild to allow natural, *in-situ*, estimation of sexual selection
73 (and its behavioural mechanism). First, we provide an overview of these findings to
74 integrate and interpret the patterns found in marine gastropods and, second, we
75 propose a general strategy that can be invoked to understand the causal drivers of
76 the observed 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,
82 for 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
84 general phenomenon in littorinids, resulting in size-dependent male mate
85 preference (e.g. *Littorina fabalis* and *Littoraria ardouiniana*; Ng & Williams, 2014;
86 Saltin, Schade, & 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

89 (Table 1). Males also, in general, copulate with larger females for longer durations
90 than with smaller females (Table 1; Erlandsson & Johannesson, 1994; Hollander,
91 Lindegarth, & Johannesson, 2005; Saur, 1990). Most species also show sexual size
92 dimorphism, with females being larger than males, but the coincidence between the
93 mating pattern and sexual size dimorphism does not hold for *Echinolittorina vidua*
94 and *Littorina littorea*, where sexes are typically of similar size (Table 1). Interestingly,
95 in one species, *Assimineea japonica*, the direction of sexual size dimorphism and also
96 the size differences between mated males and females are reversed as compared to
97 other 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),
105 indicating some degree of female influence over choice and male reproductive
106 success. A recent study has also shown that, despite being polyandrous, paternity in
107 *Littorina saxatilis* is biased towards certain fathers, suggesting the possibility of post-

108 copulatory (perhaps due to sperm competition) sexual selection for male size
109 (Johannesson et al., 2016).

110

111 **Sexual selection and size dimorphism**

112 While most studies have been confined to investigate a single mechanism at a
113 single mating stage, usually under laboratory conditions, taken together these
114 studies indicate that sexual selection on size in marine gastropods can occur at a
115 number of different times during the mating process (before, during and after
116 copulation) through a number of different mechanisms (Ng, 2013; Ng & Williams,
117 2014). The close coincidence between mating pattern and sexual size dimorphism
118 (Table 1) suggests that the mechanism that is driving sexual selection is also
119 contributing to sexual size dimorphism. A similar mechanism has been proposed in
120 black scavenger flies (*Sepsis* species), where sexual selection acting differentially on
121 males, plus increased fecundity favouring large size in females, contributed to drive
122 sexual size dimorphism (but see alternative explanations reviewed in Blanckenhorn,
123 2005).

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

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

138

139 **Material and Methods**

140 **Definitions of sexual selection**

141 Sexual selection has been considered a controversial concept since Darwin's
142 definition (Andersson, 1994; Futuyma & Kirkpatrick, 2016). In this paper, we adhere
143 to the population genetic definition where sexual selection is viewed as a
144 component of natural selection typically being caused, as proposed by Darwin, by
145 two biological mechanisms; mate competition and mate choice (Arnold & Wade,

146 1984; Endler, 1986; Lewontin, Kirk, & Crow, 1968; Merrell, 1950; Rolán-Alvarez,
147 Carvajal-Rodríguez, et al., 2015; Rolán-Alvarez & Caballero, 2000). Mate competition
148 applies its selective effects on the sex that the competition occurs within (i.e. intra-
149 sexual selection), while in contrast, mate choice exerts its selective effects on the
150 opposite sex (i.e. inter-sexual selection). The consequences of sexual selection have,
151 therefore, often been considered at different stages of the reproductive cycle,
152 depending on the study species (reviewed in Andersson, 1994), but typically are
153 subdivided into the pre-copulatory and post-copulatory stages (Eberhard, 1991). In
154 this study, we focus exclusively on pre-copulatory sexual selection (termed sexual
155 selection from now) for practical reasons, although the potential for post-copulatory
156 sexual selection has been established in several gastropod species (Johannesson et
157 al., 2016; Rolán-Alvarez, Austin, et al., 2015).

158

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

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

165 Aguilar Marine Reserve, Hong Kong (22° 12' 27" N, 114° 15' 33" E). Trail following
166 was evident when snails were awash by the rising tide during the mating season (Ng
167 et al., 2016). Specifically, this behaviour occurs when an individual (referred to as a
168 tracker) travels along the mucus path of another individual (i.e. the marker, Davies &
169 Beckwith, 1999) for more than five seconds (see Supplementary Material S1). Trail
170 following individuals were visually identified on the shore. If the male subsequently
171 mounted an individual that he followed, copulation duration was measured from the
172 moment the male had positioned himself in the copulation position until he left (see
173 Gibson, 1965, and Saur, 1990). Although it is extremely difficult to see the insertion
174 of the penis into the female's cavity *in situ*, the period during which a male remained
175 in the copulation position is considered a reliable estimate of copulation duration
176 (Saur, 1990). Females appeared to have no strategies to reject males during these
177 stages; either through preventing males from following their trails or from
178 copulating with them (e.g. such as the rejection behaviour displayed by *Littoraria*
179 *melanostoma*, Ng & Williams, 2015) and, in most cases, the females continued to
180 move and feed on the rock surface. Given this lack of response by the females, we
181 assume any variation in frequency of mounting and/or copulation duration among
182 females of different sizes was solely a result of male mate choice. Finally, all pairs
183 (*Echinolittorina malaccana*: $n = 53$; *E. radiata*: $n = 56$; *E. vidua*: $n = 43$) were collected

184 after copulation, sexed and their shell lengths (± 0.1 mm) measured using vernier
185 callipers in the laboratory.

186 To determine if there was mate choice based on snail size during trail following
187 and consequent mounting, we tested if pairs with female size $>$ male size were more
188 frequent than pairs with male size $>$ female size using a Chi-square test. In addition,
189 as an indication of male mate choice during copulation, Student's t-tests were
190 conducted to compare the copulation duration of snails in these two categories, and
191 multiple regression was used to investigate the relative contribution of male and
192 female size to the observed variation in copulation duration.

193

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

195 To test for generality in the patterns of sexual selection on size, we used published
196 material from *Littoraria flava*, *Littorina saxatilis* (Cardoso, Costa, & Loureiro, 2007;
197 Erlandsson & Rolán-Alvarez, 1998), and *Echinolittorina malaccana* and *E. radiata* (Ng
198 et al., 2016). In this study, we also incorporated unpublished data from
199 *Echinolittorina malaccana*, *E. radiata* and *E. vidua* from Cape d' Aguilar Marine
200 Reserve, Hong Kong ($22^{\circ} 12' 27''$ N, $114^{\circ} 15' 33''$ E, in June-July 2012); *Littorina*
201 *fabalis* from Abelleira, NW Spain ($42^{\circ} 47' 46.91''$ N, $9^{\circ} 1' 20.44''$ W, in July 2014 and
202 July 2016); and *L. littorea* and *L. saxatilis* (crab ecotype) from Långholmen, Sweden

203 (58°53'05.72" N, 11°07'00.67" E, in May 2014). The experimental design varied
204 slightly between locations and species, but basically consisted of the collection of
205 copulating pairs and unmated neighbouring snails (hereafter 'reference' snails, 4-10
206 individuals). The distance of these reference snails to the mating pair depended on
207 snail density and was within a 25-cm radius for *Echinolittorina malaccana*, *E. radiata*,
208 *E. vidua*, *Littorina littorea* and *L. saxatilis*, and within 10-cm for *L. fabalis*. The mating
209 pairs and reference snails were returned to the laboratory where species, sex and
210 size (as described above) were recorded.

211 Sexual size dimorphism was investigated using two-tailed Student's *t*-tests (using
212 all mating and reference individuals), and deviation from a 1:1 sex ratio was
213 examined using binomial tests (again using all the reference snails). The sexual
214 selection intensity index (standardized selection differential; *SS*), was used to
215 compare the strength of sexual selection between different populations (see Arnold
216 & Wade, 1984; Falconer & Mackay, 1996). *SS* on male and female size was measured
217 as the mean size of the mating males or females minus the mean size of reference
218 males or females, divided by the standard deviation of the size of reference males or
219 females (*SS_m* or *SS_f*; see Cardoso et al., 2007; Erlandsson & Rolán-Alvarez, 1998).
220 Sexual selection on size was tested by one-way ANOVA using the fixed factor mating
221 (mated or reference individuals) for each sex separately, with juvenile snails (either

222 with immature sexual organs or smaller than adult size (following Erlandsson &
223 Rolán-Alvarez, 1998; Mak, 1996) excluded from the analyses.

224

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

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

230 1) The first scenario is that sexual size dimorphism is just a consequence of male
231 sexual selection [see Blackerhorn 2005]. This would occur if fecundity selection
232 would always favour larger females, but sexual selection would favour larger males
233 only in certain cases (resulting in a low level of sexual size dimorphism). Under this
234 scenario a high level of sexual size dimorphism would occur exclusively when sexual
235 selection does not favour larger males (see Fig. 1). This mechanism, if it occurs in
236 most gastropod species, would predict a negative correlation between male sexual
237 selection (SS_m) and sexual size dimorphism. A variation of this explanation would be
238 that sexual selection in both sexes is the main driver of sexual size dimorphism (see
239 Blanckenhorn, 2005). In that case, differential sexual selection between sexes (i.e. SS_f
240 $> SS_m$), would result in female size being systematically larger than male size (Fig. 1).

241 We would, therefore, expect a positive correlation between differential sexual
242 selection ($SS_{f-m} = SS_{\text{female}} - SS_{\text{male}}$; or SS_f) and sexual size dimorphism across
243 populations and species.

244 2) A second evolutionary scenario is that sexual size dimorphism is pre-existing and
245 responsible for present-day levels of sexual selection, but we do not propose any
246 specific explanation for the sexual size dimorphism (as it could be caused by other
247 components of natural selection). A possible example of such a situation is when
248 differences in survivorship between sexes for size exist, causing different optima in
249 male and female size (see Blanckenhorn. 2005). Under this scenario, we propose
250 that the species-specific level of sexual selection is a consequence of certain mate
251 choices in tandem with pre-existing species-specific sexual size dimorphism. In
252 gastropods and most other species, there is positive assortative mating for size (Jiang,
253 Bolnick, & Kirkpatrick, 2013), which suggests mate choice may be based on a
254 'similarity-like' mechanism (Fernández-Meirama et al., 2017). If such similarity would
255 be displaced from the male optimum, for example if a male prefers to mate with a
256 female of similar size to himself (plus a certain constant value; as females are
257 typically larger than males in mating pairs, Table 1), then such a mechanism would
258 result in a negative correlation between SS_f (and SS_{f-m}) and sexual size dimorphism
259 (see explanation in Fig. 1). Interestingly, this prediction would never affect the

260 relationship between SS_m and sexual size dimorphism, as male mate choice will
261 affect SS_f but not SS_m .

262 The above two scenarios can only be tested when the same mechanism is
263 prevalent for most species, and if this is not the case, we would expect no
264 correlation between sexual selection and sexual size dimorphism. Using data from
265 the seven studied species (and several populations within each species), we tested
266 these alternative hypotheses for sexual selection (i.e. SS) and sexual size dimorphism
267 (Table 2). Both standardized and raw sexual size dimorphism value data were
268 investigated, but as the results were statistically very similar, we only present the
269 standardized sexual size dimorphism values. Spearman's correlation coefficient (ρ)
270 and corresponding significance tests were used to estimate the strength of the
271 sexual selection and sexual size dimorphism relationship using SPSS 23.0 (SPSS Inc.,
272 Chicago, IL, U.S.A).

273

274 **Ethical note**

275 All individuals used were captured from non-endangered populations with high
276 densities and with corresponding permission of local authorities (Xunta de Galicia
277 and the Agriculture, Fisheries and Conservation Department, Hong Kong SAR
278 Government). In addition, due to the proximity of the sampling sites to the Swire

279 Institute of Marine Science, individuals of *Echinolittorina* spp. were captured,
280 measured in the laboratory and returned alive to the sampling sites; while the
281 remaining species which were collected from distant sites, were transported to the
282 laboratory and then anesthetized (by cold temperature) before submersion in
283 alcohol.

284

285 **Results**

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

287 All cases (152) of trail following, except one, consisted of a male following a female
288 trail (i.e. females rarely followed trails to mate). Instances of males following a trail
289 of a different species were also rare (*Echinolittorina malaccana*, 6 out of 53 cases; *E.*
290 *radiata*, 6 out of 56 cases, representing ~11% of cases for both species; *E. vidua*, 0
291 out of 43 cases), and in only half of these false trail-followings did the male
292 subsequently mount and take up the copulation position. This suggests that males
293 can recognize and differentiate the species laying the mucus trail, as well as between
294 male and female mucus trails as they trail-followed and mounted many more
295 females than expected by chance (Table 3). Most conspecific mountings (> 93%)
296 resulted in copulations, but in a few cases (*E. malaccana*, one case; *E. radiata* and *E.*
297 *vidua*, two cases each) a male mounted a conspecific female without copulation, and

298 in four of these five cases (80%) the female was much smaller (2.3-3.3 mm or 32-37%
299 smaller) than the male. All species showed a significantly higher frequency of males
300 following a larger female (than their own sizes) than expected by chance (Fig. 2a),
301 suggesting a similar size-dependent male mate preference during trail following (see
302 Table 3). The same mechanism, therefore, seems to be present in the three species
303 despite their differences in sexual size dimorphism.

304 Males did not, however, copulate for significantly longer with females larger
305 than themselves as compared to females smaller than themselves, with the
306 exception of *Echinolittorina vidua*. Differential copulation duration can, therefore,
307 only be explained by size-dependent mate preference in *E. vidua* (mean duration
308 with larger females \pm SD = 10.89 ± 0.72 min, and with smaller females = 7.36 ± 0.96
309 min; $t = 2.247$, $df = 35$, $P < 0.05$, Fig. 2b), which is the only species which did not
310 exhibit sexual size dimorphism. Copulation duration, therefore, seems to be related
311 to female rather than male size (Table 4).

312

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

314 Mated females were typically larger than unmated females (indicating positive
315 sexual selection on female size), and in 13 out of 16 (>80%) comparisons these
316 differences were significant (Table 2). In males the strength of sexual selection was

317 generally weaker, less clear, and species dependant (only 7 out of 16 (44%)
318 comparisons were significant, Table 2). There were similar, positive, sexual selection
319 indices for both sexes in *Echinolittorina malaccana*, *E. radiata*, *Littorina fabalis* and
320 the sheltered ecotype of *L. saxatilis* (Table 2). For the wave ecotype of *L. saxatilis* the
321 sexual selection indices were negative, indicating smaller females were selected by
322 males, although this was only significant in one population (Table 2). Apart from this
323 one exception, the overall trend in the family Littorinidae was for positive sexual
324 selection on size in both sexes of the seven species (including the sheltered ecotype
325 of *L. saxatilis*, Table 2, overall standardized means \pm SD: males = 0.27 ± 0.153 ;
326 females = 0.32 ± 0.083).

327

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

329 Overall, the relationship between SS_f (and SS_{f-m}) and sexual size dimorphism was
330 highly negative and significant across the whole data set (Table 2, $\rho_{f=}$ -0.77, $df=$ 15,
331 $P=$ 0.001, Fig. 3; $\rho_{f-m=}$ -0.56, $df=$ 15, $P=$ 0.025). The same trend was observed using
332 the mean values within species ($\rho_{f=}$ -0.89, $df=$ 6, $P=$ 0.007, Fig. 3; $\rho_{f-m=}$ -0.79, $df=$
333 6, $P=$ 0.036) or using the seven species but maintaining the two *L. saxatilis* ecotypes
334 separately ($\rho_{f=}$ -0.71, $df=$ 7, $P=$ 0.047; $\rho_{f-m=}$ -0.74, $df=$ 7, $P=$ 0.037). All these
335 results are in full agreement with expectations from scenario 2 (i.e. sexual size

336 dimorphism was pre-existing and not driven by sexual selection but other
337 components of natural selection). The relationship between SS_m and sexual size
338 dimorphism, however, showed a pattern contrary to scenario 1, but compatible with
339 scenario 2 (see Fig. 1; $\rho_{\text{samples}} = -0.14$, $df=15$, $P= 0.613$, $\rho_{\text{species}} = 0.21$, $df= 6$, $P=$
340 0.645).

341

342 **Discussion**

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

355 Our results showed clear support for the second proposed scenario, that the
356 observed sexual size dimorphism in many marine gastropods was pre-existing and
357 not necessarily driven by sexual selection. In addition, male and female sexual
358 selection was found in many marine gastropods and may be caused by the existence
359 of a common mate choice mechanism (males preferentially mate with females of the
360 same size plus a specific value, i.e. a 'similarity-like' mechanism, Fernández-Meirama
361 et al., 2017), and such mechanism would produce a negative correlation between
362 female (but not male) sexual selection and sexual size dimorphism. This finding
363 suggests that it is the degree of sexual dimorphism which explains the observed
364 patterns in female sexual selection. The same relationship between these two
365 variables is observed even when there are populations and species that exhibit the
366 opposite trends in sexual selection or sexual size dimorphism, confirming the
367 generality of the trend. Under this scenario, species that have the largest size
368 dimorphism, even when males prefer to mate with larger females than themselves,
369 could effectively still choose relatively small females (i.e. still larger than the male)
370 from the overall female population (see Fig. 1). When we studied the mechanism of
371 sexual selection in *Echinolittorina* species with different levels of sexual size
372 dimorphism in the wild, we observed the same mechanism of male choice causing
373 female sexual selection, confirming that sexual selection cannot explain present

374 levels of sexual size dimorphism.

375 In addition to sexual selection, other selection forces can also contribute to shape
376 size traits in these snails and different selection pressures may frequently counteract
377 each other (Blanckenhorn, 2005). Fecundity selection, for example, favours large size
378 in females (larger females carry more eggs or offspring, Hughes & Answer, 1982; Ng
379 & Williams, 2012; Ross & Berry, 1991; Zahradnik et al., 2008), but variability selection
380 driven by, for example, wave action could favour smaller male size (Johannesson et
381 al., 2008). Another scenario could be that male gastropods achieve a smaller size
382 compared to females just because of differential daily activities, as searching for
383 mates has been considered to impose a large daily energetic cost, while females
384 focus preferentially on foraging and feeding (Ng et al., 2013; Rolán-Alvarez, Austin,
385 et al., 2015; Zahradnik et al., 2008), causing differential growth rates between sexes
386 (Riascos & Guzman, 2010). Distinct natural selection components or life-history traits
387 may, therefore, act differentially on males and females to drive sexual size
388 dimorphism in marine gastropods, without the need to invoke any role of sexual
389 selection.

390 Another possibility would be that the observed sexual dimorphism does not have
391 a genetic basis. It is, for example, unknown whether differences in male and female
392 body sizes in gastropods are genetic in origin. Differential ecological strategies

393 between sexes could, therefore, affect the probability of survivorship at different
394 sizes, or affect the size at adult age or growth rate differences between sexes as
395 recorded in some pulmonates (Sutton, Zhao, & Carter, 2017). The niche hypothesis,
396 which includes the former possibility, has previously been proposed as a general
397 explanation for sexual size dimorphism (Shine, 1989), but it is rather difficult to test,
398 as the ecological conditions experienced may substantially vary from one organism
399 to another. However, this phenotypic version of the niche hypothesis assumes that
400 body size differences between sexes are not genetic in origin, and this prediction
401 could be experimentally tested.

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

412 choice experiments (Erlandsson & Kostylev, 1995; Johannesson et al., 2008; Ng &
413 Williams, 2014), supporting the theory that males have the ability to assess the size
414 of females from their trails. This variety of evidence, together with the correlation
415 between sexual selection and size dimorphism found in all studied species, suggests
416 that there could be a conserved mechanism in gastropods, where males typically
417 show a fixed preference for females slightly larger than themselves, causing the
418 observed trend for sexual selection in females across species.

419 An intriguing question is, 'why would males select females based on their own
420 body sizes and not simply select the largest female?' Selecting the largest available
421 female may, in fact, not necessarily be advantageous for a male because of the risk
422 of sperm competition (Herdman, Kelly, & Godin, 2004; Wedell, Gage, & Parker, 2002).
423 Any fecundity-related benefits accruing to a male that has mated with a large female
424 may be offset by an associated fitness cost of shared paternity if large females are
425 more likely to be mated multiple times (Herdman et al., 2004). A male's strategy of
426 selecting females slightly larger than his own size during trail following may,
427 therefore, have an important implication for maximizing reproductive success
428 through investing in a range of larger females rather than the largest female
429 available (Widemo & Sæther, 1999). Another plausible reason can be related to
430 physical mating constraints, such that copulation becomes physically more difficult

431 for two individuals when their size difference exceeds a certain threshold (Arnqvist,
432 Rowe, Krupa, & Sih, 1996; Crespi, 1989). Size-dependent male mate preference
433 during trail following can, therefore, be a strategy driven by a balance between a set
434 of fitness costs and benefits (Herdman et al., 2004; Wedell et al., 2002). Empirical
435 and simulation studies will, however, be necessary to confirm this interpretation.

436 The male sexual selection pattern (SS_m from Table 2), on the other hand, can be
437 caused by male-male competition. Aggressive physical male-male contests, for
438 example, have been reported in *E. malaccana* and *E. radiata* (Ng et al., 2016) as well
439 as in several other littorinids (Gibson, 1965; Ng & Williams, 2014; Zahradnik et al.,
440 2008) and other marine gastropods (e.g. *Strombus pugilis*, Bradshaw-Hawkins and
441 Sander 1981). In the littorinid species where such contests were observed, larger
442 males more often won these 'mating battles', where the smaller males copulating
443 with females were displaced (Ng et al., 2016; Ng & Williams, 2014).

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

450 Copulating for longer could, therefore, be advantageous (in terms of fertilization
451 success) for smaller males when mating opportunities can be limited in comparison
452 to larger males. Further investigations into variation in male mate preference under
453 different levels of male-male competition are, however, needed to formally test this
454 hypothesis (see Franceschi, Lemaître, Cézilly, & Bollache, 2010).

455

456 **Conclusion**

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

468 by the way the snails move, and the multi-functional benefits of utilizing their mucus
469 trails when searching for a mating partner (Ng et al., 2013).

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

477

478 **Data accessibility**

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

481

482

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719

720 **Figure and Table legends**

721

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

738

739 Figure 2. Frequency of male trail following (as percentage of cases observed; Figure
740 2a) and copulation duration between the two mating categories (white bars: females
741 smaller than males; black bar: females larger than males; Figure 2b) in the three
742 littorinids, *Echinolittorina malaccana*, *E. radiata* and *E. vidua*, at Cape d' Aguilar
743 Marine Reserve, Hong Kong. Significantly different results are indicated by asterisks
744 (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

745

746 Figure 3. Relationship between SS_f and sexual size dimorphism (both standardized)
747 for the whole data set (light squares) and for the means within the seven species
748 (dark circles). Correlation values and statistical significances are given in the text.

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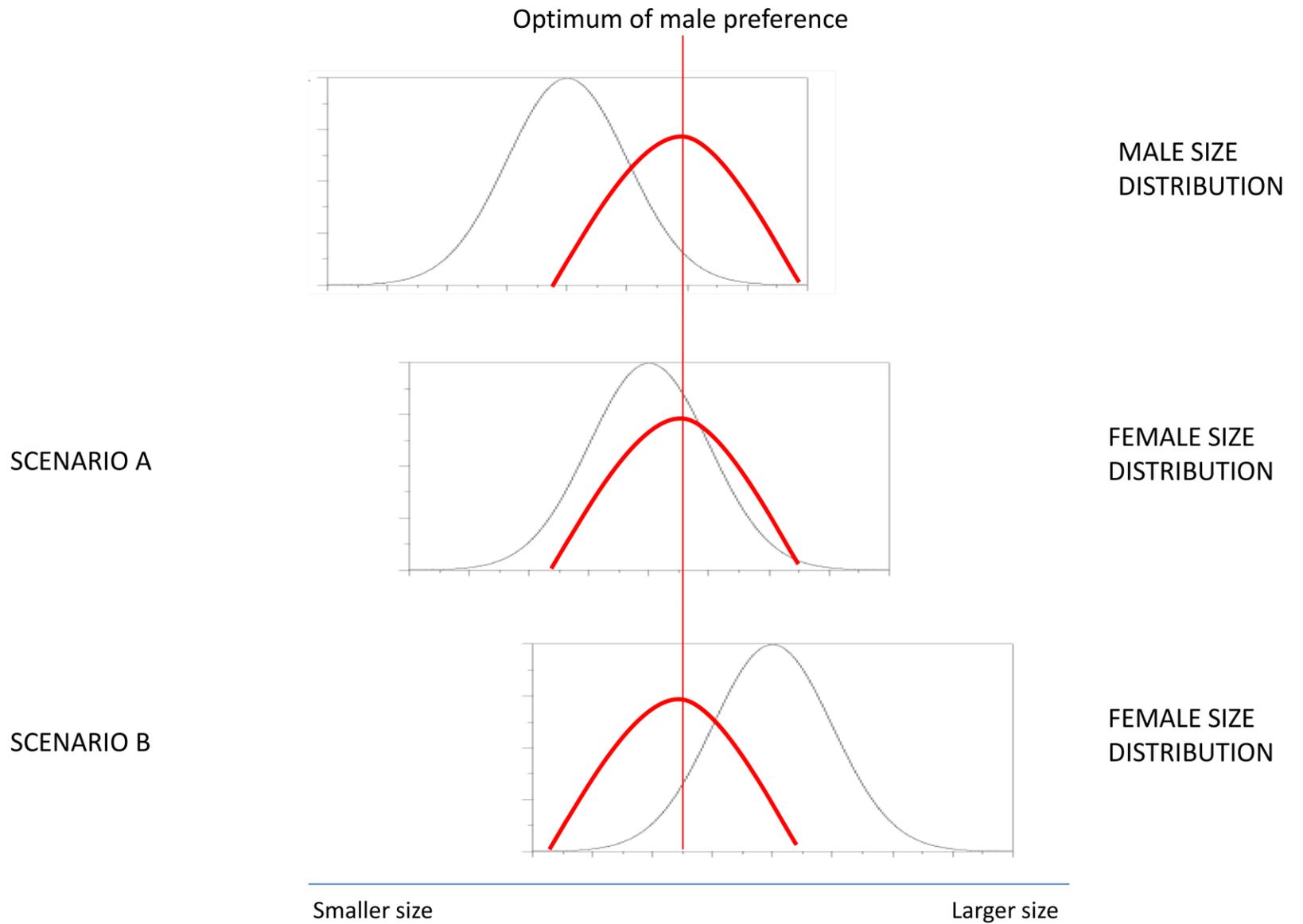
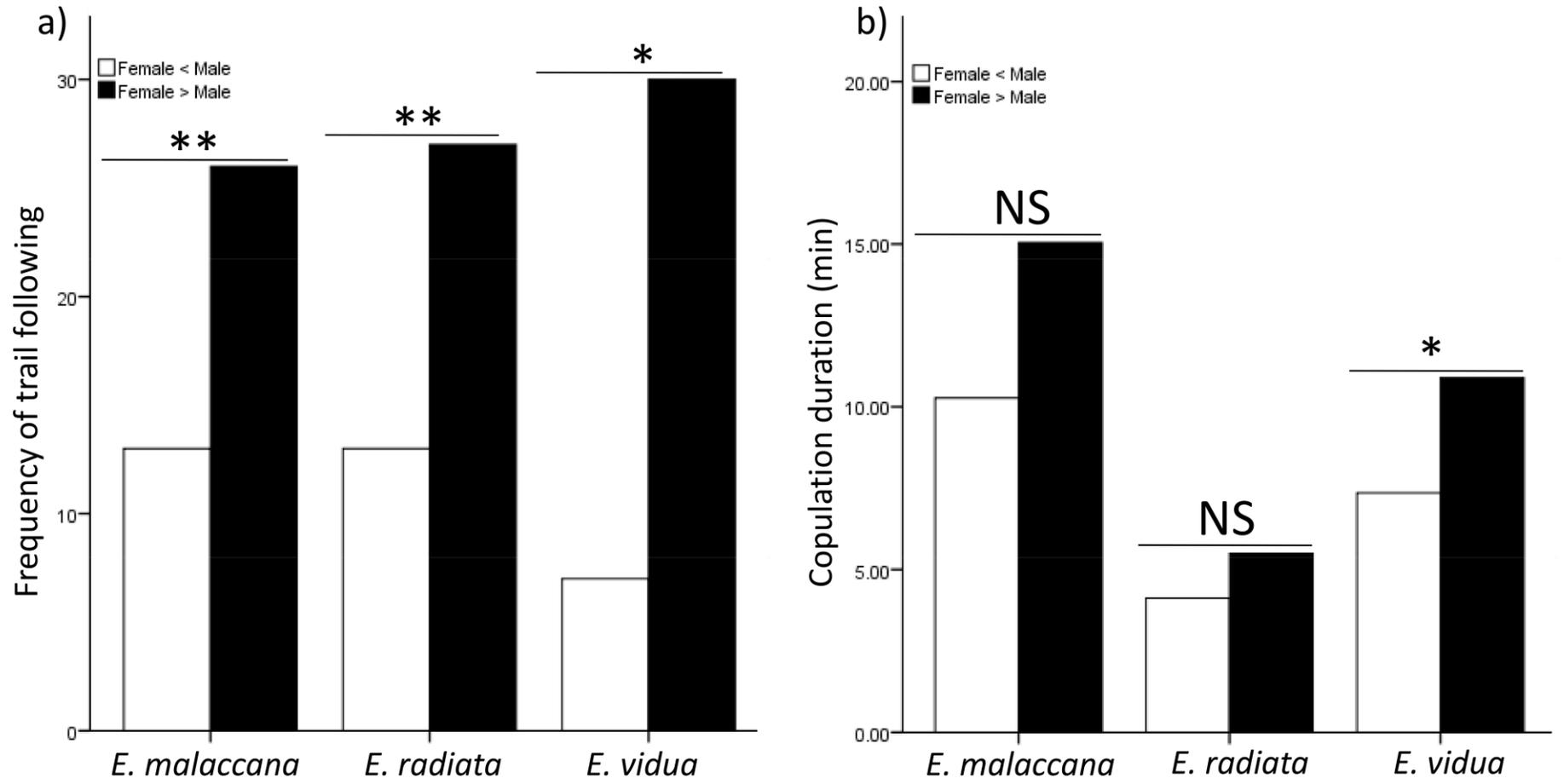


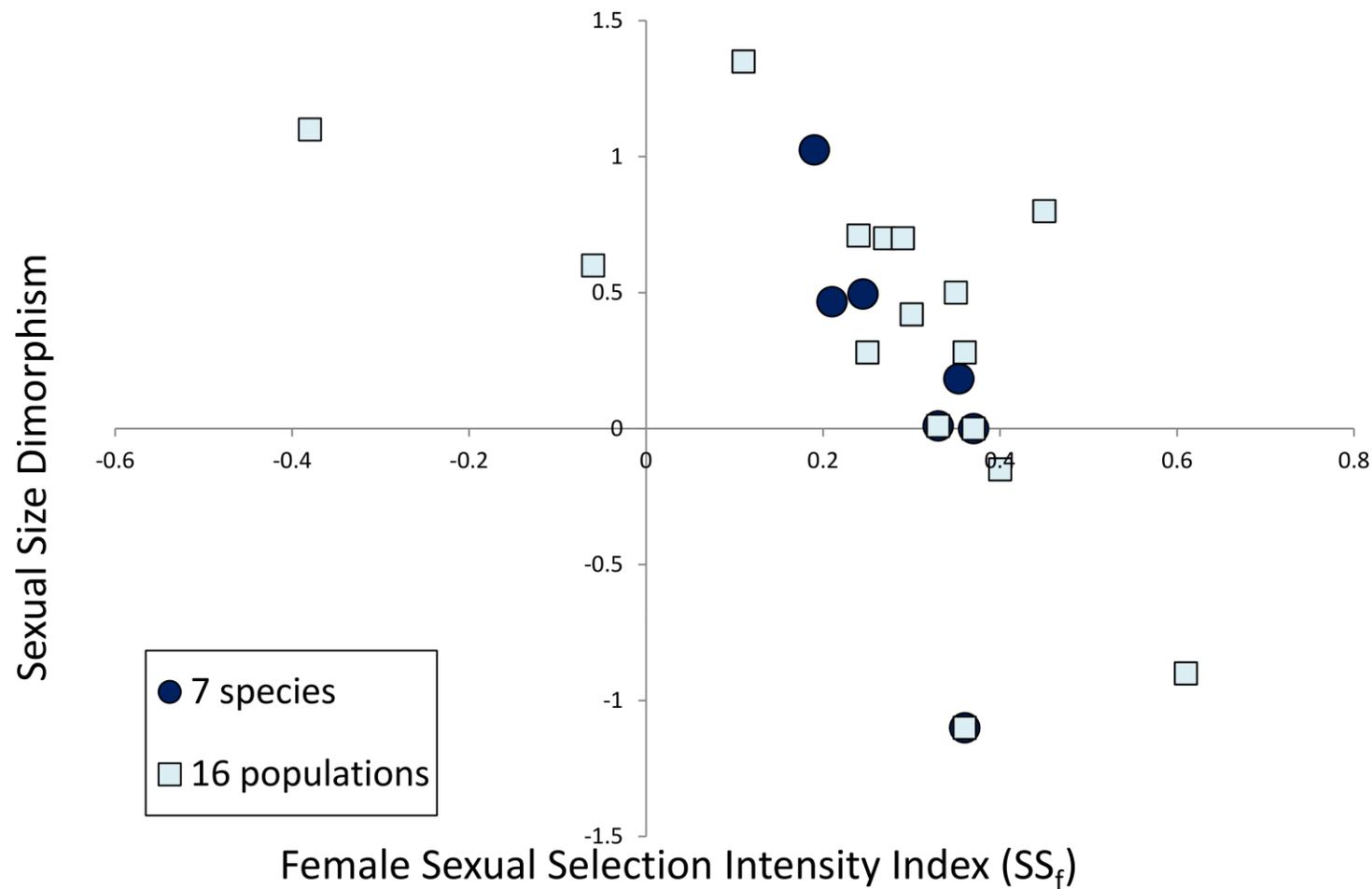
Figure 1



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Figure 2

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Figure 3.

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 WOS for publications including the words “sexual”, “selection” and “size” within Gastropoda (“Gastropoda” or “Gastropod” or “Mollusc”) (from the field “Topic”) 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.

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

Species	Locality	Reference	Nm	Nu	MALE 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 ^{***}
	Cape d' Aguilar 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 ^{**}
	Cape d' Aguilar 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>	Cape d' Aguilar ₂₀₁₂	This study	108	247	6.36 ± 1.51	6.12 ± 1.68	0.09	7.73 ± 1.62	7.47 ± 1.86	0.11
	Cape d' Aguilar ₂₀₁₅	Ng et al., 2016	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>	Cape d' Aguilar 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 ^{***}
	Abelleira ₂₀₁₄	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 ₂₀₁₆	This study	292	549	6.63 ± 0.81	6.34 ± 0.89	0.22 ^{**}	7.42 ± 0.97	7.05 ± 0.96	0.24 ^{***}
	Mean ± SD						0.22 ^{**} ± 0.00			0.24 ^{**} ± 0.01
<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 ₁₉₉₄	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 ^{***}
<i>L. saxatilis</i> _{wave}	Mean ± SD						0.39 [*] ± 0.154			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 [*]
	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

781 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
782 mated individuals and Nu = sample size of unmated (reference) individuals. Sexual selection intensity (SSm and SSf) index is the difference between mated and unmated
783 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 &
784 Rolán-Alvarez, 1998.
785

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

787

Species	Expected male- male trail following	Expected male- female trail following	Observed male- male trail following	Observed male- female 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*.

791

792

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

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

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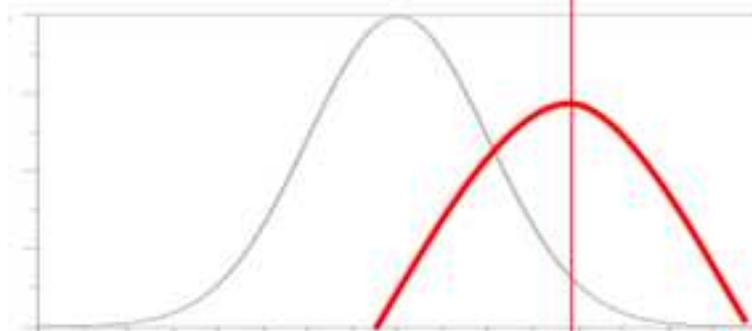
The causal relationship between sexual selection and sexual size dimorphism in marine gastropods

Acknowledgements.

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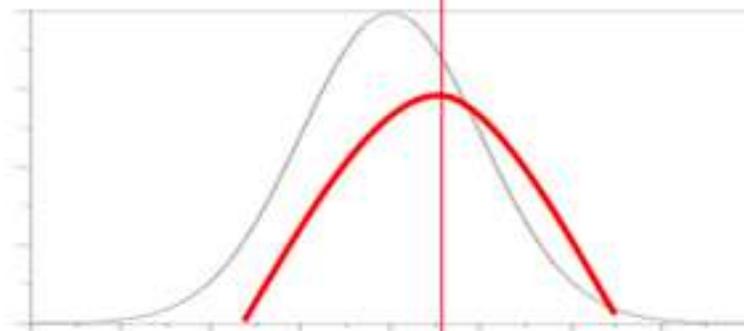
Figure 1
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Optimum of male preference



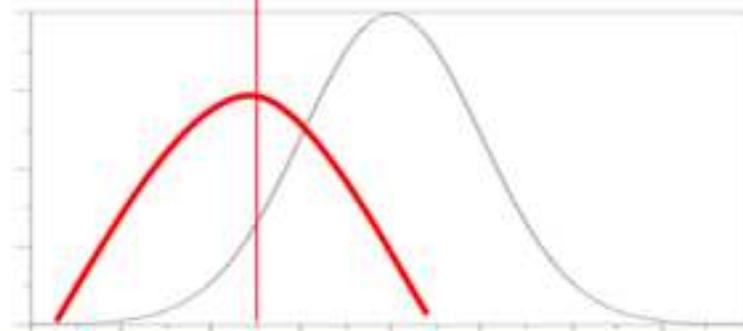
MALE SIZE DISTRIBUTION

SCENARIO A



FEMALE SIZE DISTRIBUTION

SCENARIO B



FEMALE SIZE DISTRIBUTION

Smaller size

Larger size

Figure 3
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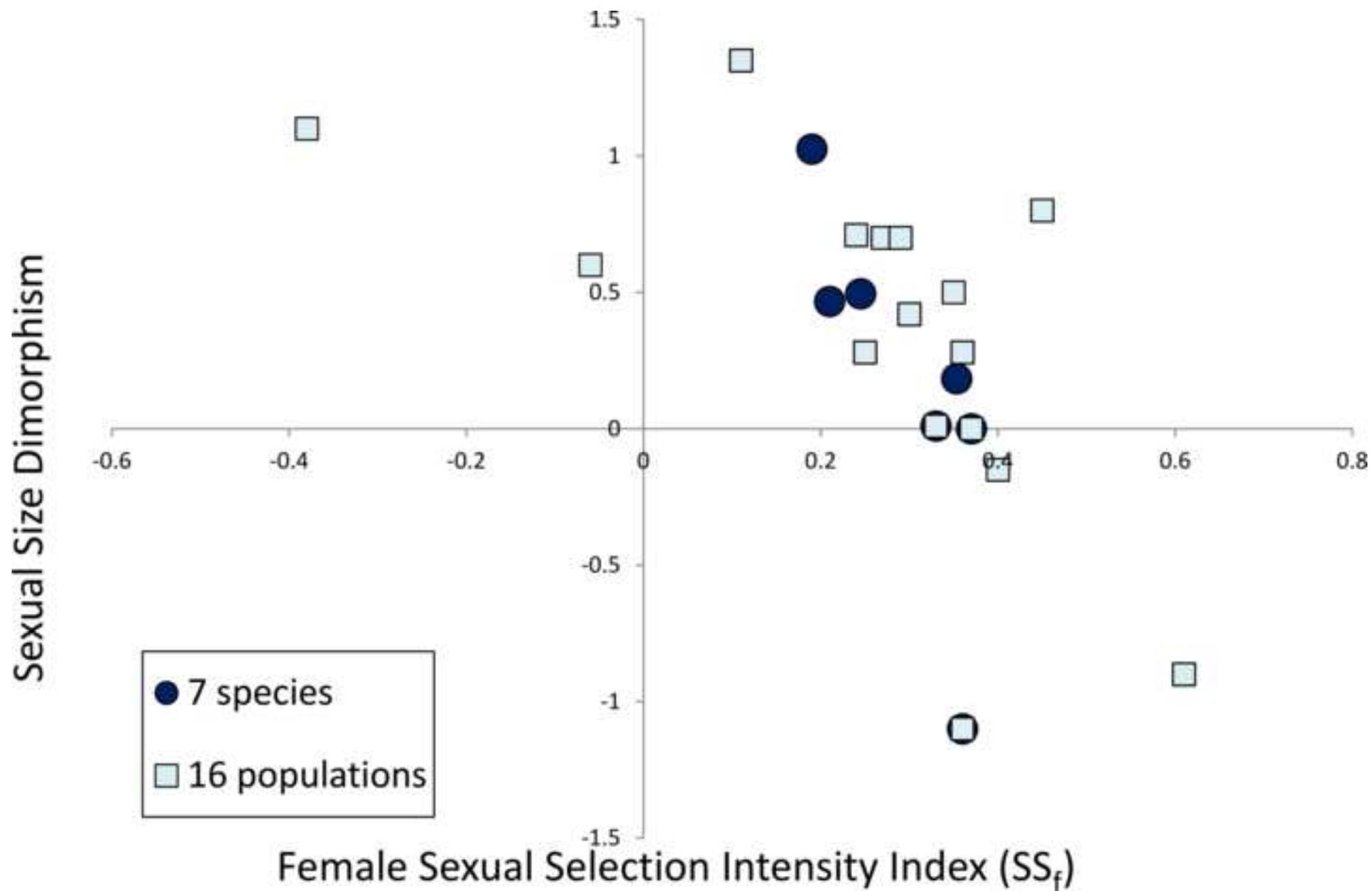
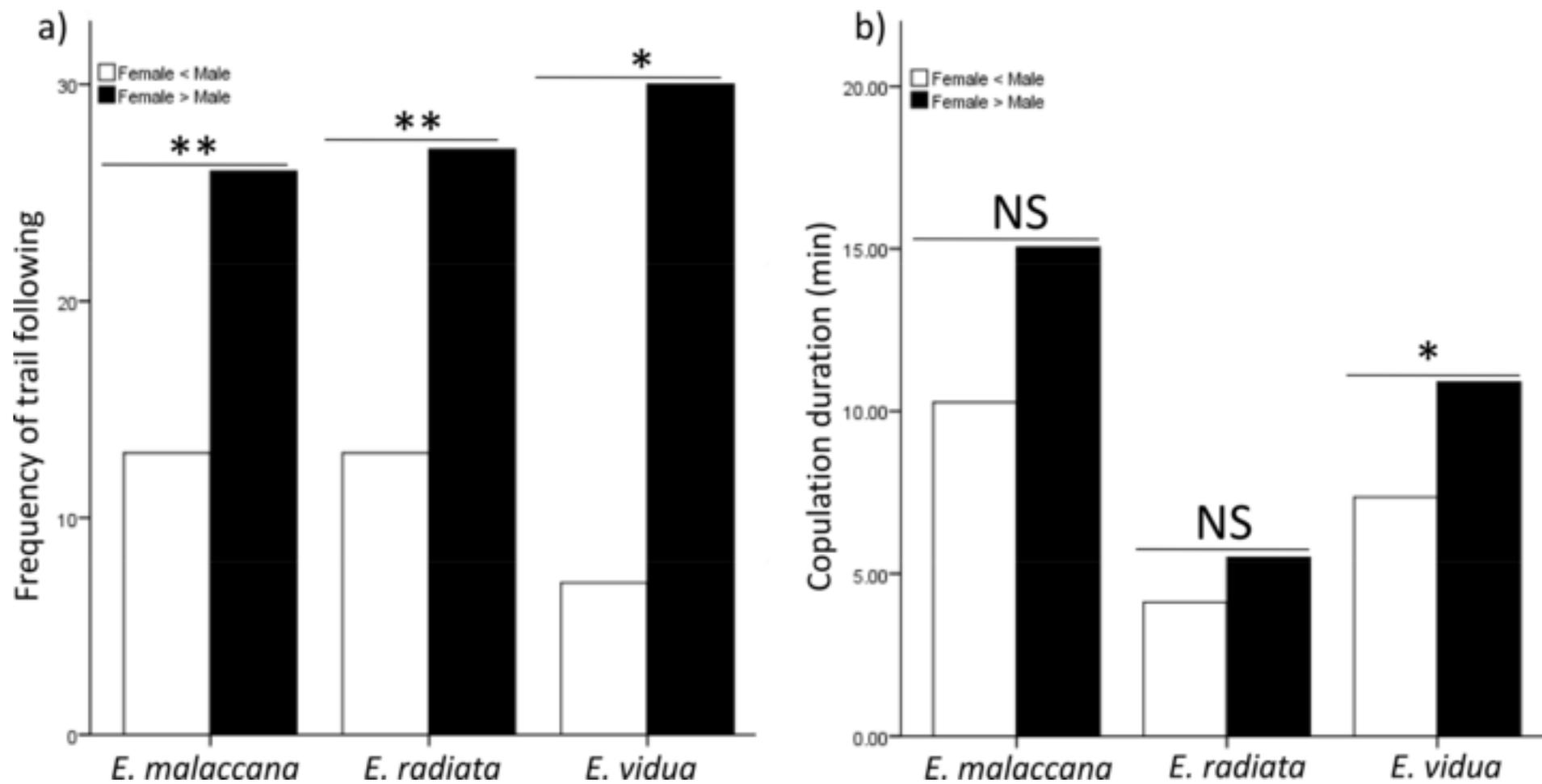


Figure
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Supplementary material for on-line publication only

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We hereby declare that the species used in this research, included in the Littorinidae family of marine gastropods, are not contemplated in any current legislation form within the European Union. Additionally, we declare that the results and main procedures of this research have been field based and therefore no animals were held in either aquariums or labs. Individuals of *Echinolittorina malacana*, *E. vidua* and *E. radiata* were collected and released back alive in the nearby area of The Swire Institute of Marine Science station in Hong Kong.

Yours sincerely, Emilio Rolán Álvarez

Vigo, 16 of October of 2018