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3 This is an author version of the contribution published on:

4 Questa è la versione dell'autore dell'opera: [**Italian**

5 **Journal of Zoology** DOI:

6 10.1080/11250003.2013.868934]

7 The definitive version is available at:

8 La versione definitiva è disponibile alla URL:

9 [<http://www.tandfonline.com/doi/abs/10.1080/11250003.2013.868934>]

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Demographic costs of sex allocation: hermaphrodites perform better in sparse populations

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Total text pages: 16

Number of tables: 0

Number of figures: 1

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Running title: reproductive load in simultaneous hermaphrodites

39 Abstract

40 *Ophryotrocha diadema* is an outcrossing, simultaneous hermaphroditic polychaete with
41 external fertilization. In isolated pairs, mature worms take turn contributing eggs upon the
42 condition that their partners reciprocate egg donation. In dense populations, these worms do
43 not reciprocate. Instead, they strongly compete for mating in their preferred male role and
44 produce few eggs. This plastic sex allocation may result in an overall different reproductive
45 performance: mean individual reproductive output will be larger in sparse than in dense
46 populations. We tested this hypothesis by measuring the individual reproductive output
47 (paternal *and* maternal offspring) of worms in sparse and dense replicated populations. In
48 dense populations, mean individual reproductive output was fourfold lower than that in sparse
49 populations. We hypothesise that such dramatic demographic costs are potentially widespread
50 in outcrossing simultaneous hermaphrodites with external fertilization and plastic sex
51 allocation. The reproductive output of hermaphroditic organisms is a function of population
52 density (i.e., the number of conspecifics) and studies on population growth and reproductive
53 performance should take this effect into account.

54

55 **Keywords:** reproductive load, tragedy of the commons, population size, mating, *Ophryotrocha*
56 *diadema*

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Comment [CR1]: I would suggest not to include this in the keyword: it is mentioned at the very end of the study, only

Comment [MCL2]: We would prefer to leave it here: there are only few examples of this phenomenon in the literature. This paper could be more easily picked up by searching engines and cited, if this term appears in the keywords

59

60 Introduction

61 Simultaneous hermaphrodites have two sexual functions and partition their reproductive
62 resources between them. Sex allocation theory predicts that they plastically adjust the proportion of
63 resources allocated to each sex as a function of mating group size (Charnov 1982). Theory predicts
64 that in sparse populations, where monogamous pairs may form, hermaphrodites increase their
65 investment in eggs and limit the male function to the production of the few sperm needed to fertilize
66 their partners' eggs. In dense populations, hermaphrodites divert resources from egg production and
67 invest more into the male function. Experiments or observations on different hermaphrodites have
68 tested this theory and have often found overall support for it, although the patterns of resource
69 allocation adjustments are highly species-specific (Raimondi & Martin 1991; Trouvè et al. 1999;
70 Schärer & Janicke 2009; Locher & Baur 2002; Hughes et al. 2002; Tan et al. 2004; Brauer et al.
71 2007). In some model systems, results show that sex allocation in hermaphrodites is plastic (i.e. it
72 changes as a function of mating opportunities), as predicted by theory. For example, the
73 hermaphroditic polychaete worms *Ophryotrocha diadema* Åkesson 1976 (Annelida: Polychaeta:
74 Dorvilleidae) have plastic female allocation that they adjust to mating opportunities, trading-off
75 with their investment in the male function. When mating opportunities are common (as in dense
76 populations), worms reduce their egg production drastically and compete for mating in the male
77 role; when mating opportunities are rare (as in sparse populations), they invest proportionally more
78 resources in egg production and, in the absence of competitors, reduce their investment into the
79 male function (Lorenzi et al. 2005, 2006). Sex allocation adjustments are the effect of sexual
80 selection acting on both sexual functions in hermaphrodites (Lorenzi and Sella, 2008; Anthes et al.
81 2010). These adjustments are typically hermaphroditic traits, and could explain why population
82 growth rates (as measured in dense, lab populations) are higher in gonochoric than hermaphroditic
83 species (Prevedelli et al. 2006).

Comment [MCL3]: We prefer to start with the definition of hermaphroditism and introduce the fact that some hermaphrodites alternate between sex roles later on in the manuscript, because in the specific literature, scientists distinguish between functions and roles. E.g. each simultaneous hermaphrodite has a male function but chooses whether it will play the male role depending on its partner(s) condition. We stay stuck to this definition throughout the manuscript.

Comment [MCL4]: Sperm has two plural forms either sperm or sperms but the first is more common in the specific literature

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Comment [MCL5]: We prefer to use the word adjustment because we already quoted this ability at line 52

Comment [MCL6]: We would prefer to stay with "common". In the first review round, Referee #2 suggested the use of the word "common" (see referee #2, who suggested the following: Minor point #5): "L63 consider 'common' instead of high (and rephrase the stuff in parentheses)"

Comment [CR7]: Explain: it is rather unclear!!

Comment [MCL8]: We clarified the text

85 If we assume a fixed budget for reproductive resources, we expect that hermaphrodites in large
86 populations would use the same amount of resources for egg production than hermaphrodites in
87 small populations, devalued of the resources diverted to increase the male function. Then we should
88 find that hermaphrodites in large populations have a lower mean reproductive success than those in
89 small populations. This reduced reproductive output should, in turn, affect population growth. We
90 tested this hypothesis in the outcrossing simultaneously hermaphroditic polychaete worm *O.*
91 *diadema* by measuring individual reproductive output of focal worms in sparse and dense
92 populations.

93

94 **Material and methods**

95 The animal model

96 *O. diadema* (Annelida, Polychaeta, Dorvilleidae) is a polychaete worm originally found in the
97 sediments of Californian harbors. Sampling from natural populations suggests that populations have
98 low densities (Premoli & Sella 1995). For example, only few *O. diadema* individuals were isolated
99 among hundreds of worms of a gonochoric *Ophryotrocha* species in the Pacific Coast (pers. comm.
100 by B. Åkesson to G.S.) and 0.1–6.6 individuals per kg⁻¹ of mussel clusters were collected in the
101 Mediterranean Sea (Simonini, pers. comm. to M.C.L.) (Schleicherová et al. 2013).
102 These worms are outcrossing simultaneous hermaphrodites with external fertilization. Before
103 maturing as hermaphrodites, they have a protandrous phase during which they can fertilize the eggs
104 laid by hermaphrodites (Sella & Lorenzi 2003). Then, they mature as hermaphrodites, and can both
105 fertilize their partners' eggs or lay eggs, but play one single role at each mating event. Eggs are laid
106 in jelly cocoons and develop into larvae that leave their cocoons 8 days later and mature into
107 simultaneous hermaphrodites in approx. 45 days. Mature hermaphrodites reproduce iteroparously
108 for 7-10 weeks (Åkesson 1976, 1982).
109 In isolated pairs, worms take turns in laying cocoons of 20-25 eggs every third day (Sella 1985,
110 1988). When more than two worms are present, they adjust their sex allocation by investing

Comment [CR9]: If mean egg production of hermaphrodites were similar in sparse and dense populations, then the amount of resources allocated to increase the male function could reduce the reproduction contribution, and hermaphrodites in dense populations would reproduce less.

Comment [MCL10]: We changed the previous sentence to clarify its meaning, following comment CR9

Deleted: Polycheta

Comment [MCL11]: We used "Californian" following the suggestion by referee #2 (see Minor comments, L78 remove 'which was' and make it 'Californian')

Comment [MCL12]: A few *O. diadema* worms were found among hundreds of worms of a (single) gonochoric species.

112 proportionally more resources into the male function (Lorenzi et al. 2005, 2006), mate
113 promiscuously (Sella & Lorenzi 2000) and can share the paternity of a single egg-cocoon with other
114 hermaphrodites (Lorenzi et al. 2013). Sex allocation adjustments are not costly in the short term
115 (Lorenzi et al. 2008) and polychaetes sense the number of conspecifics and/or potential mates
116 through waterborne chemical cues (Schleicherová et al. 2006, 2010; Minetti et al., 2013).

117 ***Experimental procedure***

118 Data were gathered from focal worms. The 'focal' worms were identified through the colour of
119 their eggs. In mature worms, eggs can be easily detected through the transparent body wall as
120 either yellow or whitish eggs. In these worms, a dominant Y allele determines a yellow-egg
121 phenotype, while the recessive y allele determines a white-egg phenotype (Sella and Marzona
122 1983). By means of this genetic marker, we can identify focal worms in a group and ascribe their
123 progeny. The focal worms had yellow eggs and their mates white eggs.

124 We carried out the experiment in glass bowls filled with 10-ml artificial sea-water and kept in a
125 thermostatic chamber at 20°C. Once a week water was replaced in the bowls and worms were fed
126 with spinach *ad libitum*.

127 To obtain a sufficient number of worms for the experiment, 24 pairs of yellow-phenotype worms
128 and 40 pairs of white ones were cultivated separately and allowed to reproduce. Their offspring
129 supplied the virgin, newly mature, yellow- and white-phenotype worms of same age to be used for
130 the experiment. At sexual maturity, two worms from each yellow-phenotype offspring (n = 48
131 worms, hereafter, 'focal worms') were randomly assigned either to sparse populations (population
132 size = 2; the population consisting of one focal, yellow-phenotype worm and one white-phenotype
133 partner, n = 24 replicates) or to dense populations (population size = 12, consisting of one focal,
134 yellow-phenotype worm and 11 white-phenotype potential partners, n = 24 replicates). With such a
135 matched-sample design, each worm in the sparse population served as a control for its sibling in the
136 dense population.

Comment [MCL13]: We clarified the text and added details.

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Comment [CR14]: Are worms under crowdy or sparse conditions kept in same volume?

Comment [MCL15]: All bowls had a volume of 10 ml (this info is reported at line 109)

138 Experimental populations were checked daily for 12 days. At the first check, focal worms' body
139 size was measured as the number of chaetigerous segments. At each check, we recorded the number
140 of yellow (laid by focal worms) and white cocoons (laid by focal partner/s), and the number of eggs
141 per cocoon. Adult worms were removed from the bowls on day 9.

142 Reproductive output of focal worms was quantified by rearing offspring until they were sexually
143 mature. Indeed, in large populations multiple potential "fathers" were present and paternity of the
144 progeny could be assigned to either the focal worms or one of their rivals only after the progenies
145 were sexually mature. When these worms matured and had eggs in their coeloms, they expressed
146 their yellow or white phenotypes and we assessed their paternity (i.e., about 45 days after egg
147 laying). Following Åkesson (1976), this marker is neutral, since there is no difference in worm
148 mortality rates before sexual maturity.

149 The ratio between the total number of cocoons produced in sparse populations and that produced in
150 dense populations was approx 1:2, leading to more larvae per unit of volume in the dense
151 population bowls. Therefore, to standardize rearing conditions, on day 9 the volume of sea water
152 was doubled in the dense population bowls.

153 The total reproductive output of focal worms was estimated as the number of offspring (both
154 maternal *and* paternal offspring) that on maturity had the yellow phenotype. Focal worms without
155 offspring were included in the calculations.

156 In order to control for the potentially confounding effect of differential egg-mortality in sparse and
157 dense populations, we estimated egg mortality as the average proportion of eggs that disappeared
158 from the cocoons in each bowl (with respect to the laid eggs).

159

160 *Statistical analyses*

161 Some replicates were excluded from [calculations](#) for various reasons (e.g., some worms died
162 altering population size). By using related worms in sparse and dense populations, we reduced the
163 overall variability due to genetic differences (Howell 2010). We used a linear mixed model (LMM)

Comment [MCL16]: We clarified the text and added details.

Comment [MCL17]: We clarified the text as requested

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165 to assess the significance of the differences in reproductive output between pairs of siblings in
166 sparse and dense populations (dependent variable: reproductive output; within-subject factor:
167 population size; random factor: family ID; covariate: body size).
168 Probabilities were two-tailed. Statistical analyses were performed using SPSS 20.0 statistical
169 package (SPSS Inc, Chicago, IL).

170

171 **Results**

172

173 ***Reproductive output of focal worms***

174 Focal worms had a dramatically lower reproductive rate in dense populations, compared to that in
175 sparse populations, with a fourfold reduction in their reproductive output (mature maternal +
176 paternal offspring) (Fig 1). The difference was highly significant, indicating that reproductive
177 output in simultaneous hermaphrodites was strongly affected by population size (LMM, population
178 size: $F_{1,35.79} = 55.557$, $P < 0.0001$; body size: $F_{1,34.99} = 4.721$, $P = 0.037$). The significant relationship
179 between body size and reproductive output which emerged in the LMM occurred only in dense
180 populations (Spearman's rho, in dense populations: $\rho = 0.496$, $P = 0.022$; in sparse populations:
181 $\rho = 0.133$, $P = 0.545$). Body size advantage in dense populations was not associated with the
182 female function (correlation between body size and egg production in dense populations: $\rho =$
183 0.180 , $P = 0.460$; in sparse populations: $\rho = 0.288$, $P = 0.231$). This suggests that larger
184 hermaphrodites in dense populations might have a higher reproductive output because they were
185 more successful in the competition for the male role.

186 Egg mortality was not significantly different between sparse and dense populations (Wilcoxon test,
187 $Z = 73.00$, total $n = 20$, $P = 0.376$), suggesting that it did not affect the results (median proportion of
188 eggs which disappeared in sparse populations: 5.56 % vs 4.86% in dense populations).

189

190 **Discussion**

Comment [MCL18]: We clarified the sentence

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Comment [MCL19]: We deleted the sentence that should have been moved to discussion. We left a short comment at the end of the sentence, to help the reader to understand the meaning of these results..

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194 In this study we document that population size (i.e. the number of conspecifics) affects the
195 reproductive output of simultaneous hermaphrodites as they produce four times more offspring in
196 sparse than in dense populations. We interpret these results as a consequence of the fact that
197 hermaphrodites have a plastic sex allocation which they adjust to mating opportunities. As
198 population size increases, mating opportunities increase as well and hermaphrodites adjust their sex
199 allocation in favour of the male function at the expense of the female function.

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200 The reduced reproductive output of the worms in dense vs. sparse populations could be the result of
201 uncontrolled density-dependent effects (e.g., mortality, oophagy, etc.) rather than a response to
202 population size (e.g., the number of conspecifics). However, this hypothesis is not supported by
203 evidence. First, egg mortality did not differ between sparse and dense populations. Second, a
204 previous study documented that *O. diadema* worms had a higher egg production in sparse than in
205 dense populations, irrespective of any density-dependent effects such as metabolite accumulation or
206 encounter probability (Lorenzi et al. 2005). Furthermore, in other experiments, we simulated large
207 population size, so that pairs of worms perceived cues as if population size were larger than two,
208 and they reduced their egg output according to the perceived, and not the real, population size
209 (Schleicherová et al. 2006, 2010). All these observations support the hypothesis that worms reduce
210 their egg output as population size increases.

Comment [MCL20]: We clarified the difference between density-dependent effects and population size

Comment [MCL21]: We used the same term as above, to highlight the difference between density-dependent effects and population size

211 It could be argued that, if worms decrease egg production in dense populations, the competition for
212 mating as males should increase and worms with more female-biased allocation will gain higher
213 reproductive success. Whilst this might be true in the short term, it might be disadvantageous in the
214 long term, because fecundity often trades off with lifespan (Stearns & Hoekstra 2000). Indeed,
215 hermaphrodites which skip the female role for long time periods live longer (Di Bona et al. 2010).

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216 Mating in sparse populations is associated with small mating groups, i.e., low numbers of partners
217 and few or no rivals over the male role. In small mating groups, hermaphrodites invest large
218 proportions of their reproductive resources into eggs, trade eggs with their partners and take turns in
219 the two sexual roles (Sella, 1985; Sella & Ramella 1999). This is an evolutionary solution to the

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224 conflict on sex roles, since both partners prefer to play the cheaper male role than the expensive
225 female role (Leonard 1993, 2005, 2006; Di Bona et al. 2010). In natural contexts, outcrossing
226 hermaphrodites may be constrained to monogamous mating regimes when they live in very sparse
227 populations, as *O. diadema* (Sella & Ramella 1999; Simonini, personal communication). In other
228 hermaphroditic species, the sizes of the populations are large but hermaphrodites are trapped in
229 monogamous mating regimes by other life-history traits. For example, the serranid fish,
230 *Hypoplectrus nigricans* is an outcrossing hermaphrodite which mates monogamously (Fischer
231 1980). Here, monogamy is constrained by the short spawning period (few hours per day), which
232 reduces the chances that paired partners desert: reproductive gains from deserting the partner may
233 be low, if most partners are paired.

234 In present study, worms in dense populations reduced their reproductive output to less than 30%
235 when compared to worms in sparse populations. Similarly, *Plasmodium chabaudi* adjust their sex
236 allocation in response to the presence of unrelated conspecifics. Reece et al. (2008) directly
237 manipulated mating-group sex ratio of these malaria parasites and measured the resulting
238 reproductive output as the number of zygotes produced. As predicted by sex allocation theory,
239 mating output was maximized at intermediate sex ratios, indicating that sex allocation in this
240 malaria parasite is likely to be under stabilizing selection and reproductive output was maximized at
241 female-biased sex ratios.

242 Overall, our study shows that the potential individual advantages in fitness due to opportunistic sex
243 allocation are countered at the population level when populations are dense; opportunistic sex
244 allocation is advantageous to the individual, but disadvantageous to the population, whose
245 reproductive rate declines. Accordingly, Prevedelli et al. (2006) found that dense populations of
246 hermaphrodites had a demographic disadvantage compared to gonochorists. Here, we highlight that
247 the demographic disadvantage of hermaphrodites is mainly due to their adaptive ability to adjust
248 their sex allocation to mating group size and, ultimately, to population size. In this perspective, our
249 study is an example of the tragedy of the commons (Hardin 1968), where traits which are

Comment [MCL22]: We changed the term using the words used by Reece et al use in their Nature paper.

Comment [CR23]: A bit confused. To be rephrased.

Comment [MCL24]: We added the explanation

250 advantageous at the individual level reduce population fitness. For example, strong cannibalism of
251 larvae and pupae by adult flour beetles is adaptive at individual level but impairs population growth
252 (Wade 1977). Similarly, hyperaggressive water-strider males gain a slightly higher mating success
253 than less aggressive males but reduce overall group mating in their pond (Chang & Sih 2013). More
254 specifically, sexual selection can diminish population reproductive rates of *Drosophila* populations
255 by imposing a “reproductive load” (Holland & Rice 1999). The reproductive load highlighted in
256 *Drosophila* was caused by antagonist sexual selection and intersexual conflicts inherent to
257 promiscuity. Similarly, sex allocation adjustments are promoted by sexual selection acting on the
258 two sexes of simultaneous hermaphrodites (Lorenzi & Sella 2008; Anthes et al. 2010; Leonard
259 2013).

260 We highlight that the demographic advantage of hermaphroditism in sparse populations (relative to
261 dense populations) is the bare outcome of sex allocation adjustments in hermaphrodites where the
262 two sexual functions interfere with each other and resources are traded off between the male and
263 female function (Lorenzi et al. 2006). Therefore, we expect that the results we obtained here could
264 be [obtained in](#) other hermaphroditic systems as well, where the two sexual functions act in
265 opposition and resources are partitioned between the male and the female function on the basis of
266 population size. We hypothesise that such dramatic demographic costs of sex allocation are
267 potentially widespread in outcrossing simultaneous hermaphrodites with external fertilization. If the
268 reproductive output of hermaphroditic organisms is a function of population size, population growth
269 studies (and their practical applications) should take the effect of sex allocation into account.

270

Comment [MCL25]: This is the term used by Chang and Sih 2013

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273 **Acknowledgements**

274 We thank Sergio Castellano and Janet Leonard for fruitful discussion on sexual selection, John
275 Pannell for comments on a previous version of the manuscript and Silvia Natta for help in the
276 laboratory. We also wish to thank two anonymuos referees who made helpful comments on a
277 previous version of the manuscript. This work was funded by MIUR (PRIN 2008 YS7AP3) and the
278 University of Turin (ex 60% to M.C.L. and G.S.).

279

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371

372 Figure legend

373 Figure 1. The reproductive output of focal worms (maternal and paternal offspring) in sparse and
374 dense populations (mean \pm s.e.).

