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Abstract: Considering the recent explosion of interest in sexual conflict, the effect of environmental conditions on the intensity of sexual conflict within populations has been largely ignored. Reproductive encounters within coelopids are characterised by sexual conflict in the form of intense male harassment, usually resulting in a vigorous pre-mating struggle. Here we investigated the effect of habitat composition and duration of exposure to oviposition sites on the level of sexual harassment by males and mating success in two species of European seaweed flies, *Coelopa frigida* and *C. pilipes*. The wrack beds inhabited by these two species are dominated by two genera of brown algae, *Fucus* and *Laminaria*, the relative proportions of which can vary considerably between wrack beds. Previous studies have shown that *Fucus* stimulates male harassment, increases copulation duration and induces females to oviposit in both species. Here we show that *Laminaria* stimulates a higher level of harassment in male *C. frigida* than *Fucus*. However, a similar effect was not observed in *C. pilipes*, with the main additional factor affecting male harassment in this species being the age of the male. Our study highlights the potential importance of environmental conditions on the intensity of sexual conflict within a population. We discuss the evolutionary significance of these observed effects in seaweed flies.

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Dear Dr Turner,

Please find attached manuscript that we hope you would consider for publication in *Animal Behaviour*. I can confirm that the research described is original and does not overlap with other published work, nor has been submitted to other journals for publication.

Yours faithfully,

Dominic Edward

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The effect of habitat composition on sexual conflict in the seaweed flies, *Coelopa frigida* and *C. pilipes*

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Editors Comments

Parts of the discussion have been reworded to make the distinction between realised and potential conflict. (Lines 240-254)

Sentence beginning line 243 and ending 244 has been deleted.

On line 268-269, “to generate sexual selection” has been deleted.

In the final paragraph reference is now made to mating rate as opposed to optimal mating rate.

In the final paragraph Simmons & Bailey 1990 has been included as a reference.

Sentence beginning on line 300 and ending on line 301 has been reworded, now on lines 298-300.

Line 308 has been changed, now line 307.

The penultimate sentence, lines 312-314 has been deleted.

Typo on line 186 has been corrected.

Figures have been reduced in Excel so as to only take up a single page in the constructed PDF file.

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Lay Summary

There are two species of seaweed fly found in Northern Europe that live in deposits of seaweed washed up on beaches at high tide. Mating in both species involves a violent struggle as females attempt to reject multiple attempts to mate made by males. We measured the number of times males attempted to mate and were successful at mating with a female under different conditions. By varying the type of seaweed that males were exposed to and how long they were exposed we found that male mating behaviour was different in the two species. Whilst the male of one species of seaweed fly was influenced by the type of seaweed, males of the other species were influenced by the length of time that they had been exposed. The variability in male mating behaviour found in seaweed flies is of interest because of the intensity of the conflict between the sexes.

1 The effect of habitat composition on sexual conflict in
2 the seaweed flies, *Coelopa frigida* and *C. pilipes*

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4

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15 *Running Headline:* **EDWARD & GILBURN: SEXUAL CONFLICT IN**

16 **COELOPIDS**

17

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20

21 *Word Count:* 3353 (excluding references and figure legends)

22 Considering the recent explosion of interest in sexual conflict, the effect of
23 environmental conditions on the intensity of sexual conflict within populations has
24 been largely ignored. Reproductive encounters within coelopids are characterised by
25 sexual conflict in the form of intense male harassment, usually resulting in a vigorous
26 pre-mating struggle. Here we investigated the effect of habitat composition and
27 duration of exposure to oviposition sites on the level of sexual harassment by males
28 and mating success in two species of European seaweed flies, *Coelopa frigida* and *C.*
29 *pilipes*. The wrack beds inhabited by these two species are dominated by two genera
30 of brown algae, *Fucus* and *Laminaria*, the relative proportions of which can vary
31 considerably between wrack beds. Previous studies have shown that *Fucus* stimulates
32 male harassment, increases copulation duration and induces females to oviposit in
33 both species. Here we show that *Laminaria* stimulates a higher level of harassment in
34 male *C. frigida* than *Fucus*. However, a similar effect was not observed in *C. pilipes*,
35 with the main additional factor affecting male harassment in this species being the age
36 of the male. Our study highlights the potential importance of environmental
37 conditions on the intensity of sexual conflict within a population. We discuss the
38 evolutionary significance of these observed effects in seaweed flies.
39

40 Following the work of Parker (1979) there has been a recent explosion of interest in
41 sexual conflict and its importance as a major force shaping the evolution of mating
42 systems (reviewed by Chapman et al. 2003; Arnqvist & Rowe 2005). Yet despite this
43 upsurge in research investigating sexual conflict, the influence of environmental
44 conditions on the intensity of conflict within populations has been largely ignored.
45 Identifying environmental influences on sexual conflict are essential if we are to fully
46 understand how mating systems operate and evolve (Ortigosa & Rowe 2002). A
47 number of studies have investigated the effects of predation risk (Sih 1988; Lima &
48 Dill 1990; Sih et al 1990) and hunger (Travers & Sih 1991) on mating behaviour but
49 very few have actually investigated its effects directly on sexual selection and sexual
50 conflict (Rowe 1992; Ortigosa & Rowe 2002).

51

52 Examples of mating systems characterised by high levels of male harassment
53 and vigorous pre-mating struggles are found in many insect taxa including sepsids
54 (Ward et al. 1992), gerrids (Rowe et al. 1994), coccinellids (Majerus 1994), carabids
55 (Takami 2002) and ichneumonids (Teder 2005). All species of seaweed fly
56 (Coelopidae) so far studied exhibit pre-mating struggles (Day et al. 1990; Crean &
57 Gilburn 1998; Crean et al. 2000). Coelopids can be found inhabiting accumulations of
58 detached seaweed deposited on the seashore after high tides or stormy weather,
59 known as wrack beds. Seaweed is known to be important for coelopid reproduction,
60 increasing male harassment of females and reducing survival in both sexes (Dunn et
61 al. 2002). Seaweed also induces oviposition in females, but otherwise does not
62 influence female reproductive behaviour, with a consistent rejection response being
63 maintained irrespective of the presence or absence of seaweed (Dunn et al. 2002).
64 Male harassment invariably results in a pre-mating struggle during which the female

65 will attempt to reject the male with a combination of shaking and kicking movements,
66 whilst at the same time curling her abdomen downwards to prevent genital contact
67 (Day et al. 1990). Thus, the presence of seaweed increases the frequency of pre-
68 mating struggles within a population of coelopids.

69

70 The two species of coelopid most commonly found on the coasts of Northern
71 Europe are *Coelopa pilipes* and *Coelopa frigida* (Phillips et al. 1995). *C. frigida* and
72 *C. pilipes* can be found in both allopatric and, more commonly, sympatric
73 populations. Both conspecific and heterospecific interactions between larvae may
74 influence the success of individuals in high density populations (Phillips et al. 1995),
75 with the mechanism of competition most likely to be purely exploitative.

76

77 The coelopid life cycle can be completed in wrack beds composed of a wide
78 variety of different seaweeds (Dobson 1974; Phillips et al. 1995). Wrack beds around
79 the UK are primarily composed of two different genera of brown algae, *Fucus* and
80 *Laminaria*. Dobson (1974) reported that *C. frigida* could be bred more successfully on
81 *Laminaria* monocultures than *C. pilipes* and also that *C. frigida* were found in greater
82 numbers in wrack beds that predominantly consisted of *Laminaria*. *C. pilipes* females
83 show a preference for ovipositing on *Fucus*, though it should be noted that both *C.*
84 *frigida* and *C. pilipes* will lay eggs on both types of seaweed (Phillips et al. 1995).

85

86 Male harassment has previously been shown to be stimulated by the presence
87 of *Fucus* in both *C. frigida* and *C. pilipes* (Dunn et al. 2002). Male harassment rate
88 determines the frequency at which pre-mating struggles occur. Therefore, the level of
89 sexual conflict within a population is affected by environmental conditions in the

90 form of the presence of seaweed. As previous studies have reported that *C. frigida*
91 larvae and adults favour *Laminaria*, it might be expected that the stimulation of male
92 harassment would also be algal specific. Additionally the duration of exposure to
93 algae might also affect the level of stimulation of male harassment. In this study we
94 compare the effects of different algal genera and duration of exposure to them on the
95 harassment and copulation success rates of male *C. frigida* and *C. pilipes*. We then
96 discuss the implications of habitat specific effects on sexual conflict and interspecific
97 competition.

98

99

METHODS

100

101 Preparation

102

103 Laboratory populations of *C. pilipes* and *C. frigida* were established from wild
104 larvae collected in February 2004 from the Forth Estuary. *C. frigida* were from
105 Whitesands, East Lothian (NT712775) and *C. pilipes* from St Monans, Fife
106 (NO521012). Virgin flies were collected and stored in 250ml flasks at 5°C with cotton
107 wool soaked in excess 5% sucrose solution. Under these conditions flies are not
108 reproductively active. Flies were maintained for no more than 5 generations in the
109 laboratory and used for mate trials within 7 days of eclosion. Seaweed (*Fucus*
110 *serratus*, *Fucus vesiculosus* and *Laminaria digitata*) was collected from Whitesands,
111 East Lothian and Cellardyke Harbour, Fife (NO577038). Seaweed was coarsely
112 minced prior to use.

113

114 Experimental Procedure

115

116 Male flies were placed into individual plastic vials (45mm dia. X 40mm) with
117 one of four different seaweed treatments - *F. serratus*, *F. vesiculosus*, *L. digitata* or a
118 control. A small amount of minced seaweed (weighing approximately 2g) was added
119 to each vial in addition to a small ball of cotton wool soaked in excess 5% sucrose
120 solution. The control group vials contained only cotton wool soaked in sucrose
121 solution. Female flies were placed into individual clear plastic containers (30mm dia.
122 X 54mm) containing a small amount of cotton wool soaked in 5% sucrose solution.
123 Flies were initially transferred into their respective containers under light CO₂
124 anaesthesia, but for the remainder of the study no anaesthesia was used. Containers
125 were subsequently stored in constant temperature rooms at 25°C prior to mate trials.
126 Female flies and control males were kept separately in a room that had no history of
127 seaweed presence. Every 24 hours additional sucrose solution was added to vials to
128 replace fluid lost by evaporation.

129

130 At intervals of 1, 2 and 3 days following preparation an equal proportion of
131 vials from each seaweed treatment group were used in mate trials. Thus a 3 x 4
132 factorial design was adopted for each fly species to determine the effect of time left on
133 the seaweed and species of seaweed upon male mating behaviour. All mate trials were
134 carried out at 25°C. Males were introduced into the vials of randomly selected females
135 and observed for up to 10 minutes or until a mount was observed. Males failing to
136 mount within 10 minutes were scored as unwilling to mount. For those that did mount
137 their given female, the outcomes of pre-mating struggles were recorded as either
138 copulation (genital coupling was observed) or female rejection (if the female managed
139 to reject the male). Flies were killed by placing them in a freezer at -25°C. Body size

140 was estimated by measuring wing length, which has been used an indicator of size in
141 most previous studies of coelopid behaviour (e.g. Day et al. 1990; Crean & Gilburn
142 1998; Crean et al. 2000; Dunn et al. 2002).

143

144 **Statistical Analysis**

145

146 Binary logistic models of willingness to mount and copulation success rate
147 (for those that mounted) were created separately using SPSS v12.0.1. Models were
148 further simplified to analyse differences within each species and between treatments.
149 Species, treatment, duration of exposure to treatment, male size, female size and the
150 interaction terms were initially included in all models as applicable. Maximal models
151 were selected based upon the Akaike Information Criterion calculated using R (R
152 Development Core Team 2006), non-significant terms being excluded from models.
153 All P-values were determined using log-likelihood chi-squares.

154

155 **RESULTS**

156

157 **Harassment Levels**

158

159 Harassment levels differed in *C. frigida* and *C. pilipes* (Chi-square test: $\chi^2_1 =$
160 53.685, $P < 0.001$) and were influenced by both treatment (Chi-square test: $\chi^2_3 =$
161 13.951, $P = 0.003$) and the number of days exposed (Chi-square test: $\chi^2_1 = 20.820$, P
162 < 0.001 ; Fig. 1.). The influence of length of exposure to treatments differed between
163 the species (Chi-square test: $\chi^2_1 = 13.222$, $P < 0.001$). *C. frigida* were not influenced
164 by length of exposure (Chi-square test: $\chi^2_1 = 0.986$, $P = 0.321$) whilst *C. pilipes* males

165 were (Chi-square test: $\chi^2_1 = 33.255, P < 0.001$). Despite a non-significant interaction
166 term, harassment by *C. frigida* males was influenced by treatment (Chi-square test: χ^2
167 $_3 = 9.942, P = 0.019$) whereas *C. pilipes* was not (Chi-square test: $\chi^2_3 = 5.644, P =$
168 0.130). The absence of a significant interaction between species and treatment may be
169 the result of a reduced data set as fewer *C. pilipes* mounted a female than *C. frigida*
170 (*C. pilipes*: 90 out of 286 (31%); *C. frigida*: 174 out of 281 (62%)). In particular, only
171 8 male *C. pilipes* mounted a female following 1 day of exposure to treatments.

172

173 Differences between *F. serratus* & *F. vesiculosus* did not contribute to the
174 altered harassment rates in *C. frigida* (Chi-square test: $\chi^2_1 = 0.030, P = 0.862$) and so
175 these treatments were combined in further analyses of male harassment. There was
176 also no significant difference between treatment with *Fucus* seaweed and the control
177 (Chi-square test: $\chi^2_1 = 1.476, P = 0.224$). Instead, the effect of different treatments for
178 *C. frigida* was the result of differences between *Laminaria* and the control (Chi-
179 square test: $\chi^2_1 = 9.549, P = 0.002$) and between *Laminaria* and *Fucus* (Chi-square
180 test: $\chi^2_1 = 5.432, P = 0.020$; Fig. 1.).

181

182 **Mating Success**

183

184 Mating success was determined primarily by an interaction between male size
185 and female size (Chi-square test: $\chi^2_1 = 6.981, P = 0.008$). This is the result of larger
186 males being able to overcome the rejection response of smaller females more easily
187 (Gilburn et al. 1992; Crean & Gilburn 1998). In addition, there was a difference in the
188 success rate between the species that was dependent upon male size (Chi-square test:
189 $\chi^2_1 = 4.890, P = 0.027$). In *C. frigida* successful males tended to be larger than

190 unsuccessful males whereas the difference was negligible in *C. pilipes*. Finally,
191 mating success was also determined by treatment (Chi-square test: $\chi^2_3 = 8.259$, $P =$
192 0.041).

193

194 There was a difference in mating success between different lengths of
195 exposure, however this was not significant at the 5% level when both species were
196 included (Chi-square test: $\chi^2_1 = 3.465$, $P = 0.063$). When considering *C. frigida* alone,
197 length of exposure had no effect on mating success (Chi-square test: $\chi^2_1 = 1.616$, $P =$
198 0.204) but the effect was significant for *C. pilipes* (Chi-square test: $\chi^2_1 = 4.006$, $P =$
199 0.045). Analogous with harassment levels, mating success in *C. pilipes* was not
200 affected by treatment (Chi-square test: $\chi^2_3 = 0.021$, $P = 0.999$), however male *C.*
201 *frigida* were influenced by treatment (Chi-square test: $\chi^2_3 = 11.549$, $P = 0.009$; Fig.
202 2.). As with the harassment levels, the absence of significant interaction terms in the
203 model including both species is likely the result of a bias in sample sizes as a higher
204 number of *C. frigida* copulations were recorded than *C. pilipes* (*C. pilipes*: 42 out of
205 90 (47%); *C. frigida*: 103 out of 174 (59%)). Two male *C. pilipes* copulated following
206 one day of exposure to treatment, increasing to only 14 after two days of exposure.

207

208 There was no difference in the effect of the different *Fucus* seaweeds on
209 mating success (Chi-square test: $\chi^2_1 = 2.438$, $P = 0.118$) and so these treatments were
210 again combined in further analyses. Treatment with either *Fucus* or *Laminaria*
211 seaweeds led to a significant increase in mating success of *C. frigida* (Chi-square test:
212 *Fucus* $\chi^2_1 = 5.114$, $P = 0.024$, *Laminaria* $\chi^2_1 = 9.348$, $P = 0.002$). Whilst *Laminaria*
213 resulted in a greater and more significant increase in mating success compared to the

214 control, there was no significant difference between the effects of *Laminaria* and
215 *Fucus* (Chi-square test: $\chi^2_1 = 1.689$, $P = 0.194$).

216

217 **DISCUSSION**

218

219 Harassment of females by male *C. frigida* was stimulated by the presence of
220 furoid seaweeds. This finding is consistent with previous studies (Dunn et al. 2002).
221 In the present study we exposed males to a second genus of brown algae, *Laminaria*,
222 also commonly found in Northern European wrack beds. This genus of seaweed was
223 found to have a greater stimulatory effect on male harassment levels than furoid
224 seaweeds. Thus, the relative proportion of *Laminaria* and *Fucus* within wrack beds is
225 likely to affect the level of male harassment by *C. frigida*, and therefore determine the
226 level of sexual conflict within each population of this species.

227

228 By contrast, seaweed species composition of a wrack bed is unlikely to affect
229 the level of sexual conflict within *C. pilipes*. Previous studies have found increased
230 mating activity in *C. pilipes* when exposed to algae (Dunn et al. 2002), however we
231 show that the duration of exposure to either algae or indeed sugar solution is the
232 primary factor determining the level of harassment within this species. Given no
233 significant interaction between duration of exposure and treatment, it is difficult to
234 state whether *C. pilipes* require a period of maturation and/or exposure to seaweed in
235 order to stimulate willingness to mate. Due to low levels of harassment in *C. pilipes*
236 relative to *C. frigida*, particularly following short periods of exposure, a much greater
237 sample size in combination with a more defined age range of flies would be required
238 to determine the influence of age as opposed to exposure.

239

240 Discovering that the intensity of male harassment is environmentally
241 determined, and varies both spatially and temporally, has important implications for
242 attempts to measure the intensity of selection occurring as a result of sexual conflict.
243 A single measurement of the intensity of realised conflict might not be a true
244 reflection of the level within a population. Several measurements might be required at
245 different time points and under different environmental conditions in order to gain a
246 clear estimate of the average level of, and variability in, the intensity of conflict
247 occurring with a population.

248

249 Habitat variation can also influence the outcome of sexual selection. For
250 example turbidity inhibits mate choice in cichlid fish (Seehausen et al. 1997) and
251 availability of breeding sites determines the strength of sexual selection in the
252 European lobster and sand goby (Forsgren et al. 1996; Debuse et al. 2003). Population
253 density has been identified as fundamental to the evolutionary outcome of sexual
254 selection and sexual conflict (Martin & Hosken 2003; Kokko & Rankin 2006). We
255 show that environmental variation also impacts upon the mating system of coelopids.
256 Whilst the proximal mechanism for this association requires further investigation, we
257 suggest either a developmental or competitive advantage conferred to *C. frigida* by
258 enhanced reproduction in *Laminaria* deposits.

259

260 The discovery that genus of seaweed is likely to determine the level of male
261 harassment within a wrack bed poses the question what effect seaweed composition
262 within a wrack bed might also have on female behaviour. *C. frigida* females might
263 temporarily avoid areas of wrack beds with high proportions of *Laminaria* until they

264 are ready to mate in order to reduce harassment by males. Female *C. frigida* may also
265 be predicted to alter their reluctance to mate when exposed to different seaweeds. As
266 *Laminaria* is their preferred oviposition medium, the presence of this seaweed might
267 increase resistance to male harassment if female reluctance has evolved through mate
268 assessment (Eberhard 1996; Teder 2005). The opposite may be observed if reluctance
269 to mate has evolved in order to avoid costs associated with mating (Rowe 1992; Rowe
270 et al 1994; Blanckenhorn et al 2000; Dunn et al. 2002). In this case, female reluctance
271 might be expected to be reduced in the presence of *Laminaria* as increased levels of
272 male harassment are likely to increase the costs of resistance.

273

274 Different patterns of female reluctance are also predicted in *C. pilipes* by the
275 mate assessment and reduced mating rate hypotheses. The mate assessment
276 hypothesis predicts that female resistance should intensify as male harassment
277 increases over time. By contrast, the reduced mating rate hypothesis, predicts the
278 opposite, a reduction in female resistance over the same time period that male
279 harassment rate increases, as a result of increased costs to resistance. Thus, the spatial
280 and temporal effects found on male harassment levels provide us with alternative
281 predictions for the mate assessment and reduced mating rate hypotheses. The
282 generation of alternative predictions for these hypotheses has proved difficult and
283 their separation has become one the most controversial areas of research within sexual
284 conflict. This study has enabled us to generate additional alternative predictions that
285 can be tested in future studies.

286

287 Experimental manipulation of mating rates has been fundamental to the study
288 and understanding of processes underlying sexual conflict. This can be achieved with

289 relative ease by altering either the operational sex ratio (Arnqvist 1992; Rowe 1992;
290 Vepsalainen & Savolainen 1995; Rowe & Arnqvist 2002; Wigby & Chapman 2004)
291 or population density (Arnqvist 1992; Martin & Hosken 2003; Hardling & Kaitala
292 2005). In addition, other ecological factors such as food deprivation (Simmons &
293 Bailey 1990; Rowe 1992; Sih & Krupa 1992; Ortigosa & Rowe 2002), predation (Sih
294 & Krupa 1992; Sih 1994) and mating history (Shuker & Day 2001; Ortigosa & Rowe
295 2003) have also been found to influence the extent of sexual conflict. However, in the
296 majority of studies there is a bias towards manipulation of female mating rates, with a
297 relative inability to alter male mating behaviour (Sih & Krupa 1992; Ortigosa & Rowe
298 2002; Rowe & Arnqvist 2002). The greater reproductive investment made by females
299 in a majority of taxa would likely explain the wider variation in susceptibility to the
300 costs of mating when exposed to different environmental stresses. For example, in the
301 water striders (Gerridae) female hunger is found to influence mating frequency whilst
302 male hunger does not (Rowe 1992; Ortigosa & Rowe 2002). Similarly, a male biased
303 OSR does not affect the mating rate of male gerrids. It has been suggested that
304 optimal male mating rates are relatively high and constant among gerrids in relation to
305 females (Rowe & Arnqvist 2002). Although one study (Lauer et al. 1996) found that
306 male mating insistence, but not mating rate, was positively correlated with male
307 density. It therefore appears that interspecific variation in male mating rate is not
308 sufficient to explain behavioural covariation, which is instead most likely the result of
309 variation in female mating rates (Rowe & Arnqvist 2002). In *Coelopa* a contrasting
310 system is observed, with variation in male mating rates and mating success occurring
311 both inter- and intraspecifically. Through the manipulation of male mating behaviour,
312 coelopids provide an ideal model system in future comparative studies and population
313 crosses.

314

315

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320

321

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1 The effect of habitat composition on sexual conflict in
2 the seaweed flies, *Coelopa frigida* and *C. pilipes*

3

4

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15 *Running Headline:* **EDWARD & GILBURN: SEXUAL CONFLICT IN**

16 **COELOPIDS**

17

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22 Considering the recent explosion of interest in sexual conflict, the effect of
23 environmental conditions on the intensity of sexual conflict within populations has
24 been largely ignored. Reproductive encounters within coelopids are characterised by
25 sexual conflict in the form of intense male harassment, usually resulting in a vigorous
26 pre-mating struggle. Here we investigated the effect of habitat composition and
27 duration of exposure to oviposition sites on the level of sexual harassment by males
28 and mating success in two species of European seaweed flies, *Coelopa frigida* and *C.*
29 *pilipes*. The wrack beds inhabited by these two species are dominated by two genera
30 of brown algae, *Fucus* and *Laminaria*, the relative proportions of which can vary
31 considerably between wrack beds. Previous studies have shown that *Fucus* stimulates
32 male harassment, increases copulation duration and induces females to oviposit in
33 both species. Here we show that *Laminaria* stimulates a higher level of harassment in
34 male *C. frigida* than *Fucus*. However, a similar effect was not observed in *C. pilipes*,
35 with the main additional factor affecting male harassment in this species being the age
36 of the male. Our study highlights the potential importance of environmental
37 conditions on the intensity of sexual conflict within a population. We discuss the
38 evolutionary significance of these observed effects in seaweed flies.
39

40 Following the work of Parker (1979) there has been a recent explosion of interest in
41 sexual conflict and its importance as a major force shaping the evolution of mating
42 systems (reviewed by Chapman et al. 2003; Arnqvist & Rowe 2005). Yet despite this
43 upsurge in research investigating sexual conflict, the influence of environmental
44 conditions on the intensity of conflict within populations has been largely ignored.
45 Identifying environmental influences on sexual conflict are essential if we are to fully
46 understand how mating systems operate and evolve (Ortigosa & Rowe 2002). A
47 number of studies have investigated the effects of predation risk (Sih 1988; Lima &
48 Dill 1990; Sih et al 1990) and hunger (Travers & Sih 1991) on mating behaviour but
49 very few have actually investigated its effects directly on sexual selection and sexual
50 conflict (Rowe 1992; Ortigosa & Rowe 2002).

51

52 Examples of mating systems characterised by high levels of male harassment
53 and vigorous pre-mating struggles are found in many insect taxa including sepsids
54 (Ward et al. 1992), gerrids (Rowe et al. 1994), coccinellids (Majerus 1994), carabids
55 (Takami 2002) and ichneumonids (Teder 2005). All species of seaweed fly
56 (Coelopidae) so far studied exhibit pre-mating struggles (Day et al. 1990; Crean &
57 Gilburn 1998; Crean et al. 2000). Coelopids can be found inhabiting accumulations of
58 detached seaweed deposited on the seashore after high tides or stormy weather,
59 known as wrack beds. Seaweed is known to be important for coelopid reproduction,
60 increasing male harassment of females and reducing survival in both sexes (Dunn et
61 al. 2002). Seaweed also induces oviposition in females, but otherwise does not
62 influence female reproductive behaviour, with a consistent rejection response being
63 maintained irrespective of the presence or absence of seaweed (Dunn et al. 2002).
64 Male harassment invariably results in a pre-mating struggle during which the female

65 will attempt to reject the male with a combination of shaking and kicking movements,
66 whilst at the same time curling her abdomen downwards to prevent genital contact
67 (Day et al. 1990). Thus, the presence of seaweed increases the frequency of pre-
68 mating struggles within a population of coelopids.

69

70 The two species of coelopid most commonly found on the coasts of Northern
71 Europe are *Coelopa pilipes* and *Coelopa frigida* (Phillips et al. 1995). *C. frigida* and
72 *C. pilipes* can be found in both allopatric and, more commonly, sympatric
73 populations. Both conspecific and heterospecific interactions between larvae may
74 influence the success of individuals in high density populations (Phillips et al. 1995),
75 with the mechanism of competition most likely to be purely exploitative.

76

77 The coelopid life cycle can be completed in wrack beds composed of a wide
78 variety of different seaweeds (Dobson 1974; Phillips et al. 1995). Wrack beds around
79 the UK are primarily composed of two different genera of brown algae, *Fucus* and
80 *Laminaria*. Dobson (1974) reported that *C. frigida* could be bred more successfully on
81 *Laminaria* monocultures than *C. pilipes* and also that *C. frigida* were found in greater
82 numbers in wrack beds that predominantly consisted of *Laminaria*. *C. pilipes* females
83 show a preference for ovipositing on *Fucus*, though it should be noted that both *C.*
84 *frigida* and *C. pilipes* will lay eggs on both types of seaweed (Phillips et al. 1995).

85

86 Male harassment has previously been shown to be stimulated by the presence
87 of *Fucus* in both *C. frigida* and *C. pilipes* (Dunn et al. 2002). Male harassment rate
88 determines the frequency at which pre-mating struggles occur. Therefore, the level of
89 sexual conflict within a population is affected by environmental conditions in the

90 form of the presence of seaweed. As previous studies have reported that *C. frigida*
91 larvae and adults favour *Laminaria*, it might be expected that the stimulation of male
92 harassment would also be algal specific. Additionally the duration of exposure to
93 algae might also affect the level of stimulation of male harassment. In this study we
94 compare the effects of different algal genera and duration of exposure to them on the
95 harassment and copulation success rates of male *C. frigida* and *C. pilipes*. We then
96 discuss the implications of habitat specific effects on sexual conflict and interspecific
97 competition.

98

99

METHODS

100

101 **Preparation**

102

103 Laboratory populations of *C. pilipes* and *C. frigida* were established from wild
104 larvae collected in February 2004 from the Forth Estuary. *C. frigida* were from
105 Whitesands, East Lothian (NT712775) and *C. pilipes* from St Monans, Fife
106 (NO521012). Virgin flies were collected and stored in 250ml flasks at 5°C with cotton
107 wool soaked in excess 5% sucrose solution. Under these conditions flies are not
108 reproductively active. Flies were maintained for no more than 5 generations in the
109 laboratory and used for mate trials within 7 days of eclosion. Seaweed (*Fucus*
110 *serratus*, *Fucus vesiculosus* and *Laminaria digitata*) was collected from Whitesands,
111 East Lothian and Cellardyke Harbour, Fife (NO577038). Seaweed was coarsely
112 minced prior to use.

113

114 **Experimental Procedure**

115

116 Male flies were placed into individual plastic vials (45mm dia. X 40mm) with
117 one of four different seaweed treatments - *F. serratus*, *F. vesiculosus*, *L. digitata* or a
118 control. A small amount of minced seaweed (weighing approximately 2g) was added
119 to each vial in addition to a small ball of cotton wool soaked in excess 5% sucrose
120 solution. The control group vials contained only cotton wool soaked in sucrose
121 solution. Female flies were placed into individual clear plastic containers (30mm dia.
122 X 54mm) containing a small amount of cotton wool soaked in 5% sucrose solution.
123 Flies were initially transferred into their respective containers under light CO₂
124 anaesthesia, but for the remainder of the study no anaesthesia was used. Containers
125 were subsequently stored in constant temperature rooms at 25°C prior to mate trials.
126 Female flies and control males were kept separately in a room that had no history of
127 seaweed presence. Every 24 hours additional sucrose solution was added to vials to
128 replace fluid lost by evaporation.

129

130 At intervals of 1, 2 and 3 days following preparation an equal proportion of
131 vials from each seaweed treatment group were used in mate trials. Thus a 3 x 4
132 factorial design was adopted for each fly species to determine the effect of time left on
133 the seaweed and species of seaweed upon male mating behaviour. All mate trials were
134 carried out at 25°C. Males were introduced into the vials of randomly selected females
135 and observed for up to 10 minutes or until a mount was observed. Males failing to
136 mount within 10 minutes were scored as unwilling to mount. For those that did mount
137 their given female, the outcomes of pre-mating struggles were recorded as either
138 copulation (genital coupling was observed) or female rejection (if the female managed
139 to reject the male). Flies were killed by placing them in a freezer at -25°C. Body size

140 was estimated by measuring wing length, which has been used an indicator of size in
141 most previous studies of coelopid behaviour (e.g. Day et al. 1990; Crean & Gilburn
142 1998; Crean et al. 2000; Dunn et al. 2002).

143

144 **Statistical Analysis**

145

146 Binary logistic models of willingness to mount and copulation success rate
147 (for those that mounted) were created separately using SPSS v12.0.1. Models were
148 further simplified to analyse differences within each species and between treatments.
149 Species, treatment, duration of exposure to treatment, male size, female size and the
150 interaction terms were initially included in all models as applicable. Maximal models
151 were selected based upon the Akaike Information Criterion calculated using R (R
152 Development Core Team 2006), non-significant terms being excluded from models.
153 All P-values were determined using log-likelihood chi-squares.

154

155 **RESULTS**

156

157 **Harassment Levels**

158

159 Harassment levels differed in *C. frigida* and *C. pilipes* (Chi-square test: $\chi^2_1 =$
160 53.685, $P < 0.001$) and were influenced by both treatment (Chi-square test: $\chi^2_3 =$
161 13.951, $P = 0.003$) and the number of days exposed (Chi-square test: $\chi^2_1 = 20.820$, P
162 < 0.001 ; Fig. 1.). The influence of length of exposure to treatments differed between
163 the species (Chi-square test: $\chi^2_1 = 13.222$, $P < 0.001$). *C. frigida* were not influenced
164 by length of exposure (Chi-square test: $\chi^2_1 = 0.986$, $P = 0.321$) whilst *C. pilipes* males

165 were (Chi-square test: $\chi^2_1 = 33.255$, $P < 0.001$). Despite a non-significant interaction
166 term, harassment by *C. frigida* males was influenced by treatment (Chi-square test: χ^2
167 $_3 = 9.942$, $P = 0.019$) whereas *C. pilipes* was not (Chi-square test: $\chi^2_3 = 5.644$, $P =$
168 0.130). The absence of a significant interaction between species and treatment may be
169 the result of a reduced data set as fewer *C. pilipes* mounted a female than *C. frigida*
170 (*C. pilipes*: 90 out of 286 (31%); *C. frigida*: 174 out of 281 (62%)). In particular, only
171 8 male *C. pilipes* mounted a female following 1 day of exposure to treatments.

172

173 Differences between *F. serratus* & *F. vesiculosus* did not contribute to the
174 altered harassment rates in *C. frigida* (Chi-square test: $\chi^2_1 = 0.030$, $P = 0.862$) and so
175 these treatments were combined in further analyses of male harassment. There was
176 also no significant difference between treatment with *Fucus* seaweed and the control
177 (Chi-square test: $\chi^2_1 = 1.476$, $P = 0.224$). Instead, the effect of different treatments for
178 *C. frigida* was the result of differences between *Laminaria* and the control (Chi-
179 square test: $\chi^2_1 = 9.549$, $P = 0.002$) and between *Laminaria* and *Fucus* (Chi-square
180 test: $\chi^2_1 = 5.432$, $P = 0.020$; Fig. 1).

181

182 **Mating Success**

183

184 Mating success was determined primarily by an interaction between male size
185 and female size (Chi-square test: $\chi^2_1 = 6.981$, $P = 0.008$). This is the result of larger
186 males being able to overcome the rejection response of smaller females more easily
187 (Gilburn et al. 1992; Crean & Gilburn 1998). In addition, there was a difference in the
188 success rate between the species that was dependent upon male size (Chi-square test:
189 $\chi^2_1 = 4.890$, $P = 0.027$). In *C. frigida* successful males tended to be larger than

190 unsuccessful males whereas the difference was negligible in *C. pilipes*. Finally,
191 mating success was also determined by treatment (Chi-square test: $\chi^2_3 = 8.259$, $P =$
192 0.041).

193

194 There was a difference in mating success between different lengths of
195 exposure, however this was not significant at the 5% level when both species were
196 included (Chi-square test: $\chi^2_1 = 3.465$, $P = 0.063$). When considering *C. frigida* alone,
197 length of exposure had no effect on mating success (Chi-square test: $\chi^2_1 = 1.616$, $P =$
198 0.204) but the effect was significant for *C. pilipes* (Chi-square test: $\chi^2_1 = 4.006$, $P =$
199 0.045). Analogous with harassment levels, mating success in *C. pilipes* was not
200 affected by treatment (Chi-square test: $\chi^2_3 = 0.021$, $P = 0.999$), however male *C.*
201 *frigida* were influenced by treatment (Chi-square test: $\chi^2_3 = 11.549$, $P = 0.009$; Fig.
202 2.). As with the harassment levels, the absence of significant interaction terms in the
203 model including both species is likely the result of a bias in sample sizes as a higher
204 number of *C. frigida* copulations were recorded than *C. pilipes* (*C. pilipes*: 42 out of
205 90 (47%); *C. frigida*: 103 out of 174 (59%)). Two male *C. pilipes* copulated following
206 one day of exposure to treatment, increasing to only 14 after two days of exposure.

207

208 There was no difference in the effect of the different *Fucus* seaweeds on
209 mating success (Chi-square test: $\chi^2_1 = 2.438$, $P = 0.118$) and so these treatments were
210 again combined in further analyses. Treatment with either *Fucus* or *Laminaria*
211 seaweeds led to a significant increase in mating success of *C. frigida* (Chi-square test:
212 *Fucus* $\chi^2_1 = 5.114$, $P = 0.024$, *Laminaria* $\chi^2_1 = 9.348$, $P = 0.002$). Whilst *Laminaria*
213 resulted in a greater and more significant increase in mating success compared to the

214 control, there was no significant difference between the effects of *Laminaria* and
215 *Fucus* (Chi-square test: $\chi^2_1 = 1.689$, $P = 0.194$).

216

217 **DISCUSSION**

218

219 Harassment of females by male *C. frigida* was stimulated by the presence of
220 furoid seaweeds. This finding is consistent with previous studies (Dunn et al. 2002).
221 In the present study we exposed males to a second genus of brown algae, *Laminaria*,
222 also commonly found in Northern European wrack beds. This genus of seaweed was
223 found to have a greater stimulatory effect on male harassment levels than furoid
224 seaweeds. Thus, the relative proportion of *Laminaria* and *Fucus* within wrack beds is
225 likely to affect the level of male harassment by *C. frigida*, and therefore determine the
226 level of sexual conflict within each population of this species.

227

228 By contrast, seaweed species composition of a wrack bed is unlikely to affect
229 the level of sexual conflict within *C. pilipes*. Previous studies have found increased
230 mating activity in *C. pilipes* when exposed to algae (Dunn et al. 2002), however we
231 show that the duration of exposure to either algae or indeed sugar solution is the
232 primary factor determining the level of harassment within this species. Given no
233 significant interaction between duration of exposure and treatment, it is difficult to
234 state whether *C. pilipes* require a period of maturation and/or exposure to seaweed in
235 order to stimulate willingness to mate. Due to low levels of harassment in *C. pilipes*
236 relative to *C. frigida*, particularly following short periods of exposure, a much greater
237 sample size in combination with a more defined age range of flies would be required
238 to determine the influence of age as opposed to exposure.

239

240 Discovering that the intensity of male harassment is environmentally
241 determined, and varies both spatially and temporally, has important implications for
242 attempts to measure the intensity of selection occurring as a result of sexual conflict.
243 A single measurement of the intensity of realised conflict might not be a true
244 reflection of the level within a population. Several measurements might be required at
245 different time points and under different environmental conditions in order to gain a
246 clear estimate of the average level of, and variability in, the intensity of conflict
247 occurring with a population.

248

249 Habitat variation can also influence the outcome of sexual selection. For
250 example turbidity inhibits mate choice in cichlid fish (Seehausen et al. 1997) and
251 availability of breeding sites determines the strength of sexual selection in the
252 European lobster and sand goby (Forsgren et al. 1996; Debuse et al. 2003). Population
253 density has been identified as fundamental to the evolutionary outcome of sexual
254 selection and sexual conflict (Martin & Hosken 2003; Kokko & Rankin 2006). We
255 show that environmental variation also impacts upon the mating system of coelopids.
256 Whilst the proximal mechanism for this association requires further investigation, we
257 suggest either a developmental or competitive advantage conferred to *C. frigida* by
258 enhanced reproduction in *Laminaria* deposits.

259

260 The discovery that genus of seaweed is likely to determine the level of male
261 harassment within a wrack bed poses the question what effect seaweed composition
262 within a wrack bed might also have on female behaviour. *C. frigida* females might
263 temporarily avoid areas of wrack beds with high proportions of *Laminaria* until they

264 are ready to mate in order to reduce harassment by males. Female *C. frigida* may also
265 be predicted to alter their reluctance to mate when exposed to different seaweeds. As
266 *Laminaria* is their preferred oviposition medium, the presence of this seaweed might
267 increase resistance to male harassment if female reluctance has evolved through mate
268 assessment (Eberhard 1996; Teder 2005). The opposite may be observed if reluctance
269 to mate has evolved in order to avoid costs associated with mating (Rowe 1992; Rowe
270 et al 1994; Blanckenhorn et al 2000; Dunn et al. 2002). In this case, female reluctance
271 might be expected to be reduced in the presence of *Laminaria* as increased levels of
272 male harassment are likely to increase the costs of resistance.

273

274 Different patterns of female reluctance are also predicted in *C. pilipes* by the
275 mate assessment and reduced mating rate hypotheses. The mate assessment
276 hypothesis predicts that female resistance should intensify as male harassment
277 increases over time. By contrast, the reduced mating rate hypothesis, predicts the
278 opposite, a reduction in female resistance over the same time period that male
279 harassment rate increases, as a result of increased costs to resistance. Thus, the spatial
280 and temporal effects found on male harassment levels provide us with alternative
281 predictions for the mate assessment and reduced mating rate hypotheses. The
282 generation of alternative predictions for these hypotheses has proved difficult and
283 their separation has become one the most controversial areas of research within sexual
284 conflict. This study has enabled us to generate additional alternative predictions that
285 can be tested in future studies.

286

287 Experimental manipulation of mating rates has been fundamental to the study
288 and understanding of processes underlying sexual conflict. This can be achieved with

289 relative ease by altering either the operational sex ratio (Arnqvist 1992; Rowe 1992;
290 Vepsalainen & Savolainen 1995; Rowe & Arnqvist 2002; Wigby & Chapman 2004)
291 or population density (Arnqvist 1992; Martin & Hosken 2003; Hardling & Kaitala
292 2005). In addition, other ecological factors such as food deprivation (Simmons &
293 Bailey 1990; Rowe 1992; Sih & Krupa 1992; Ortigosa & Rowe 2002), predation (Sih
294 & Krupa 1992; Sih 1994) and mating history (Shuker & Day 2001; Ortigosa & Rowe
295 2003) have also been found to influence the extent of sexual conflict. However, in the
296 majority of studies there is a bias towards manipulation of female mating rates, with a
297 relative inability to alter male mating behaviour (Sih & Krupa 1992; Ortigosa & Rowe
298 2002; Rowe & Arnqvist 2002). The greater reproductive investment made by females
299 in a majority of taxa would likely explain the wider variation in susceptibility to the
300 costs of mating when exposed to different environmental stresses. For example, in the
301 water striders (Gerridae) female hunger is found to influence mating frequency whilst
302 male hunger does not (Rowe 1992; Ortigosa & Rowe 2002). Similarly, a male biased
303 OSR does not affect the mating rate of male gerrids. It has been suggested that
304 optimal male mating rates are relatively high and constant among gerrids in relation to
305 females (Rowe & Arnqvist 2002). Although one study (Lauer et al. 1996) found that
306 male mating insistence, but not mating rate, was positively correlated with male
307 density. It therefore appears that interspecific variation in male mating rate is not
308 sufficient to explain behavioural covariation, which is instead most likely the result of
309 variation in female mating rates (Rowe & Arnqvist 2002). In *Coelopa* a contrasting
310 system is observed, with variation in male mating rates and mating success occurring
311 both inter- and intraspecifically. Through the manipulation of male mating behaviour,
312 coelopids provide an ideal model system in future comparative studies and population
313 crosses.

314

315

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320

321

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1 **Figure Captions**

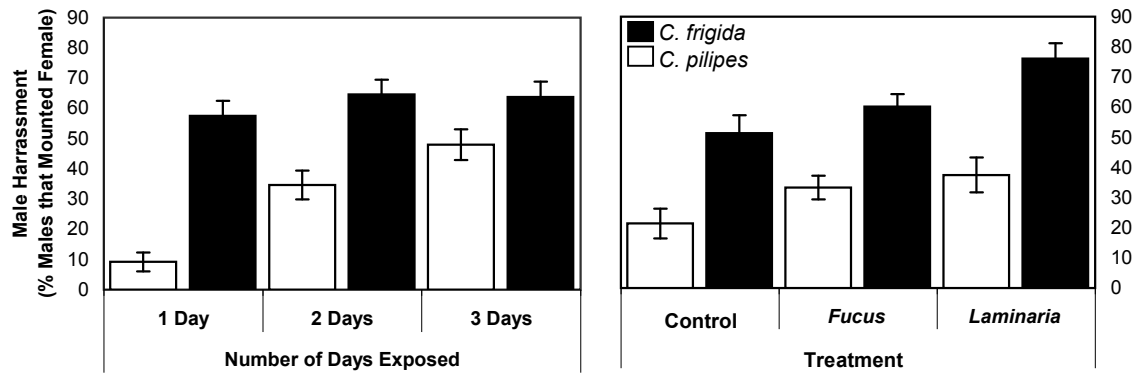
2

3 **Figure 1.** The effect of duration of exposure and different treatments on harassment
4 levels in *C. frigida* & *C. pilipes*.

5

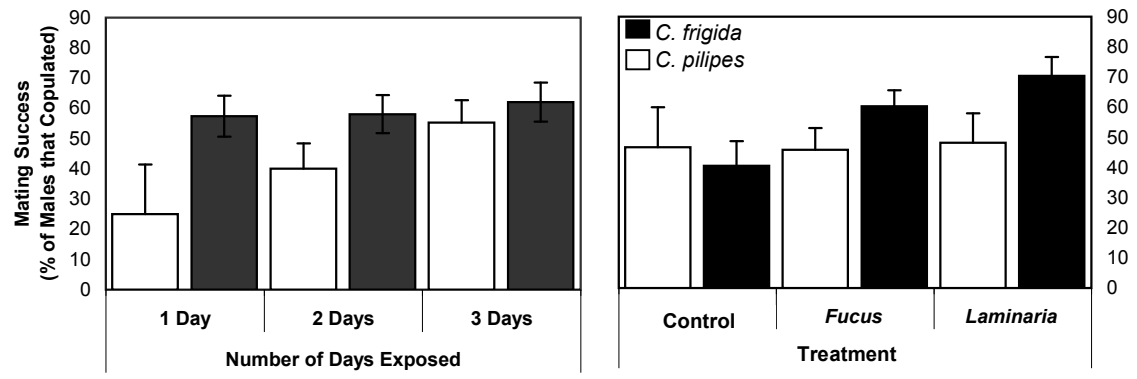
6 **Figure 2.** The effect of duration of exposure and different treatments on mating
7 success of male *C. frigida* & *C. pilipes*.

Figure 1



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Figure 2



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