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Published in:
Environmental Pollution

Publication date:
2017

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Citation for published version (APA):

De Jong, K., Amorim, M. C. P., Fonseca, P. J., Fox, C. J., & Heubel, K. U. (2017). Noise can affect acoustic communication and subsequent spawning success in fish. *Environmental Pollution*. Advance online publication. <https://doi.org/10.1016/j.envpol.2017.11.003>

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1 Noise can affect acoustic communication and subsequent spawning success in fish

2 Running head: Noise and spawning

3

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17

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20

21 Keywords: Acoustic communication; Aquatic noise pollution; Multimodal courtship;

22 Spawning success; Vocal fish.

23

24 **Abstract**

25 There are substantial concerns that increasing levels of anthropogenic noise in the oceans
26 may impact aquatic animals. Noise can affect animals physically, physiologically and
27 behaviourally, but one of the most obvious effects is interference with acoustic
28 communication. Acoustic communication often plays a crucial role in reproductive
29 interactions and over 800 species of fish have been found to communicate acoustically. There
30 is very little data on whether noise affects reproduction in aquatic animals, and none in
31 relation to acoustic communication. In this study we tested the effect of continuous noise on
32 courtship behaviour in two closely-related marine fishes: the two-spotted goby (*Gobiusculus*
33 *flavescens*) and the painted goby (*Pomatoschistus pictus*) in aquarium experiments. Both
34 species use visual and acoustic signals during courtship. In the two-spotted goby we used a
35 repeated-measures design testing the same individuals in noise and control treatments in
36 alternating order. For the painted goby we allowed females to spawn, precluding a repeated-
37 measures design, but permitting a test of the effect of noise on female spawning decisions.
38 Males of both species reduced acoustic courtship, but only painted gobies also showed less
39 visual courtship in the noise treatment compared to the control. Female painted gobies were
40 less likely to spawn in the noise treatment. Thus, our results provide experimental evidence
41 for negative effects of noise on acoustic communication and spawning success. Spawning is a
42 crucial component of reproduction and fitness. Therefore, even though laboratory results
43 should not be extrapolated directly to field populations, our results suggest that reproductive
44 success may be sensitive to noise pollution. Because abundant small-sized fish, such as
45 gobies, have been identified as keystone species in coastal environments, effects of noise
46 pollution on their reproduction could have ecosystem-level impacts.

47

48 **Capsule**

49 This paper provides experimental evidence that noise can affect reproductive communication
50 and the subsequent spawning success, potentially reducing fitness in common marine fish.

51

52 **Introduction**

53 One of the main pollutants attracting increasing attention in the marine environment is
54 anthropogenic noise from activities such as shipping and offshore developments
55 (Slabbekoorn *et al.*, 2010; Radford *et al.*, 2014; Kunc *et al.*, 2016; Hawkins & Popper,
56 2017). As a result, ocean noise levels have increased, for example, by as much as 12 dB in 40
57 years in the North Eastern Pacific (Hildebrand, 2009). Noise can affect aquatic animals
58 physically, physiologically and behaviourally (reviewed in Shannon *et al.*, 2016; Kunc *et al.*,
59 2016). However, little is known about how noise could affect spawning behaviour and
60 spawning success, even though spawning success is an important component of fitness and
61 reproductive success, and a stage of reproduction that directly reflects reproductive decisions
62 of both parents (Andersson 1994, Barbosa and Magurran 2006). Fishes are key components
63 of most aquatic ecosystems and impacts on this group of receptors are of global concern
64 (FAO, 2016). The frequency-range of anthropogenic noise often overlaps with fish-hearing
65 and has been found to affect fish physiology and a range of behaviours (Slabbekoorn *et al.*,
66 2010; Popper *et al.*, 2014; Radford *et al.*, 2014; Kunc *et al.*, 2016; Hawkins & Popper, 2017).
67 Affected behaviours include crucial behaviours to survival such as foraging (Magnhagen *et*
68 *al.* 2017) and predator avoidance (Simpson *et al.* 2016). Reproduction is vital to population
69 sustainability, however, reproduction is also very sensitive to stress and changes in
70 environmental conditions (Billard *et al.*, 1981; Bonga, 1997). Acoustic communication is
71 known to play an important role in mating behaviour and reproduction in some fishes
72 (Myrberg & Lugli, 2006). For example, sound production is common in many gadoids and is
73 thought to synchronise gamete release in haddock, *Melanogrammus aeglefinus* (Casaretto *et*

74 *al.*, 2014; Hawkins & Amorim, 2000) and in cod (*Gadus morhua*; Rowe & Hutchings, 2006).
75 Acoustic signals may even be essential for mate attraction or to induce spawning behaviour
76 as seen in the Lusitanian toadfish (*Halobatrachus didactylus*), where mating success depends
77 on acoustic courtship performance by the males (Amorim *et al.*, 2016). Noise has been shown
78 to decrease acoustic communication active space (Alves *et al.* 2016) and affect calling
79 behaviour (Picciulin *et al.*, 2012; Holt & Johnston, 2015) Although evidence of effects of
80 noise have been found on other aspects of reproduction, such as parental behaviour (Bruitjtes
81 & Radford, 2014; Nedelec *et al.*, 2017), larval survival (Nedelec *et al.*, 2017) and larval
82 development (Nedelec *et al.*, 2015), we are aware of only one peer-reviewed paper that
83 reports potential effects of noise on spawning success. Sierra-Flores *et al.* (2015) describe the
84 differences in spawning success between fish exposed to six hours of low-frequency sound
85 sweeps randomly distributed over the day for three months, relative to a control group. The
86 noise-exposed population produced fewer eggs, with lower fertilization rates and higher
87 levels of the stress-hormone cortisol in eggs. However, because the treatment was not
88 replicated, the authors were unable to test whether this difference was caused by the noise
89 treatment.

90

91 Noise may not only affect signalling behaviour in the acoustic sensory modality. For
92 example, in cuttlefish, noise disturbance has been shown to affect visual signalling behaviour
93 (Kunc *et al.*, 2014). Such a carry-over effect from one modality into another could be due to
94 stress or distraction (Chan *et al.*, 2010; Kunc *et al.*, 2014). Many animals rely on more than
95 one modality to signal their attractiveness: e.g. sound plus visual cues (Rowe, 1999; Hebets
96 & Papaj, 2005). A main hypothesis for the function of multi-modal signals is that one
97 modality may be a back-up for a loss of signal efficacy in another modality (Bradbury &
98 Vehrencamp, 1998; Hebets & Papaj, 2005). Such a back-up signal could mitigate effects of

99 noise on acoustic signalling. However, if noise has a detrimental effect across modalities, its
100 effects on fish reproduction could be even more dramatic. Therefore, we tested whether noise
101 affects reproductive communication in the visual and the acoustic modalities of two species
102 of common coastal marine fish: the two-spotted goby (*Gobiusculus flavescens*) and the
103 painted goby (*Pomatoschistus pictus*).

104

105 We used a controlled experimental approach to test whether noise could potentially affect
106 courtship behaviour and the resulting spawning success in two species of small marine fish.

107 While field studies on free-range animals with realistic sound sources provide the most direct
108 tests of effects of different noise-sources (Hawkins and Popper, 2017), they often suffer from
109 limited replication, due to logistic and financial limitations (Ware et al, 2015; Schröder et al,
110 2012; Streever et al., 2016; McCauly et al. 2017). Another important advantage of laboratory
111 studies is that all non-treatment parameters can be controlled, which enables a direct test of
112 treatment effects (Slabbekkoorn, 2016). A controlled environment may also reduce
113 behavioural variation within treatments, increasing the power of a test on a limited number of
114 individuals (Slabbekkoorn, 2016; Neo et al. 2016). However, in the laboratory as well as in
115 the field, a thorough understanding of the study system and its natural environment is needed
116 to allow for natural behaviour to occur (Magnhagen et al., 2017; de Jong et al., 2012).

117 Therefore, we used two species of fish from a family that has been studied extensively both
118 in the laboratory and in the field and that is known to show their full repertoire of courtship
119 and spawning behaviour in an aquarium set-up (Forsgren et al.). We aimed for an increase in
120 noise-levels in both pressure and particle motion within a frequency range that overlapped
121 with the calls of our study species rather than trying to mimic any particular noise source,
122 which is very hard to accomplish in small aquaria (Parvelescu, 1967, Nedelec et al., 2016).

123

124 As experimental noise we used a low-frequency continuous multi-tonal sound in the
125 frequency range of these species courtship calls. Low frequency harmonics are a common
126 feature of the sound-signature of small boats, which are a main noise source in the coastal
127 areas where gobies breed (Miller, 1986). Continuous noise is expected to affect
128 communication more than stress-levels in animals (Wright et al. 2007). We expected noise to
129 affect acoustic courtship either positively, if males try to compensate for a loss of
130 communication efficacy by increasing the number of calls, or negatively, if males “give up”
131 due to a reduced chance of success. An increase in visual courtship would be evidence for the
132 back-up hypothesis, while a decrease in visual courtship would evidence a carry-over effect
133 into different modalities. We also hypothesised that females would be less willing to spawn
134 in noisy conditions if communication was impaired.

135

136 **Material and methods**

137 *General design*

138 We exposed male gobies to a control or an added noise treatment for three days. On day four
139 we introduced females and allowed the males to court. We compared both visual and acoustic
140 courtship behaviour between the treatments.

141

142 *Study species*

143 The two-spotted goby (*G. flavescens*) and the painted goby, (*P. pictus*), are closely related
144 coastal marine species (Huyse *et al.*, 2004). The two-spotted goby is a semi-pelagic species
145 inhabiting shallow water along rocky shores and the painted goby is a benthic species
146 inhabiting shallow gravel and sand substrate areas in the Eastern Atlantic Ocean and in some
147 areas of the Mediterranean Sea (Miller, 1986). Both are species with a polygamous mating
148 system and paternal care of the eggs. Two-spotted goby males defend a nest in empty shells,

149 in algae or in crevices (Miller, 1986; Gordon, 1983; Skolbekken & Utne-Palm, 2001), while
150 painted goby males build nests under bivalve shells, by shovelling sand in a pile over the
151 shell (Bouchereau *et al.*, 2003). Males of both species show visual displays (Two-spotted
152 gobies: (Amundsen & Forsgren, 2001; de Jong *et al.*, 2009), painted gobies: Amorim &
153 Neves, 2007) and produce sound during courtship (Two-spotted gobies: de Jong *et al.* 2016a;
154 painted gobies: Amorim & Neves, 2007). Females enter the nest after courtship and lay eggs
155 in a single layer on the substrate, after which they leave all parental care to the male (Gordon,
156 1983; Bouchereau *et al.*, 2003). Males only court acoustically when the female is close
157 (Pedroso *et al.* own observations) and such quiet low-frequency goby sounds do not
158 propagate far in shallow water (*Padogobius martensii*: Lugli and Fine, 2007), suggesting that
159 reflections from tank walls are not likely to interfere with acoustic communication in these
160 species.

161

162 *Catching and husbandry*

163 Two-spotted gobies were captured in June 2014 with hand nets during low tide close to the
164 shore at Dunstaffnage, Scotland (56° 27' N 5° 26.5' W), and transferred to the aquarium in a
165 cool box. In the laboratory, males and females were kept separately in large single sex stock
166 tanks (100 l.) with sand substrate under a 12 h light: 12 h dark regime. Each tank was
167 provided with a continuous flow of seawater. The temperature in the aquaria fluctuated with
168 the ambient seawater temperatures. Fish were fed daily *ad libitum* with frozen *Artemia*
169 nauplii.

170

171 Painted gobies were caught January and February 2015 with hand nets in intertidal pools at
172 Parede (38° 41'N, 9° 21'W), Portugal, during spring low tides and transferred to the
173 laboratory in a container with aeration. In the laboratory, males and females were kept

174 separately in 18 l. stock tanks with sand substrate under a 12 h: 12 h dark/ light regime.
175 Artificial saltwater (32-35‰) was supplied to the stock tanks with a separate recirculation
176 system for each of the experimental tanks. Both the stock aquaria and the experimental
177 aquaria were kept at 16 °C. Fish were fed twice a day *ad libitum* with a mix of chopped
178 mussel, clams and shrimp.

179

180 Experimental aquaria for both species contained a 3 cm layer of sand and a nest made from a
181 plastic tube with a chimney to accommodate the hydrophone (Fig. 1; see Amorim *et al.*, 2013
182 for details of the nest and recording setup). We used glass aquaria of similar dimensions
183 (two-spotted gobies: 50x35x30cm, wall thickness: 8 mm, painted gobies: glass, 51x36x31
184 cm, wall thickness: 5 mm). The inside of the nest was covered with a plastic sheet to prevent
185 males from entering the chimney and for later removal and photography of the eggs in the
186 nest. Nests were filled half-way with sand to weigh them down. For two-spotted gobies a
187 horizontal tile was added as a floor, because males do not occupy nests with a sand floor. A
188 noise-egg (de Jong *et al.*, 2016b) was placed just behind the nest in a cloth bag and weighed
189 down with a stone. In the control treatments, the egg was switched off (Fig. 1). The
190 experimental aquaria were insulated from floor transmitted low-frequency noise. For two
191 spotted gobies, we used 4 layers of floor insulation, one layer of sand and rubber feet, for
192 painted gobies, we used two layers of shock absorbing rubber interspaced between two 3 cm
193 thick stone slabs.

194

195 The two-spotted goby experiment was carried out in the Scottish Association for Marine
196 Science (SAMS) aquarium. Each male was presented with two females constrained in a
197 transparent container with holes on the fourth day of a treatment (Fig. 1a). Males were
198 exposed to both treatments in a repeated measures design. Ten males were first exposed to

199 the control treatment, while another six males were first exposed to the added noise treatment
200 ($N = 16$).

201

202 The painted goby experiment was carried out in January and February 2015 at the University
203 of Lisbon. Here, each male was presented with two free-swimming females and we recorded
204 female spawning behaviour, as well as male courtship behaviour (Fig. 1b). Because a
205 spawning event may affect subsequent courtship behaviour (Jamieson & Colgan, 1989), this
206 precluded the use of a repeated measures design. We therefore compared different males
207 between the treatments (Added-noise: $N = 20$, control: $N = 16$).

208

209 *Noise treatments*

210 The “noise egg”, consisting of an electromotor in a waterproof container (de Jong *et al.*,
211 2016b), was used to generate a constant low frequency multi-tone with a fundamental
212 frequency of around 100 Hz and several strong harmonics (Fig. 2). For the painted goby, the
213 noise level was 100 ± 1 dB re 1 μ Pa ($N = 16$) in the control treatments compared to 125 ± 6
214 dB re 1 μ Pa ($N = 20$) in the added noise treatments. The contribution of frequencies above
215 1000 Hz (outside the range of fish hearing; was small (see Suppl. Material for details). The
216 difference between treatments was 5 dB larger in the two-spotted goby set-up than the
217 average of the painted goby set-up (104 vs 134 dB re 1 μ Pa; see Suppl. Table S1), although
218 we have to note we only made one measurement in the two-spotted goby set-up. Particle
219 acceleration, measured with an accelerometer (Klein *et al.*, 2013), was elevated on average
220 by 20 dB at 200 Hz compared to ambient recordings in the male nest (Fig. 3). The harmonic
221 structure of this experimental noise allowed us to unambiguously quantify the number of
222 calls in the added noise treatment as well as in the control treatment (Fig. 4).

223

224 Males of both species were allowed to acclimatize to the treatment (Added noise or Control)
225 for three days. During this period they were allowed to court twice with different constrained
226 females. Painted gobies built nests by shovelling sand in a pile over the plastic tube. We
227 scored the size of the nest twice daily during the five days of the experiment on a scale from
228 0 (no nest building) to 10 (plastic tube completely covered with sand).

229

230 *Experimental trials*

231 *(i) Two spotted gobies*

232 Two-spotted goby females were constrained in a plastic container with holes during the trial.
233 To reduce disturbance of the females between the acclimatisation period and the courtship
234 trial, we introduced two-spotted goby females into the experimental aquarium directly in the
235 container (Table 1). This was, however, a confined space and the acclimatisation was
236 therefore limited to 30 minutes, with the females at 30 cm from the nest, after which we
237 started the trial by moving the container closer to the nest (~2 cm from the nest opening). We
238 recorded visual and acoustic courtship during the 30 min trial. Male sounds were recorded
239 with a HTI-96 Min hydrophone (sensitivity -201 dB re: 1V/ μ Pa, frequency response 2 Hz –
240 30 kHz), which came as standard hydrophone with an SM2+ recorder (Wildlife Acoustics
241 Inc, Concord, Massachusetts, USA, 48kHz), placed inside the nest chimney. Visual courtship
242 was recorded on video (Sony HDR-CX280E, Sony, Tokyo, Japan). Males were weighed to
243 the nearest 0.1 g and measured to the nearest 0.5 mm after the experiment to reduce handling-
244 time before and during the experiment. Male size was on average 45.4 mm (range: 41 – 53)
245 and male weight 0.68 g (range: 0.43 – 1.01). All males were used in both treatments.

246

247 *(ii) Painted gobies*

248 Females were allowed to acclimatise to the treatment and interact with the male behind a
249 transparent divider overnight at a distance of 30 cm from the nest. We then added an opaque
250 divider for an hour before we started the experimental trial to obtain a resting period without
251 courtship. After the female acclimation period, we started a trial by lifting the divider to
252 release the females. We recorded acoustic and visual courtship during the 30 min of a trial
253 and noted whether and when the female entered the nest. Male sounds were recorded using a
254 hydrophone inside the nest chimney (B & K 8104, Brüel & Kjær, Naerum, Denmark;
255 sensitivity -205 dB re: 1V/ μ Pa; frequency response 0.1 Hz - 180 kHz). The signal from the
256 hydrophone was conditioned through a Mediator Sound Level Meter (B & K 2238, Brüel &
257 Kjær, Naerum, Denmark) and recorded at 8 kHz, 16 bit, to a laptop via an A/D converter
258 (Edirol UA-25, Roland, Osaka, Japan) with Adobe Audition 3.0 (Adobe Systems Inc.,
259 Mountain View, CA, USA). Visual courtship was recorded on video (Sony HDR-CX280E,
260 Sony, Tokyo, Japan). After the observations, the male and female fish were left in the
261 aquarium for one more day and the nest checked for eggs every three hours during day light
262 with a handheld torch. The next day, we ended the trial and weighed males to the nearest 0.1
263 g, measured them to the nearest 0.5 mm and took a picture of the eggs on the plastic sheet.
264 Male size was on average 41.6 mm (range: 35.5 – 48.0) and weight was on average 0.63 g
265 (range: 0.4 – 0.94). There were no significant differences between the treatments in male total
266 length and male weight ($P > 0.2$ for both).

267

268 *Analyses*

269 Sound analyses were undertaken using PRAAT version 6.0.19. (Boersma & Weenink, 2017).
270 For both species we counted the number of drums and thumps made during the 30 min of a
271 trial.

272

273 Visual courtship was scored from a silent video by an observer that was blind to the
274 treatment. For the two-spotted goby we counted the number of fin displays, quivers, lead
275 swims and fast approaches during the first 10 minutes of the trial. For the painted goby, the
276 minimum time for a female to enter the nest to spawn was as short as one minute. Because all
277 visual courtship was completed before spawning, we only report counts for the first minute of
278 visual courtship. We scored visual courtship building on the description by Amorim and
279 Neves (2007): We counted the frequency of hops (components of an approach), jumps
280 (components of an eight-display), quivers, leads, and fast approaches (including nudges). We
281 also noted the number of swims (movement over longer distances).

282

283 Statistical analyses were performed in R v3.3.1 (R core team, 2016). Frequencies of visual
284 behaviours per species were combined using principal component analyses (PCA) to avoid
285 multiple tests on correlated behaviours (Tabachnik and Fidell, 2001). Only components with
286 an eigenvalue > 1 were used. Median calling frequencies (sounds per 30 min) and principal
287 component scores for visual behaviours were compared between treatments with non-
288 parametric tests because the high number of zero's precluded the use of linear models. We
289 used the Wilcoxon Signed Rank Test for the repeated measures design (two-spotted gobies)
290 and the Mann-Whitney Test for independent data (painted gobies). Binomial data (whether or
291 not a male built a nest and whether or not a female spawned; painted gobies) were modelled
292 using generalized linear models (GLM: binomial family; R packages: lme4 (R core team,
293 2016), MASS (Venables & Ripley, 2002). Male weight and the interaction between treatment
294 and male weight were included in the models as covariates. We provide estimates and
295 confidence intervals for treatment effects in the text, for full models see Suppl. Table S2-S3).
296 If confidence intervals do not overlap with 0, the effect is significant ($P < 0.05$).

297

298 *Ethics*

299 All experiments were performed in compliance with laws of Portugal and the UK. After
300 consulting the appropriate authority (UK Home Office Inspector for Animals (Scientific
301 Procedures) Act 1986), we were informed that licences were not required for the capture of
302 the fish or the procedures used in these experiments in the UK. In Portugal, we operated
303 under a permit for catching painted gobies from the National Defense Ministry (Autoridade
304 Marítima Nacional - Capitania do Porto de Cascais). Permit nr. 550/2013.

305

306 **Results**

307 *Male nest building in painted goby*

308 We found no significant effect of noise treatment on nest building in the painted goby.
309 Fourteen of 20 males built a nest in the added noise treatment, compared to fourteen of
310 sixteen in the control treatment (Estimate effect added noise treatment (logit): -4.6, *CI*: -2.3 -
311 15.2, *N* = 36; Suppl. Table S2). The maximum score of the nest over the whole trial did not
312 differ significantly between treatments (*W* = 205, *P* = 0.15, *N* = 36), but there was a trend
313 towards males weighing longer before they started building a nest in the added noise
314 treatment (*W* = 107, *P* = 0.08, *N* = 36; Median (IQR): Control treatment: 2.5 (1.0 – 2.8),
315 Added noise: 2.5 (2.5 – 4.5) days).

316

317 *Male acoustic courtship behaviour*

318 Males did not start calling until the female was close to or in the nest. Both goby species
319 produced two different call types: a drum and a thump (sensu Amorim and Neves 2007; Fig.
320 5). Two-spotted gobies produced an average of 5 ± 11.1 (mean \pm SD, *N* = 16) drums and $58 \pm$
321 122.0 (*N* = 16) thumps in 30 minutes, while painted gobies produced 39 ± 56.3 (*N* = 36)
322 drums and 7 ± 15.6 (*N* = 36) thumps in 30 minutes, overall. Combined, this meant that there

323 was no significant difference in the mean ($W = 247.5$, $P = 0.42$, $N = 52$), but there was a
324 difference in the variance (Levene's test: $F_{1,50} = 6.73$, $P = 0.012$) of the frequency of acoustic
325 behaviours between the species.

326

327 Males of both species produced significantly fewer drums in the added noise compared to the
328 control treatments (Fig. 6a-b; Two-spotted gobies: $V = 89.5$, $P = 0.002$, $N = 16$; Painted
329 gobies: $W = 228.5$, $P = 0.025$, $N = 36$). There was no significant difference in the number of
330 thumps between the added-noise and the control treatment (Fig. 6.c-d; Two-spotted gobies: V
331 $= 4$, $P = 0.86$, $N = 16$; Painted gobies: $W = 153.5$, $P = 0.81$, $N = 36$) although few males
332 produced thumps at all (6 of 16 two spotted gobies, 8 of 36 painted gobies).

333

334 *Male visual courtship behaviour*

335 Painted gobies were much more active than two-spotted gobies and displayed on average 19
336 ± 9.2 (mean \pm SD, $N = 36$) behaviours in the first minute, while two-spotted gobies displayed
337 2.3 ± 2.08 (mean \pm SD, $N = 16$) behaviours per minute. Thus, there was a significant
338 difference in the mean ($W = 4$, $P < 0.0001$, $N = 52$) and in the variance (Levene's test: $F_{1,50} =$
339 11.7 , $P = 0.001$) of the frequency of visual behaviours between the species.

340

341 Visual courtship display did not differ between noise treatments for the two-spotted goby.

342 The PCA produced one principal component with an eigenvalue > 1 . All recorded behaviours
343 were strongly positively correlated with this component (loadings > 0.7 ; Table 2). We
344 excluded fast approaches from the analyses, because these only occurred once. The scores on
345 the PC1 did not differ significantly between the treatments (Figure 7a; $V = 82.5$, $P = 0.469$, N
346 $= 16$).

347

348 In contrast, painted goby males in the added noise treatment displayed significantly less
349 visual courtship than males in the control treatment. The PCA produced three principal
350 components with an eigenvalue > 1 (Table 3). PC1 was positively correlated with the
351 advanced courtship behaviours: jumps and leads; PC2 was positively correlated with hops
352 and negatively correlated with fast approaches, which are two different ways to approach a
353 female; PC3 was positively correlated with quivers and negatively correlated with swims
354 (Table 3). Scores on PC1 and PC2 were significantly higher for males in the control treatment
355 compared to males in the noisy treatment, while the scores on PC3 did not differ between the
356 treatments. (Figure 7 b-d; Wilcoxon Rank Sum Test: PC1: $W = 226$, $p = 0.04$, PC2: $W = 224$,
357 $p = 0.04$, PC3: $W = 204$, $p = 0.17$)

358

359 *Female spawning behaviour*

360 We found a significant effect of added noise on spawning behaviour in female painted
361 gobies. Eleven of sixteen males received eggs in the control treatment (69%), while nine of
362 twenty males received eggs in the added-noise treatment (45 %). As a result, there was a
363 significant effect of noise on the likelihood that males received eggs (estimate of effect added
364 noise treatment (logit): -9.1, *CI*: -20.0 - 1.3, $N = 36$; Suppl. Table S3). There was no
365 significant effect of treatment on the area covered with eggs ($W = 194.5$, $P = 0.26$, $N = 36$),
366 nor on the latency till the first female spawned (All males: $W = 120.5$, $P = 0.18$, $N = 36$; Only
367 successful males: $W = 42.5$, $P = 0.60$, $N = 20$).

368

369 **Discussion**

370 The results of our aquarium experiment show that males of two closely related species of
371 gobies altered acoustic courtship signals in response to exposure to continuous added noise.
372 Male painted gobies also significantly reduced visual courtship in the noise treatment

373 although male two-spotted gobies did not. Female painted gobies were less likely to spawn in
374 the noise treatment. Thus, acoustic and visual communication as well as the subsequent
375 spawning success can be negatively affected by noise.

376

377 Anthropogenic noise varies in intensity, distribution, persistence and timescale depending on
378 source (McGregor *et al.*, 2013). Pile driving and seismic surveys produce impulsive
379 intermittent sounds, while boat noise often fluctuates and noise from operational wind-farms
380 is largely continuous. Of these sources our experimental noise is most comparable to boat and
381 windfarm noise, due to the low-frequency-harmonic component and more continuous nature
382 of these sounds (de Jong *et al.*, 2016b). While windfarms operate continuously, ship noise
383 may vary from intermittent to a continuous source of noise, depending on traffic intensity
384 (Havilland-Howell *et al.*, 2007). Intermittent noise may allow animals to compensate for
385 noisy periods by calling more during subsequent silent intervals (Picciulin *et al.*, 2012). Thus,
386 intermittent noise will provide more opportunities to communicate acoustically than
387 continuous noise. Continuous noise, on the other hand, will likely affect animals more
388 through the masking of acoustic signals and cues than through direct stress (Wright *et al.*
389 2007). In fish, stress, as measured by cortisol-levels, was indeed found not to be influenced
390 by continuous noise, in contrast to intermittent and fluctuating noise (Nichols *et al.*, 2015;
391 Wright *et al.*, 2007; Wysocky *et al.*, 2006). Moreover, fish may even habituate to impulsive
392 sounds if exposed for long enough (Radford *et al.*, 2016). The fact that we found effects of
393 noise on reproductive behaviour in both sexes on the third day of exposure to continuous
394 noise, therefore, suggests that the effects of noise we found were caused by an impact on
395 communication, rather than a direct effect of noise-induced stress.

396

397 A reduction in male calling rate during noise exposure is consistent with previously published
398 field data from Atlantic croaker (Luczkovich, 2012) and Oyster toadfish (Luczkovich, 2016),
399 where calls were recorded less frequently at sites with high boat traffic (but see Piciullin *et*
400 *al.*, 2012). Because our experimental noise allowed us to quantify the number of calls under
401 both control and added-noise conditions (Fig. 4), we are confident that the reduction in
402 detected calls was caused by males calling less frequently rather than a reduced likelihood of
403 detection under noisier conditions. However, we did not find a difference in the number of
404 thumps between the treatments, although their infrequency led to less statistical power to
405 detect differences compared with drum frequency. Differences in the response of different
406 call types to noise have previously been found in birds, mammals and reptiles (Pohl *et al.*,
407 2013; Luo *et al.*, 2016; Brumm & Zolinger, 2017).

408

409 Male painted gobies displayed less visual courtship, as well as acoustic courtship in the added
410 noise treatment, but we could not detect a treatment effect on male two-spotted goby visual
411 displays. This may have been due to a reduced power of the test, because two-spotted goby
412 males displayed less visual courtship overall and the variation between males was larger. On
413 the other hand, male behaviour may have been affected by a difference in male–female
414 interactions between the experiments. Painted goby females were free to use the full range of
415 the aquarium, while two-spotted goby females were confined close to the male. The latter
416 location may have signalled high female interest in both treatments, thus sustaining an
417 elevated male visual courtship rate under noisy conditions.

418

419 The reduction in male visual courtship under noisy conditions in the painted goby could have
420 been due to a cross-modal effect. Indeed, noise has previously been shown to affect signalling
421 behaviour across modalities in cuttlefish (Kunc *et al.*, 2014) and hermit crabs (Walsh *et al.*,

422 2017), as well as in terrestrial animals (tree frogs; Troianowski *et al.*, 2017). From the
423 sensory compensation hypothesis, an increase in visual signalling would have been expected
424 (Hartman & Abrahams, 2000; Hebets & Papaj, 2005). However, in the current study neither
425 species tried to compensate for the deterioration of acoustic communication by increased
426 visual signalling.

427

428 Female painted gobies were less willing to spawn in the added noise treatment, which may be
429 due to a direct effect of stress or an effect of reduced male signalling on reproductive success
430 (see Halfwerk *et al.*, 2011 for an example in birds). Females had been acclimatised to the
431 treatment for a day before the trial started, while cortisol regained normal levels after half an
432 hour in response to continuous noise in two species of fish (Nichols *et al.*, 2015; Wysocky *et al.*,
433 2006). Therefore, we assume that a direct effect of noise-induced stress may have been
434 less important than the decrease in male acoustic signalling in the noise treatment and the
435 masking effect of noise on the remaining calls, even though our data do not allow us to
436 directly falsify either of these hypotheses. In addition to masking, females may have been
437 distracted from male courtship by noise (Chan *et al.* 2010). Song characteristics of male
438 painted gobies have been previously correlated to male quality (Amorim *et al.*, 2013) and
439 may allow females to distinguish between closely-related cryptic species. (Pedroso *et al.*,
440 2012). Such information may be crucial to females spawning decisions, leading to fewer
441 spawning events in noisier conditions.

442

443 Overall we found reduced male courtship behaviour and reduced female spawning in
444 response to an experimental low-frequency continuous noise. The increased noise-levels in
445 our study did not significantly affect overall activity (PC3; Fig. 7d) or nest building in the
446 painted goby. This could indicate that field populations behaving outwardly normal could

447 still be affected in their reproductive behaviour and reproductive success. The difference of
448 25 - 30 dB between the added-noise and the control treatments is comparable to increases in
449 noise-levels by shipping (e.g. Magnhagen *et al.*, 2017; Nedelec *et al.*, 2017) and the noise
450 levels used in this study were within the range found in UK coastal waters (Merchant *et al.*
451 2016). However, because the structure of our continuous experimental noise was different
452 from real sources of anthropogenic noise, the levels used in our experiment cannot be directly
453 translated to thresholds for the onset of effects of specific noise-sources.

454

455 Combinations of controlled laboratory experiments and field studies have shown that noise-
456 induced changes in communication can lead to population-level effects in the terrestrial
457 environment (Birds: Halfwerk *et al.*, 2011; Schröder *et al.*, 2012; Potvin and Macdougall-
458 Shackleton, 2015), frogs (Kaiser *et al.*, 2011). In the aquatic environment, effects of noise
459 have been found on sensitive crucial developmental stages (Nedelec *et al.*, 2014; Nedelec *et*
460 *al.*, 2017; McCauley *et al.*, 2017), stress levels (Nichols *et al.* 2015) and crucial behaviours to
461 survival such as resource assessment (Walsh *et al.* 2017), foraging (Magnhagen *et al.* 2017),
462 and predator avoidance (Simpson *et al.* 2016). We provide experimental evidence of a
463 negative effect of noise on communication and spawning success in a common marine fish in
464 a controlled environment. Spawning is the most clearly quantifiable investment in a specific
465 mating and, as such, directly related to fitness. Moreover, long term reduction in egg
466 production is expected to lead to a mean decrease in the population, even if yearly variations
467 in spawning stock are often not correlated to recruitment (Houde, 2002; Szuwalski *et al.*,
468 2014). Small apparently abundant marine fish, such as gobies, are thought to play vital roles
469 in coastal marine foodwebs (Pockberger *et al.*, 2015); so reductions in their reproductive
470 output could have wider ecosystem consequences. Data from aquarium experiments cannot

471 be translated into population-level effects directly; rather, our data stress the urgency of
472 studies that address population-level effects in natural populations.

473

474 **Acknowledgements**

475 We would like to thank the Scottish Association for Marine Science (SAMS) and the
476 University of Lisbon for providing laboratory space. Christine Beveridge, Maria Gouveia,
477 Catarina Rosa, Sarah Schröder and Joana Vincente for help with catching, husbandry and
478 logistics; Ben Wilson for the use of equipment and Denise Risch for advice on the SPL-
479 analyses. We would like to thank Adrian Klein for kindly providing us with particle motion
480 sensors and help with the analyses of the PM-data.

481

482 **Funding**

483 KdJ was funded by the: Alexander von Humboldt Foundation (NLD /1150888STP), and her
484 stay in Oban was funded by ASSEMBLE (227799). The Volkswagen Foundation funded
485 KUH (84 846 / 92 002). The Science and Technology Foundation, Portugal funded M.C.P.A.
486 (strategic projects UID/MAR/04292/2013 to MARE) and P.J.F. (UID/BIA/00329/2013 to
487 cE3c). Competing interests: none.

488

489 **References**

490

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- 673
- 674 **Supporting Information**
- 675 **Supplementary Table S1.** Sound Pressure Levels in the different treatments.

676 **Supplementary Table S2.** Results from a binomial GLM testing the effects of noise on
677 whether or not a male built a nest in the painted goby.

678 **Supplementary Table S3.** Results from a binomial GLM testing the effects of noise on
679 whether or not a male received eggs in the painted goby.

680 **Supplementary Figure S1.** Sound levels throughout an aquarium

681 **Supplementary Figure S2.** Frequency of visual courtship behaviours of male two-spotted
682 gobies in an added noise vs. a control treatment.

683 **Supplementary Figure S3.** Frequency of visual courtship behaviours of male painted gobies
684 in an added noise vs. a control treatment.

685 **Figures and tables**

Time	Two-spotted gobies	Painted gobies
Day 1	Male introduced	Male introduced
Day 2	SF presented (5 min)	SF in, scored nest building
Day 3	SF presented (5 min)	SF out, scored nest building
		TF introduced behind partition
Day 4	TF introduced 30 min. before trial	Scored nest building
	Trial: Females constrained	Trial: TF free to spawn
	Changed treatment / end	Checked spawning
Day 5	Repeat from Day 2	Checked spawning
		End
End	Weighed and measured males and TF	Weighed and measured males and TF

686 Table 1. Timelines of two experiments testing effects of noise on spawning in two goby

687 species, SF = stimulus females, TF = test females.

688

Recorded behaviours	PC1
Fin displays	0.77
Quivers	0.87
Leads	0.83
Eigenvalue	2.05

689

690 **Table 2.** Loadings on the first principal component from a PCA on male two-spotted goby

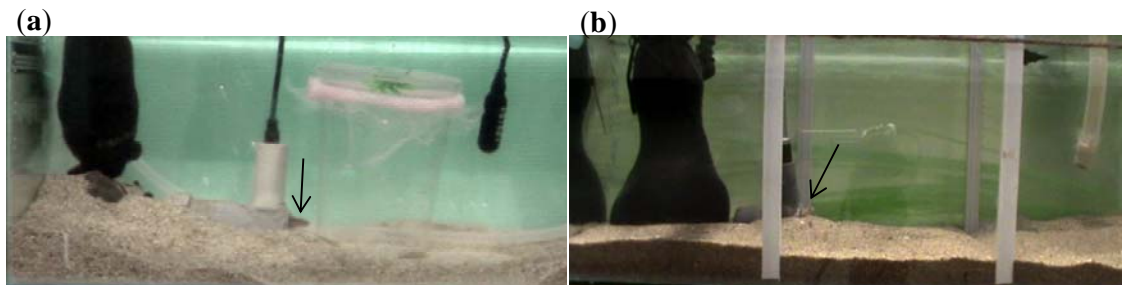
691 visual courtship. Loadings > 0.5 are printed in bold.

692

Recorded behaviours	PC1	PC2	PC3
Jumps (eights)	0.98		
Leads	0.97		
Hops		0.79	
Fast approaches		-0.80	-0.20
Quivers			0.84
Swims		-0.31	-0.78
Eigenvalues	2.21	1.29	1.22

693

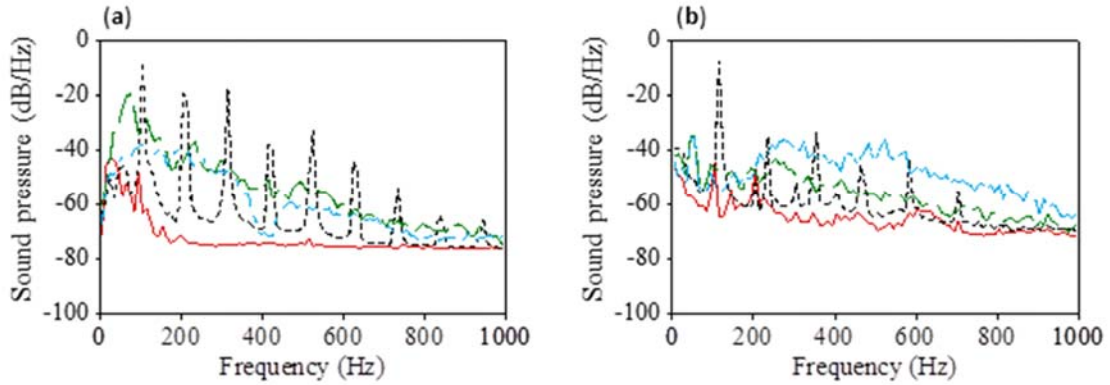
694 **Table 3.** Loadings on the first three principal components from a PCA on male painted goby
695 visual courtship. Loading < 0.2 are suppressed, loadings > 0.5 are printed in bold.



696

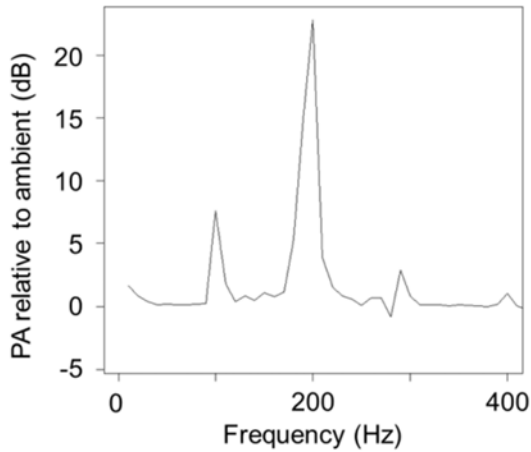
697 **Figure 1.** Side view of the experimental set-up to test the effects of noise on two-spotted
698 gobies (a) and painted gobies (b). The noise-egg is covered in cloth behind the nest and the
699 hydrophone is in the nest chimney. Goby males are in both nests with their heads out (see
700 arrow). For the two-spotted goby set-up females were constrained in a transparent container 2
701 cm from the nest opening.

702



703

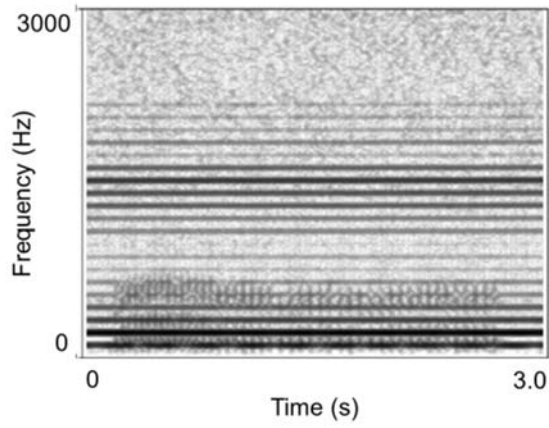
704 **Figure 2.** Representative examples of goby calls compared to added noise and control
 705 treatments for (a) the two-spotted goby and (b) the painted goby. Red continuous line: control
 706 treatment, black dotted line: noise treatment, green dashed line: goby thump, blue dashed-
 707 dotted line: goby drum.



708

709 **Figure 3.** The difference in particle acceleration (PA) between noise and control treatments
 710 measured in the nest chimney. Shown is the average difference from the six aquaria used in
 711 the experimental set-up for painted gobies.

712

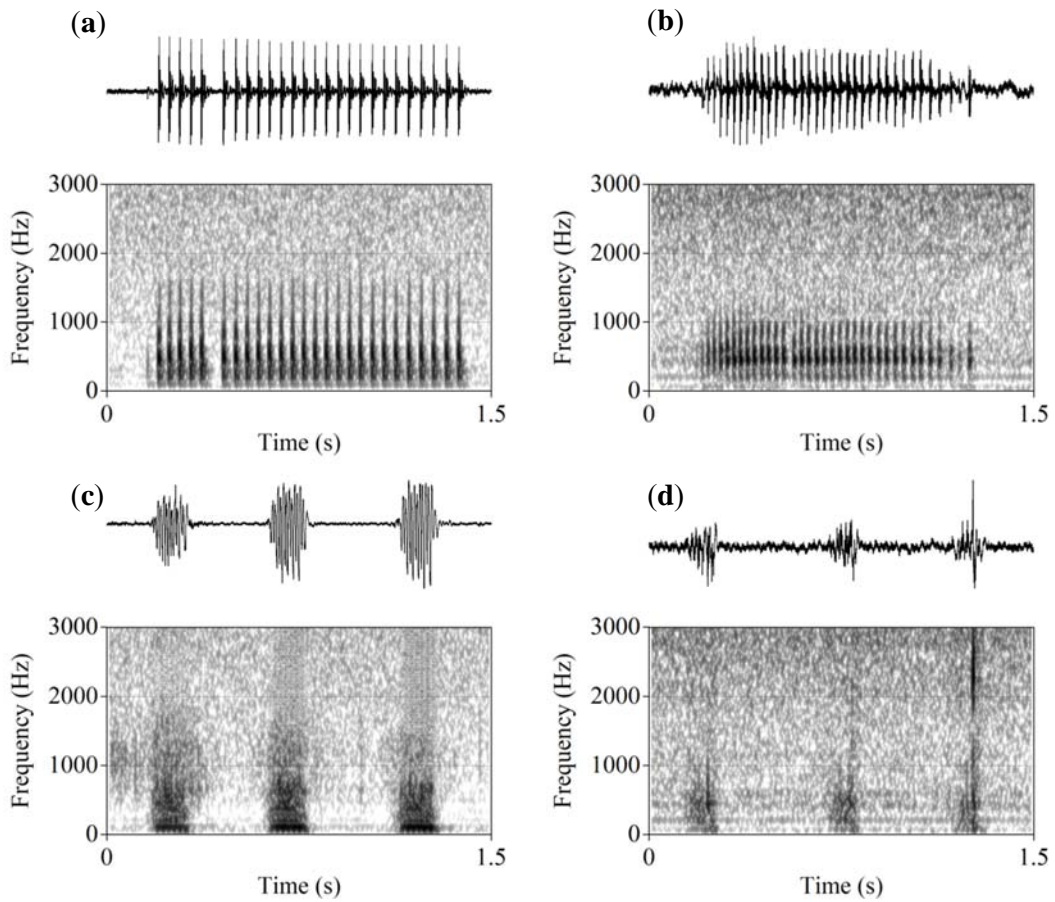


713

714 **Figure 4.** A spectrogram of a goby call in a noise treatment showing how calls can be

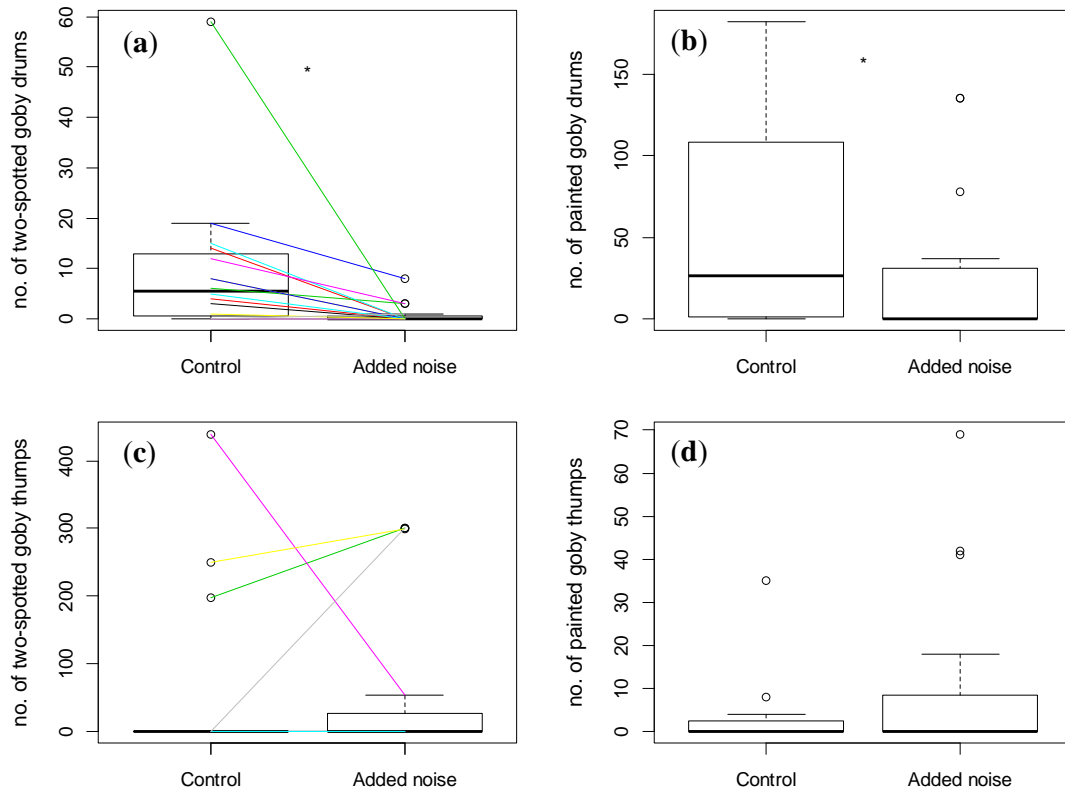
715 counted in the noise treatments.

716



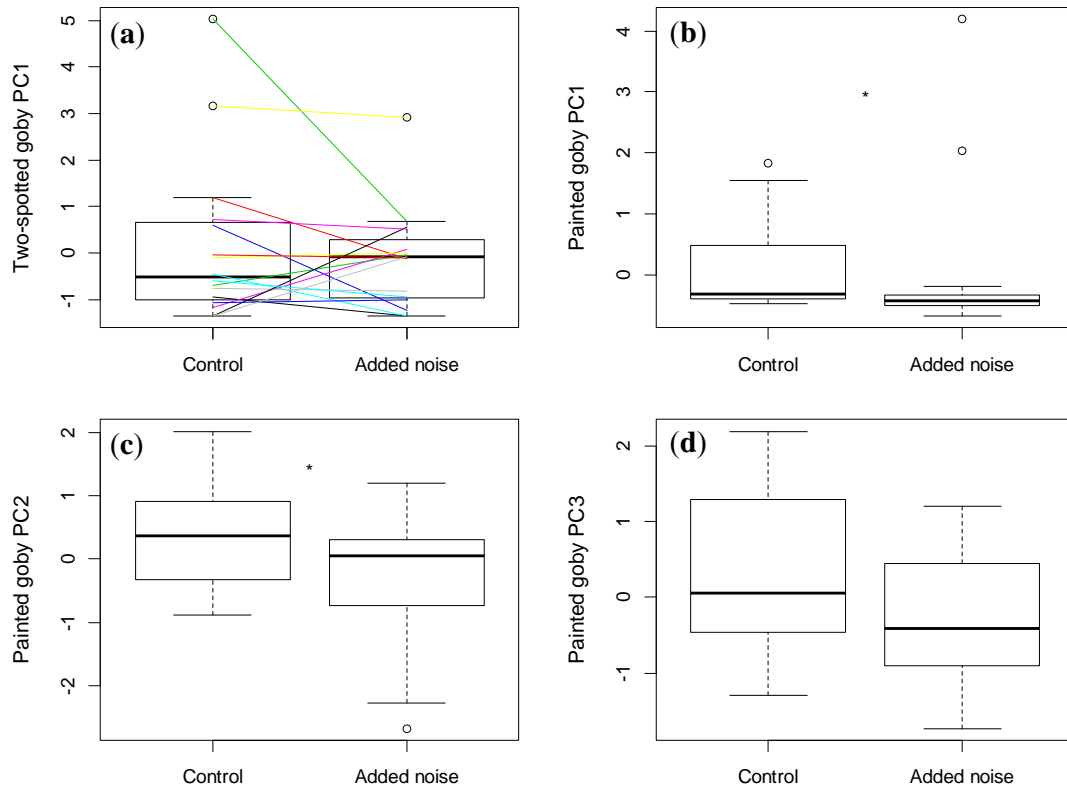
717

718 **Figure 5.** Courtship sounds of two species of gobies. Wave forms (top) and spectrograms
 719 (bottom) of (a) a two-spotted goby drum, (b) a painted goby drum, (c) a two-spotted goby
 720 thump and (d) a painted goby thump.



721

722 **Figure 6.** Acoustic courtship behaviour of two goby species compared between an added
 723 noise treatment and a control treatment. (a) Number of two-spotted goby drums, (b) painted
 724 goby drums, (c) two-spotted goby thumps and (d) painted goby thumps. The boxplots
 725 represent medians (black line), the 25th to 75th percentiles (boxes) and outliers (circles). For
 726 two-spotted gobies (left panels) we added lines to represents individual males. For painted
 727 gobies, we did not use a repeated measures design.



728

729 **Figure 7.** Visual courtship behaviour of two goby species compared between an added noise

730 treatment and a control treatment. Scores from a principal component analysis on (a) two-

731 spotted goby PC1, (b) painted goby PC1, (c) painted goby PC2 and (d) painted goby PC3.

732 The boxplots represent medians (black line), the 25th to 75th percentiles (boxes) and outliers

733 (circles). For two-spotted gobies (a) we added coloured lines to represents individual males.

734 For painted gobies, we did not use a repeated measures design.