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1 **Fear contagion in zebrafish: a behaviour affected by familiarity**

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15 **Running head** : emotional contagion in fish

16 **Key-words**: alarm response; animal model; emotional contagion; empathy

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26 **Abstract**

27 Emotional contagion has recently been described in fish but whether it is affected by
28 familiarity is not known. We tested whether the sight of a distressed conspecific elicited fear
29 in zebrafish, and whether this was modulated by familiarity. Groups of six zebrafish were
30 housed together in the same tanks for 7 days to create familiar conditions. The behaviour of
31 individual fish was then recorded in paired tanks within sight of either a familiar or an
32 unfamiliar individual, before and after distilled water or an alarm substance was added to the
33 demonstrator, but not to the observer. As expected, addition of distilled water did not elicit
34 any behavioural change in either the demonstrator or the observer. However, addition of an
35 alarm cue triggered anti-predatory behaviours in the demonstrator, which caused the
36 expression of equivalent anti-predatory behaviours in the observer, suggesting the existence
37 of fear contagion. Furthermore, the extent of fear contagion and behavioural matching was
38 affected by familiarity, and observers matched the behaviour of the demonstrators more
39 closely when they watched a distressed familiar neighbour than when they watched an
40 unfamiliar fish. Our results have implications for fish welfare because they show that fish can
41 become stressed by simply watching others become stressed. They also have implications for
42 experimental design because fish housed in separate tanks cannot be assumed to be
43 statistically independent if they can eavesdrop on their neighbours.

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51 INTRODUCTION

52 Emotional contagion can be defined as the instantaneous matching of emotional state between
53 an observer and a demonstrator (Nakahashi & Ohtsuki, 2018). This phenomenon has been
54 explained through the perception-action mechanism, which postulates that the perception of a
55 demonstrator's state triggers a neural, unconscious and automatic representation of the same
56 state in the observer, causing an equivalent expression of behaviours (Preston & de Waal,
57 2002). Emotional contagion is considered a component and evolutionary precursor of
58 empathy (Preston & de Waal, 2002), and has been demonstrated in humans, birds and
59 mammals (Gonzalez-Liencre, Juckel, Tas, Friebe, & Brüne, 2014; Reimert, Bolhuis, Kemp,
60 & Rodenburg, 2014), and recently, also in fish (Oliveira, Idalencio, Kalichak, dos Santos
61 Rosa, Koakoski, de Abreu et al., 2017).

62 To evaluate emotional contagion studies have typically focussed on negative
63 emotional states such as stress, pain and fear (Carnevali, Montano, Statello, Coudé,
64 Vacondio, Rivara, et al., 2017). A common measure of fear in rodents is freezing behaviour,
65 which can be triggered by a mild electric shock (Lezak, Missig, & Carlezon Jr, 2017;
66 Pisansky, Hanson, Gottesman, & Gewirtz, 2017). Fear elicited in this way propagates from
67 frightened demonstrators to naïve observers, resulting in increased frequency of freezing
68 (Jeon, Kim, Chetana, Jo, Ruley, Lin, et al., 2010; Knapp, Overstreet, & Breese, 2007;
69 Knapska, Walasek, Nikolaev, Neuhäusser-Wespy, Lipp, Kaczmarek, et al., 2006) and
70 activation of the amygdala in the observer (Knapska et al., 2006). Moreover, it seems that
71 how an emotion is shared between individuals is modulated by contextual aspects such as
72 kinship, familiarity and social closeness (Liévin-Bazin, Pineaux, Clerc, Gahr, von Bayern, &
73 Bovet, 2018; Preston & de Waal, 2002). For instance, when mice are paired with distressed
74 demonstrators they tend to freeze if they had been reared together, but become more active if
75 they come from different cages (Gonzalez-Liencre et al., 2014). In addition, pain perception

76 in mice is more intense when observers are familiar with demonstrators than when they are
77 strangers (Langford, Crager, Shehzad, & Smith, 2006). Observers typically respond
78 differently to signals sent by familiar and unfamiliar conspecifics (Gonzalez-Liencre et al.,
79 2014; Jeon et al., 2010), a strategy thought to be adaptive as it can help avoid sensory
80 overload (Hutchinson, 2005) and focuses attention on signals emitted by those neighbours
81 that matter the most, including ‘nasty neighbours’ and ‘dear enemies’ (Müller & Manser,
82 2007).

83 A recent study has provided evidence for fear contagion in zebrafish (Oliveira et al.,
84 2017), which suggests that this phenomenon may be conserved among social vertebrates.
85 However, to what extent fear contagion in fishes can be affected by the degree of familiarity
86 between demonstrators and observers is not known. Familiarity can broadly be defined as the
87 ability to discriminate between individuals based on previous interactions, and is influenced
88 by the time of interaction and the size of the group among fishes (Griffiths, 2003).

89 Here we used dyadic behavioural tests to assess if fear contagion was affected by
90 familiarity in zebrafish. To this end, demonstrators were exposed to either distilled water or
91 an alarm substance, known to cause a fear response on this species (Speedie & Gerlai, 2008).
92 If there was fear contagion, an observer watching a frightened demonstrator might be
93 expected to match its behaviour and present an equivalent fear response (Meyza, Bartal,
94 Monfils, Panksepp, & Knapska, 2017), which in the case of zebrafish is characterised by an
95 increase in swimming velocity, length of freezing bouts, and frequency of diving (Kalueff,
96 Gebhardt, Stewart, Cachat, Brimmer, Chawla, et al., 2013). This is in contrast to a simple
97 startle response, which in adult zebrafish typically consists of short bursts of locomotor
98 activity, zigzagging and swimming away from the source of the startle (Chanin, Fryar, Varga,
99 Raymond, Kyzar, Enriquez, et al., 2012).

100 Zebrafish are highly social (Gerlai, 2010), can discriminate familiar from unfamiliar
101 fish after only 20 min of interaction (Hinz, Kobbenbring, Kress, Sigman, Müller, & Gerlach,
102 2013; Madeira & Oliveira, 2017), and form cohesive groups under threat (Speedie & Gerlai,
103 2008). Therefore, we also hypothesized that observers would show a heightened fear response
104 to the sight of distressed demonstrators when they had been reared together (i.e. were
105 familiar), than when they had been reared apart (i.e. were unfamiliar).

106

107 **METHODS**

108 *Experimental fish and husbandry conditions*

109 Two-month old, laboratory-reared zebrafish (*Danio rerio*) of homogeneous size were sourced
110 from a local supplier and kept in four 50 L tanks (density = 2 fish/L) connected to a
111 recirculation system for four months before testing. Water quality was maintained by
112 mechanical, biological and chemical filtration, in addition to UV disinfection. Water
113 temperature was kept at $28 \pm 1^\circ\text{C}$, pH at 7.2 and ammonia and nitrite at recommended
114 optimal levels for the species. Photoperiod was set at 12D:12L with the help of fluorescent
115 lights (150 lumens) with the start of the light phase set at 7:00 hrs. Fish were fed commercial
116 pellets twice a day (Nutricom Pet, 38% protein, 4% lipids) and *Artemia salina* once daily.

117

118 *Development of familiar and unfamiliar groups*

119 Seven days prior to testing, a sample of 156 adults of both sexes was collected haphazardly
120 from the four stock tanks, mixed, and allocated at random in groups of six to 26 x 20 L glass
121 aquaria (40L x 20W x 25 H cm). Aquaria were filled with system water, were fitted with a
122 sponge filter to maintain water quality, and had their bottom and sides covered with white
123 plastic sheets to prevent visual contact with others groups. Food was offered twice a day, as
124 above.

125

126 *Acclimation period*

127 After seven days of being reared in groups of six, two individuals from either the same or
128 different aquaria (i.e. familiar or unfamiliar conditions) were transferred to two 2L test
129 aquaria (20L x 10W x 20H cm) placed side by side and left to acclimatize for 18 hours prior
130 to testing, one fish serving as a 'demonstrator' and the other as an 'observer'. To ensure that
131 unfamiliar fish would not become 'familiar' during the acclimation period, a removable

132 divider was placed between the two test aquaria, so that demonstrator and observer had no
133 visual contact until the divider was lifted just prior to testing. Similarly, and in order to avoid
134 a potential disruption of familiar dyads, these were acclimated without dividers, in full sight
135 of each other. To test if this could have affected their subsequent behaviour, we tested 8
136 additional familiar dyads acclimatized with dividers, and compared their behaviour to
137 familiar dyads acclimatized without dividers.

138

139 *Testing of fear contagion*

140 Following the 18h acclimation period, the divider preventing visual contact was removed and
141 the demonstrator and observer were simultaneously recorded (Sony DCR-SX45 Digital VCR)
142 for 5 min (basal behaviour). A syringe connected to a small silicon tube was then used to
143 remotely deliver 2 ml of either distilled water or an alarm substance to the demonstrator
144 (delivery being allocated at random), and their behaviours were recorded at 10 minute
145 intervals over an hour. To obtain the alarm substance, one zebrafish from the stock tanks was
146 euthanized by an overdose of clove oil (2 ml/L water), and approximately 1 cm² of skin from
147 each flank was removed, macerated in 10 ml of distilled water and filtered. Fresh alarm
148 substance was prepared every morning before testing.

149 We tested 48 dyads exposed to alarm substance (24 unfamiliar and 24 familiar) and
150 20 dyads exposed to distilled water (10 unfamiliar and 10 familiar). In addition, 20
151 demonstrators (10 exposed to alarm substance and 10 exposed to distilled water) were tested
152 without observers to ascertain which behaviours were modified by the addition of the alarm
153 substance, and to what extent the presence of an observer influenced the demonstrator's
154 behaviour. We chose to test more fish exposed to alarm substance than to distilled water
155 because the behaviour of controls was well established and less variable, and this helped us to
156 evaluate fear contagion more fully and reduce the number of fish used in the experiments

157 (Kramer & Font, 2017). Fish were only used once in the experiments, either as observers or
158 demonstrators. Twelve (unrelated) fish were used for the preparation of the alarm substance
159 (10 ml was obtained from each fish).

160 We used *ZebTrack* (Pinheiro-da-Silva, Silva, Nogueira, & Luchiari, 2017) to extract
161 from the video recordings six behavioural metrics that have previously been shown to
162 accurately describe the fear response in zebrafish (Kalueff et al., 2013), namely : (1) mean
163 swimming speed, (2) maximum swimming speed, (3) total distance travelled, (4) time spent
164 freezing, (5) swimming depth (i.e. distance from the tank bottom), and (6) mean distance to
165 the conspecific's tank. In response to a threat, zebrafish tend to increase their swimming
166 speed, spend more time in freezing bouts, dive to the bottom of the tank, and swim closer to a
167 conspecific's tank, denoting a greater urge to shoal (Kalueff et al., 2013).

168

169 *Statistical Analyses*

170 Statistical analysis was conducted in R v. 3.4.3 (R Core Team 2013). Our experiment
171 conformed to a fully factorial 2 x 2 x 2 BACI design (before-after-control-impact) and we
172 modelled the behaviour of the observer (dependent variable) as a linear mixed effect model
173 using the *lme4* (Bates, Mächler, Bolker, & Walker, 2014) and *lmerTest* (Kuznetsova,
174 Brockhoff, & Christensen, 2017) R packages. We used as fixed effects (predictors) the
175 behaviour of the demonstrator, the time (before or after the stressor was added), the
176 familiarity (familiar vs unfamiliar dyad) and the stressor type (alarm substance vs distilled
177 water), and considered the dyad identity as a random effect to control for variation among test
178 arenas and account for potential non-independence of observations. For each behavioural
179 metric, we started with a maximal model with all main effects and interactions and used the
180 *step* and *dredge* functions in the *MuMIn* package (Bartoń, 2013) to arrive at a minimal
181 adequate model via Maximum Likelihood on the basis of single deletion tests and relative

182 changes in AICc values. The most plausible model (within 2 AICc) was refitted by REML
183 and the model adequacy and assumptions were checked by examining plots of fitted vs
184 residuals, fitted vs observed values, as well as plots of random effects and standardized fixed
185 effects using the *sjPlot* package (Lüdecke, 2016). We report standardized fixed effect
186 estimates. One dyad had missing values so there were 134 observations corresponding to 67
187 dyads.

188 To better assess the extent to which observers were able to match the behaviour of
189 demonstrators, we also carried out a principal component analysis (PCA) using the *prcomp*
190 function in R, and modelled the scores along the first two first principal components (which
191 together explained 94% of variation) as a function of fish type (observer or demonstrator) and
192 degree of familiarity.

193

194 *Ethical Note*

195 All experimental procedures were authorized by Animal Ethics Committee permit CEUA
196 042/2015 granted by Universidade Federal do Rio Grande do Norte, and permit IP-1516-8
197 granted by the Animal Welfare and Ethical Review Body of Swansea University.

198

199

200 RESULTS

201 Inspection of temporal data indicated that the response of single fish to alarm cues was rapid
202 and did not persist for more than 10 minutes after the administration of the alarm substance,
203 probably due to habituation (Appendix, Fig. A1). We, therefore, concentrated the analysis on
204 the first 10 minutes after addition of the stimuli. In addition, the response of familiar fish to
205 alarm substance was not affected by whether they were visually isolated or not during the 18
206 hrs of acclimation (**Appendix, Table A1**).

207

208 *Swimming speed*

209 Demonstrators in the dyadic tests increased their average swimming speed when an alarm
210 substance was added, but not when distilled water was added (Fig. 1a,c). Observers
211 responded by increasing their speed when the demonstrator was familiar (Fig. 1b), but by
212 decreasing it when the demonstrator was unfamiliar (Fig. 1d; estimate demonstrator = 0.40, P
213 = 0.002; estimate time = 0.93, $P < 0.001$; estimate familiarity x time = 1.18, $P < 0.001$;
214 estimate time x stressor = 0.53, $P = 0.002$; estimate familiarity x time x stressor = -0.60, P
215 < 0.001). Fear contagion, hence, was affected by familiarity.

216

217 *Freezing behaviour*

218 Demonstrators spent more time freezing when an alarm substance was added, but not when
219 distilled water was added (Fig. 2a,c). The time observers spent freezing increased when an
220 alarm substance was added to the demonstrator (Fig. 2b,d; estimate = 0.85, $P < 0.001$), and
221 also with time (estimate = 0.90, $P < 0.001$), but observers froze on average for significantly
222 less time than demonstrators (estimate = -0.19, $P = 0.024$). There was a significant interaction
223 between time and stressor (estimate = 0.63, $P < 0.001$) as observers only increased the time
224 spent freezing over basal values when the alarm substance was added, not when distilled

225 water was added. Familiarity, hence, did not influence the freezing response, which was very
226 strong under both conditions.

227

228 *Distance from the tank bottom (swimming depth)*

229 Changes in swimming depth in response to distilled water and alarm substance are shown in
230 Figure 3. Following the addition of the alarm substance, demonstrators moved closer to the
231 bottom of the tank, a behaviour not seen when distilled water was added (Fig. 3a,c). Familiar
232 observers mimicked this behaviour (Fig. 3b), tracking what the demonstrator did (estimate =
233 0.19, $P = 0.033$), a response not seen for unfamiliar observers (Fig. 3d). Swimming depth
234 increased over basal values (estimate = 0.23, $P = 0.011$), as well as with the addition of the
235 alarm cue (estimate = 0.31, $P = 0.018$). There were significant interactions between
236 demonstrator's depth and familiarity (estimate = 0.25, $P = 0.03$), demonstrator's depth and
237 time (estimate = 0.25, $P = 0.002$), familiarity and time (estimate = 0.44, $P < 0.001$),
238 familiarity and stressor (estimate = 0.54, $P < 0.001$), and familiarity x time x stressor
239 (estimate = 0.28, $P = 0.003$). There was, hence, evidence of fear contagion which was also
240 affected by familiarity.

241

242 *Distance to the conspecific's tank (proximity to the demonstrator)*

243 Following the addition of the alarm substance to the demonstrators (Fig. 4a,c), observers
244 swam closer to the demonstrators (Fig. 4b,d; estimate = 0.60, $P < 0.001$), something that did
245 not happen when distilled water was added. Distance to the demonstrators decreased over
246 basal values (estimate = 0.43, $P = 0.005$), but increased with familiarity (estimate = 0.16, $P =$
247 0.031) and was also affected by the interactions between demonstrator and time (estimate =
248 0.36, $P = 0.03$), and between time and stressor (estimate = -0.49, $P < 0.001$). There was thus

249 evidence of fear contagion with respect to proximity to the other fish's tank, and this was also
250 influenced by familiarity.

251 *PCA Analysis*

252 PCA analysis showed that the behaviour of demonstrators and observers was similar and
253 showed little variation among individuals when the fish were not stressed (i.e. when distilled
254 water was added or before an alarm cue was added, Fig. 5a-c, Fig. 5e-g). The first component
255 (PC1) accounted for 62.8 – 94.0% of the variation and described freezing behaviour (loading
256 = -0.99), while PC2 accounted for 2.6-17.2% of the variation and described mostly variation
257 in swimming speed (loading = 0.93), proximity to the conspecific (loading = 0.28), and
258 distance to the bottom of the tank (loading = -0.24). No statistical difference between
259 observer and demonstrators was found along PC1 ($F_{3,90} = 0.847$, $P = 0.472$), but there was a
260 marked difference along PC2 ($F_{3,90} = 68.52$, $P < 0.001$; Fig. 5d,h), which depended on the
261 type of fish (estimate for observer = 1.21, SE = 0.64, $P = 0.009$), the extent of familiarity
262 (estimate for unfamiliar = -7.72, SE = 0.91, $P < 0.001$), and their interaction (estimate for
263 observer x unfamiliar = 15.44, SE = 1.29, $P < 0.001$). Thus, observers were better able to
264 match the fear response of demonstrators when they were familiar (Fig. 5d) than when they
265 were unfamiliar (Fig. 5h).

266

267 DISCUSSION

268 Our study provides novel evidence in support for the existence of fear contagion in fish, and
269 suggests that this is influenced by familiarity, as shown for mammals (Gonzalez-Liencrez et
270 al., 2014; Jeon et al., 2010). They add support to the idea that fish are not only capable of
271 identifying and reacting to the behaviour of conspecifics (Jesuthasan & Mathuru, 2008; Rey,
272 Huntingford, Boltana, Vargas, Knowles, & Mackenzie, 2015), but that they are sufficiently
273 flexible to modify their response depending on the identity of their neighbours.

274 The alarm response of zebrafish has been characterised by an increase in swimming
275 speed interspersed with freezing bouts and diving to the bottom (Kalueff et al., 2013). To
276 trigger a fear response we added an alarm substance to the water, *schreckstoff*, (von Frisch,
277 1938), a well-established stressor for zebrafish (Jesuthasan & Mathuru, 2008). As expected,
278 the addition of the alarm substance triggered a flight response in zebrafish, which swam
279 faster, moved to deeper waters, and included longer bouts of freezing behaviour, something
280 not observed when distilled water was added. Such anti-predatory behaviours were matched
281 to a large extent by the observers, even though they had no direct exposure to the stressor.
282 Thus, the mere sight of a distressed conspecific was enough to trigger in the observer a fear
283 response similar to that experienced and displayed by the demonstrator.

284 Emotional contagion appears common in social mammals, where it has been
285 demonstrated for pain, fear and distress (Carnevali et al., 2017; Gonzalez-Liencrez et al.,
286 2014; Jeon et al., 2010; Langford et al., 2006), emotions that are relevant for survival and that
287 are thought to enhance inclusive fitness. For example, in group-living animals, the sharing
288 among conspecifics of an emotional state, such as fear, can generate a coordinated group
289 response that can increase the probability of escaping from predators (Briefer, 2018;
290 Nakahashi & Ohtsuki, 2018). Although less well studied, emotional contagion has also been
291 reported for non-mammalian species, including birds (Briefer, 2018; Liévin-Bazin et al.,

292 2018), and the zebrafish, where two recent studies have provided evidence of emotional fever
293 (Rey et al., 2015), and fear contagion (Oliveira et al., 2017), two responses once thought to be
294 restricted to mammals.

295 Our study shows that fear contagion is modulated by familiarity in zebrafish, since
296 three of the four responses examined varied depending on whether the observers were
297 watching individuals they were familiar with. Compared to unfamiliar demonstrators,
298 observers responded to the sight of familiar demonstrators by matching their swimming
299 speeds more closely, and by moving closer to the bottom. These results suggest that
300 familiarity in zebrafish may serve to strengthen the extent of emotional contagion, as
301 observed in birds and mammals. For example, mice spend more time freezing when they are
302 watching a familiar cage-mate in distress than when they are watching an unfamiliar one
303 (Gonzalez-Liencre et al., 2014). Similarly, the reaction of cockatiels to a conspecific's
304 distress call depends on their degree of affiliation, and a stronger response is elicited when the
305 call is emitted by a partner than by other individuals (Liévin-Bazin et al., 2018). Observers in
306 our study also moved closer to the demonstrators when they were in distress, but - perhaps
307 unexpectedly, this was more pronounced when they were paired with unfamiliar
308 demonstrators.

309 Among fishes, several studies have shown that familiarity increases shoal cohesion
310 (Chivers, Brown, & Smith, 1995; Lachlan, Crooks, & Laland, 1998) and facilitates social
311 learning (Swaney, Kendal, Capon, Brown, & Laland, 2001), and our study shows that
312 familiarity also affects fear contagion, which may explain why association with familiar fish
313 is generally adaptive (Griffiths, 2003). For example, familiar brown trout respond 14% faster
314 than unfamiliar fish to a predator attack (Griffiths, Brockmark, Höjesjö, & Johnsson, 2004),
315 most likely because they can interpret signals from familiar fish more accurately. Familiarity
316 in our study was established rapidly, after only seven days of cohabitation, which is

317 consistent with previous results in zebrafish (Madeira & Oliveira, 2017) and other species
318 (Griffiths, 2003), where individuals were able to recognize familiar neighbours after short
319 periods of interaction. Although it is possible that some individuals in our study may have
320 become familiar in the stock tanks (i.e., before the 7 day cohabitation experiment), this is
321 unlikely as the group size was too large (100 fish/tank) for individual recognition (Griffiths &
322 Magurran, 1997), fish were mixed and allocated at random to 26 groups, and this would not
323 explain why familiar and unfamiliar fish behaved so differently.

324 The advantages of familiarity may be accrued through visual recognition, but also
325 through chemical cues (Griffiths, 2003), as fish can recognize the metabolites of conspecifics
326 released in the water (Ward, Webster, Magurran, Currie, & Krause, 2009). Zebrafish can use
327 both chemical and visual cues for individual recognition (Hinz et al., 2013), but our study
328 indicates that visual signals alone are enough to trigger fear contagion, as familiar fish
329 behaved differently from unfamiliar fish when no chemical signals were exchanged between
330 observers and demonstrators. The strong shoaling behaviour of zebrafish may help explain
331 the evolution of fear contagion on this species. Unlike social learning, which is thought to
332 have evolved to facilitate the long-term transmission and storage of information (Brown &
333 Laland, 2003), fear contagion may have evolved to deal with rapid, short-term signals and
334 swift responses, such as the anti-predatory response (Nakahashi & Ohtsuki, 2015).

335 It has been proposed that behavioural contagion should be heightened when the
336 demonstrator displays abnormal or extreme behaviours (Nakahashi & Ohtsuki, 2015).
337 Freezing is an extreme behaviour that can be induced by alarm substances and is commonly
338 seen in many fish species in response to predators (Miklosi, Csanyi, & Gerlai, 1997; Roberts,
339 Taylor, & Garcia de Leaniz, 2011; Roberts & Garcia de Leaniz, 2011; Speedie & Gerlai,
340 2018). In this sense, the increased duration in freezing bouts following exposure to the alarm
341 substance was highly contagious in our study, but it was not affected by familiarity. We

342 suggest that for zebrafish, freezing behaviour constitutes a more robust signal of danger than
343 bottom dwelling or an increase in swimming speed. Hence, it may be adaptive for an
344 individual to freeze when another one is freezing, regardless of the sender's identity. On the
345 other hand, changes in swimming speed or in the position in the water column form part of
346 the normal behaviour of zebrafish (Kalueff et al., 2013) and may represent less extreme, and
347 hence less reliable, signals.

348 Our results show that zebrafish can not only distinguish between familiar and
349 unfamiliar conspecifics by visual cues alone, but that they can also eavesdrop on their
350 neighbours living in separate tanks and adjust their behaviour accordingly. This may have
351 implications for fish welfare if, for example, fish can become stressed simply by watching
352 their neighbours become stressed. In the wild, eavesdropping may be adaptive as it allows
353 zebrafish to acquire information on predatory threat from shoal neighbours (Abril-De-Abreu,
354 Cruz, & Oliveira, 2015; Oliveira et al., 2017), but the implications for fish welfare in
355 captivity deserve further attention. In livestock, contagion of negative emotions such as fear
356 and anxiety can impair the behaviour and health of the group (Reimert, Bolhuis, Kemp, &
357 Rodenburg, 2013), and our study suggests that the same could happen in zebrafish.

358 Our results also have implications for experimental design because fish housed in
359 separate tanks may not be assumed to be statistically independent (Colegrave & Ruxton,
360 2017) if their behaviour is affected by that of others. Visual isolation of tanks, therefore, must
361 be ensured to prevent eavesdropping. Ultimately, our study indicates that fish - just like
362 mammals, are capable of recognizing and sharing emotions and place them into the right
363 context. Future studies might benefit from investigating if emotional contagion also occurs in
364 relation to positive stimuli (such as access to food, mates, or enriched habitats) as this could
365 perhaps be used to improve welfare.

366

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373 statistical analyses. All authors approved the final submission.

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505 **Appendix**

506 **Table A1.** Behaviour of familiar zebrafish held with and without a divider during the acclimatization period.

Behaviour	Demonstrator								
	Basal			After 10 min			Basal		
	without divider	with divider	<i>p</i>	without divider	with divider	<i>p</i>	without divider	with divider	<i>p</i>
Average speed (cm/s)	2.6 ± 0.9	3.3 ± 2.3	0.07	4.7 ± 3.0	4.1 ± 1.6	0.82	3.0 ± 1.3	3.1 ± 1.9	0.92
Maximum speed (cm/s)	42.1 ± 14.8	53.7 ± 12.5	0.05	39.2 ± 27.9	44.0 ± 16.4	0.66	41.6 ± 14.9	46.5 ± 13.3	0.66
Total dist. travelled (cm)	199.3 ± 110.3	294.1 ± 100.9	0.07	192.7 ± 107.5	250.1 ± 75.8	0.23	199.5 ± 134.1	263.2 ± 105.7	0.23
Freezing (s)	27.9 ± 16.8	15.5 ± 9.9	0.08	192.4 ± 136.9	180.5 ± 38.9	0.82	28.2 ± 13.9	16.9 ± 9.1	0.08
Distance from bottom (cm)	5.1 ± 2.9	3.9 ± 2.8	0.34	2.0 ± 1.8	1.4 ± 0.4	0.38	5.5 ± 3.1	3.1 ± 1.7	0.34
Distance to other tank (cm)	7.2 ± 2.6	5.5 ± 0.9	0.11	6.3 ± 2.5	6.6 ± 2.9	0.79	6.8 ± 2.3	6.3 ± 1.8	0.11

507

508 p-values correspond to student t-tests between groups held with or without a divider before (basal) and 10 mi
 509 added

510

511

512

513

514

515 **Figure captions**

516 **Figure A1.** Temporal variation in the response of single zebrafish (i.e. demonstrators without
517 observers) to the addition of either distilled water (blank) or an alarm cue. Shown are means
518 (± 95 CI) for swimming speed (a), distance to the tank bottom (b), duration of freezing bouts
519 (c), maximum swimming speed (d), distance to the other tank (e), and total distance travelled
520 (f).

521

522 **Figure 1.** Changes in the swimming speed (mean ± 95 CI) of observers (b, d) before and after
523 distilled water or an alarm cue were delivered to the demonstrators (a, c), but not to the
524 observers, in dyadic tests involving familiar (a, b) and unfamiliar pairs (c, d).

525

526 **Figure 2.** Changes in the freezing behaviour (mean ± 95 CI) of observers (b, d) before and
527 after distilled water or an alarm cue were delivered to the demonstrators (a, c), but not to the
528 observers, in dyadic tests involving familiar (a, b) and unfamiliar pairs (c, d).

529

530 **Figure 3.** Changes in the swimming depth (mean ± 95 CI) of observers (b, d) before and after
531 distilled water or an alarm cue were delivered to the demonstrators (a, c), but not to the
532 observers, in dyadic tests involving familiar (a, b) and unfamiliar pairs (c, d).

533

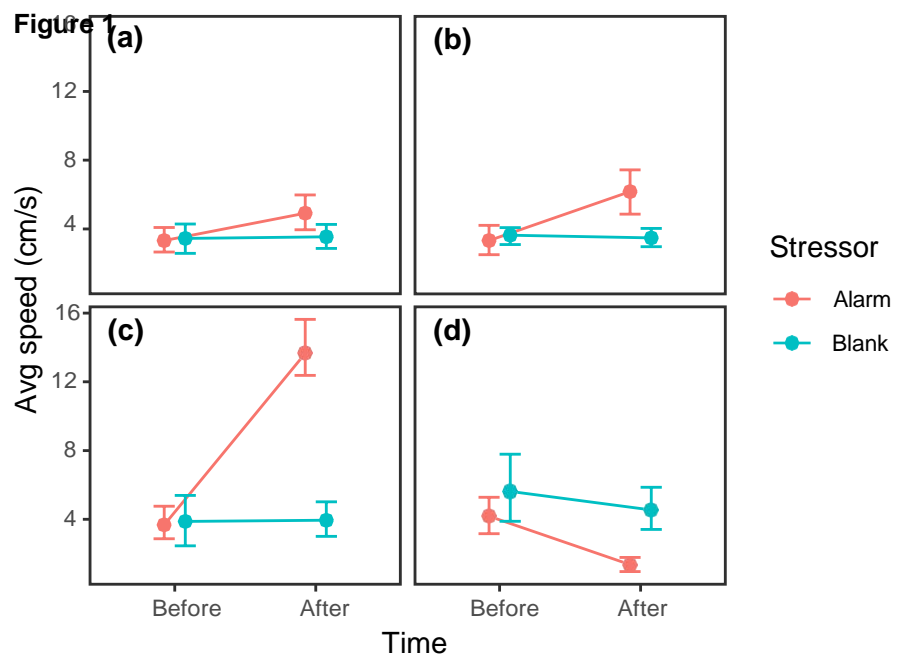
534 **Figure 4.** Changes in the distance to the conspecific's tank (mean ± 95 CI) of observers (b, d)
535 before and after distilled water or an alarm cue were delivered to the demonstrators (a, c), but
536 not to the observers, in dyadic tests involving familiar (a, b) and unfamiliar pairs (c, d).

537

538 **Figure 5.** Variation along the first two principal components (means ± 95 CI) describing the
539 behaviour of familiar (a-d) and unfamiliar (e-h) dyads of zebrafish before and after distilled

540 water (a-b, e-f) or an alarm cue (c-d, g-h) were delivered to the demonstrators (red), but not to
541 the observers (green).

Figure 1



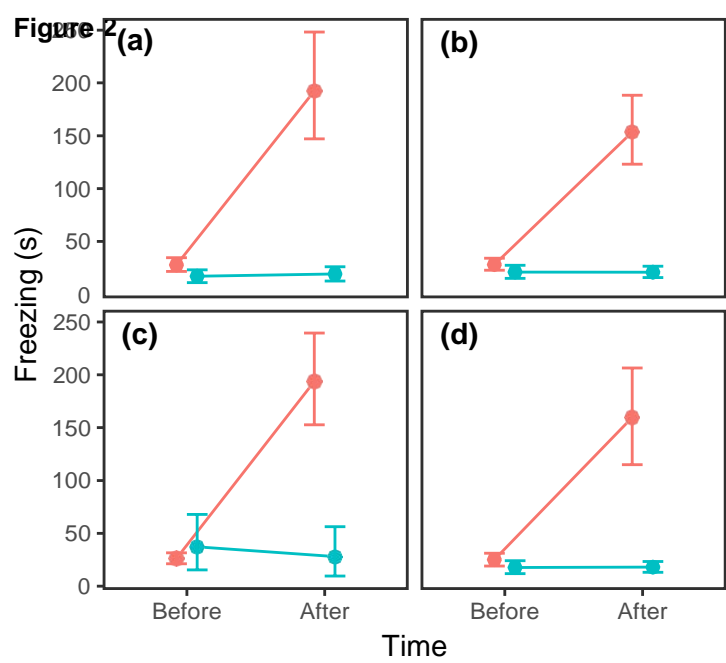


Figure 3

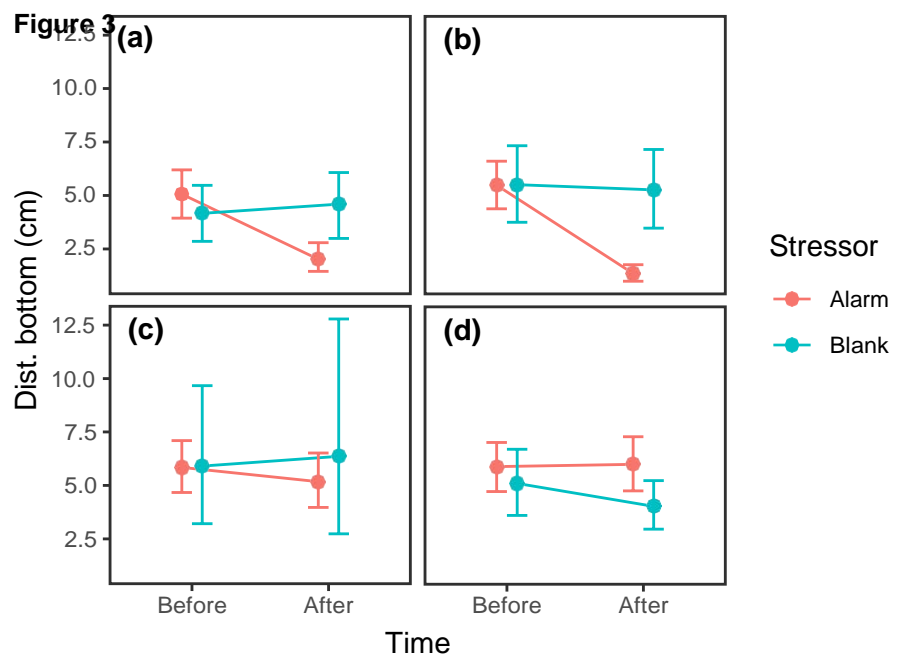


Figure 4

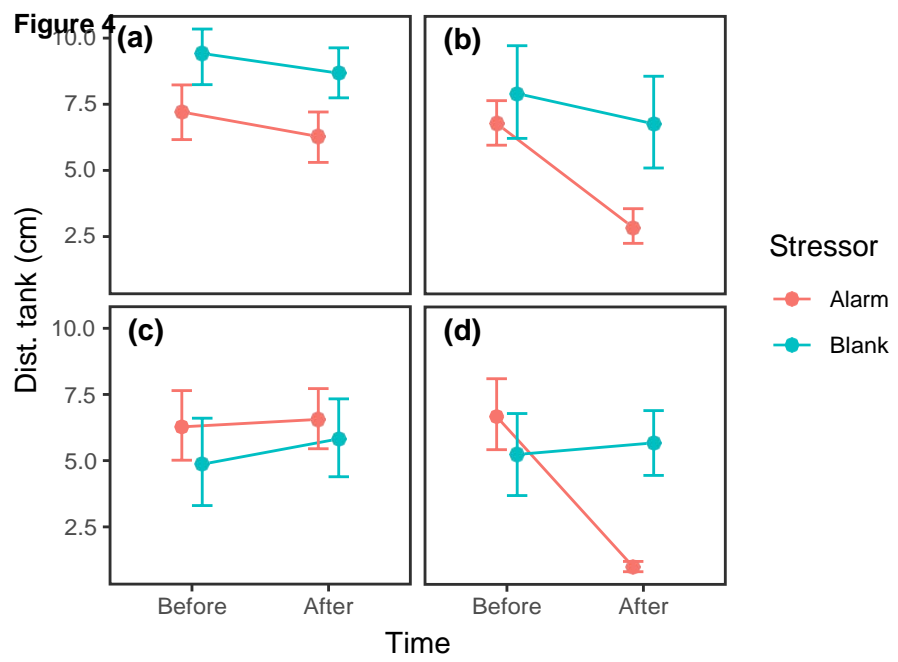


Figure 5

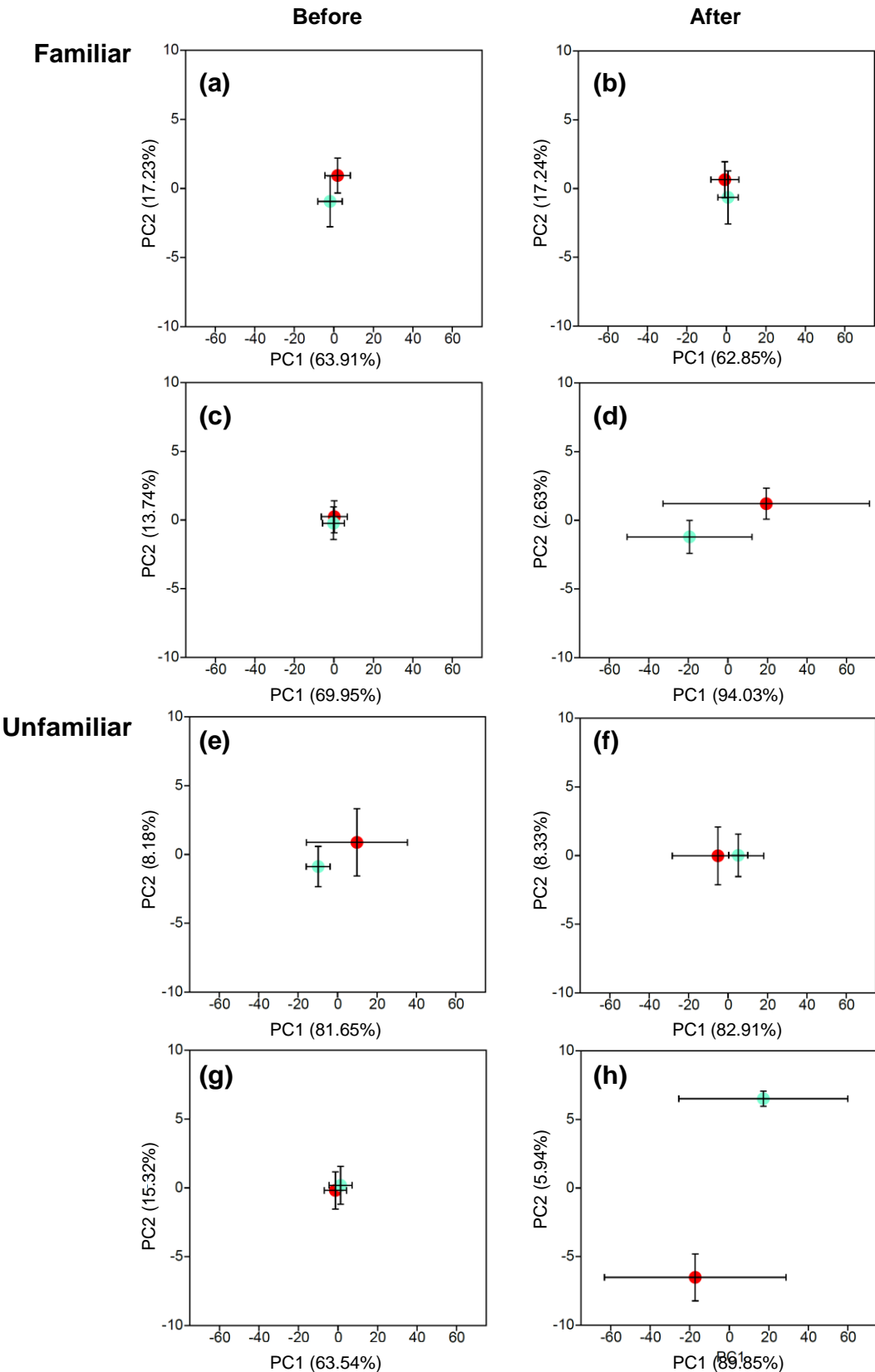
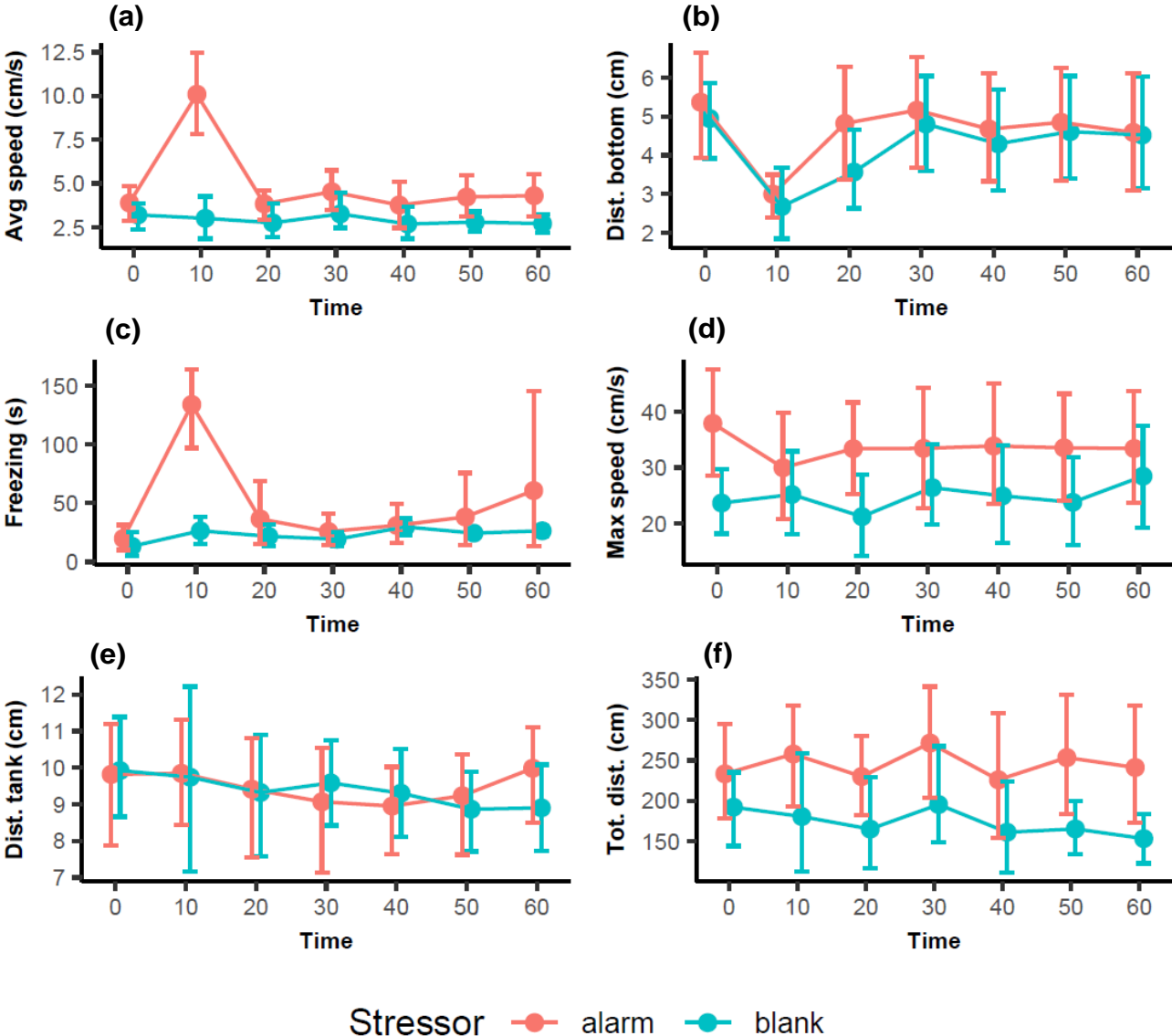


Figure A1



Animal Welfare Note

All experimental procedures were authorized by the Animal Ethics Committee of Universidade Federal do Rio Grande do Norte (permit CEUA 042/2015) and Swansea University's Animal Welfare and Ethical Review Body (permit IP-1516-8).