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Validation of a novel immersive Virtual Reality setup with responses of wild-caught freely-moving coral reef fish

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Abstract

Virtual Reality (VR) enables standardised stimuli to invoke behavioural responses in animals, however, in fish studies VR has been limited to either basic virtual stimulation projected below the bowl for freely-swimming individuals or a simple virtual arena rendered over a large field-of-view for head-restrained individuals. We developed a novel immersive VR setup with real-time rendering of animated 3D scenarios, validated in a proof of concept study on the behaviour of coral reef post-larval fish. Fish use a variety of cues to select a habitat during the recruitment stage, and to recognize conspecifics and predators, but which visual cues are used remains unknown. We measured behavioural responses of groups of five convict surgeonfish (*Acanthurus triostegus*) to simulations of habitats, static or moving shoals of conspecifics, predators, and non-aggressive heterospecifics. Post-larval fish were consistently attracted to virtual corals and conspecifics presented statically, but repulsed by their predators (bluefin jacks, *Caranx melampygus*). When simulated shoals passed nearby repeatedly, they were again attracted by conspecifics showing a tendency to follow the shoal, whereas they moved repeatedly to the back of the passing predator shoal. They also discriminated between species of similar sizes: they were attracted more to conspecifics than butterflyfish (*Forcipiger longirostris*), and repulsed more by predators than parrotfish (*Scarus psittacus*). The quality of visual simulations was high enough to identify between visual cues – size, body shape, colour pattern – used by post-larval fish in species recognition. Despite a tracking technology limited to fish 2D positions in the aquarium, preventing the real-time updating of the rendered viewpoint, we could show that VR and modern tracking technologies offer new possibilities to investigate fish behaviour through the quantitative analysis of their physical reactions to highly-controlled scenarios.

Keywords. Virtual Reality; proof of concept; post-larval; reef fish; visual recognition; conspecific; predator; habitat; recruitment

35 Introduction

36 Animal behaviours such as foraging, habitat choice, predator avoidance, social behaviour and
37 mate choice, are studied for multiple reasons including to understand how they have been shaped by
38 natural selection or how they are impacted by internal and external stimuli. Animal behavioural
39 studies face two challenges: one is related to the subject's own understanding of the task to be
40 performed, and the other is linked to measuring its response (Drew, 2019). One way to solve the first
41 challenge is to use a task that requires a natural reaction to a stimulation presented in an ecological
42 context, and for which the understanding is implicit. Conventional experimental approaches used live
43 stimulus animals or environments, but they suffered from a lack of control and standardization as
44 neither the behaviour of stimulus animals nor the local environment can be completely controlled.
45 For instance, testing behavioural dominance in response to an opponent requires trials with multiple
46 opponents of known dominance and applying a correction factor (Alatalo et al., 1991; Mills et al.,
47 2007). Furthermore, experiments with live stimulus animals often require long methodological
48 preparation, which limits the number of possible manipulations (Neri, 2012). As a result, stimuli have
49 been artificially designed to provide repeatable behavioural observations (Carmichael, 1952) and
50 have evolved from simple pictures and physical models to Virtual Reality (VR) that enables
51 standardised manipulations of stimulus behaviours or environments. The second challenge, response
52 measurement, has been solved using video-based tracking systems of freely moving focal animals,
53 which assumes that the behavioural response lies in the kinematics of the animal, e.g. position,
54 orientation, speed, spatial dispersion. Therefore VR provides a good methodological compromise
55 between a perfectly controlled but not ecologically valid stimulation, and a realistic natural situation
56 with little to no parameter control. Although VR simulators have been widely used over the last 25
57 years to elucidate the perceptual, sensorimotor and cognitive mechanisms underlying human spatial
58 orientation in the environment (e.g. Tarr & Warren, 2002; Vidal et al., 2004; Mossio et al., 2008; Vidal
59 et al., 2009; Vidal & Bühlhoff, 2009), only recently have they been adapted to investigate animal
60 behaviour, ranging from mice to fruit flies and zebrafish (Harvey et al., 2009; Stowers et al., 2017; for
61 a discussion, see Drew, 2019).

62 The first studies of fish visual behaviour that used pre-recorded video stimuli in mating
63 preference tasks date from the end of the nineties, with either manipulated real-videos for
64 (Rosenthal & Evans, 1998) or synthetically generated videos of 3D animated fish (Künzler & Bakker,
65 1998). Ten years later, the same team showed that computer animations of artificial fish allow
66 manipulating movement, body shape and skin-colour to investigate preferences in the cichlid
67 *Pelvicachromis taeniatus* (Baldauf et al., 2009). The survival potential of prey group formation and
68 movement was measured through the response of real predatory bluegill sunfish (*Lepomis*
69 *macrochirus*) to virtual prey projections (Ioannou et al., 2012). Both experiments used one or two

70 screen monitors to display the virtual images. Since then, technology has greatly improved, and VR
71 has led to considerable advances in the understanding of the neural bases of zebrafish visual
72 behaviour (Portugues & Engert, 2009; Dunn et al., 2016), shoaling behaviour and social interactions
73 (Larsch & Baier, 2018; Huang et al., 2020; Harpaz et al., 2021), as well as decision making (Barker &
74 Baier, 2015). However, to date the use of VR to study fish behaviour has been restricted to zebrafish
75 larvae, either moving freely in a bowl responding to basic virtual stimulation projected below such as
76 moving dark disks, a checker board or grass bottom, coupled with infrared 3D tracking (Stowers et
77 al., 2017), or with head-restrained zebrafish responding to conspecifics in a simple virtual arena
78 covering 180° of the visual field and rotating based on tail movements (Huang et al., 2020).
79 Therefore, modern VR technology including realistic rendering and immersion in a large 3D volume
80 has not been adapted to fish studies yet, despite the limitless number of findings that can be
81 generated in terms of quantitative animal behaviour and their ecological implications. Here, we carry
82 out a proof of concept study on a new setup for freely-moving fish within an aquarium with an
83 immersive full-field rendering of virtual scenes using projections not only from below, but on all five
84 sides (except the top). We propose that our VR setup has considerable future potential for all types
85 of behavioural studies on fish species at any stage of their life-cycle. Here, our methodology is tested
86 on the behaviour of post-larval coral reef fish exposed to multiple scenarios during their recruitment.

87 In all marine environments, one of the main mysteries of fish ecology is how larvae recruit
88 onto the relatively rare patches of coastal habitats (for review, see Doherty, 2002; Barth et al., 2015).
89 The life-cycle of most reef fish species starts with a planktonic larval phase, lasting several weeks,
90 followed by recruitment and a sedentary reef phase for juveniles and adults (Leis & McCormick,
91 2002). At the end of the pelagic phase, this recruitment relies on the detection of a suitable habitat
92 which will facilitate larval survival and growth (Doherty, 2002; Lecchini & Galzin, 2003). Simultaneous
93 to that choice, species-specific changes in morphology and physiology, metamorphosis, occur. These
94 changes are linked to ecological shifts with modifications of diet and diel activity period (McCormick
95 et al., 2002; Besson et al., 2017; Holzer et al., 2017) but also of the sensory systems (Lecchini et al.,
96 2005; Tettamanti et al., 2019). Many studies have highlighted the role of sensory and swimming
97 mechanisms in larval habitat selection, most focusing on the role of chemical (e.g. Atema et al., 2002;
98 Vail & McCormick, 2011; Coppock et al., 2013; Lecchini et al., 2013) and acoustic cues (e.g. Tolimieri
99 et al., 2004; Montgomery et al., 2006; Holles et al., 2013; Parmentier et al., 2015). However, vision is
100 a well-developed sense in coral reef fish larvae (Myrberg & Fuiman, 2002), effective to up to 10 m for
101 *Plectropomus leopardus* post-larval fish at recruitment (Leis & Carson-Ewart, 1999). Once larvae are
102 close to a reef, visual cues of conspecifics become important in the recruitment process (Booth,
103 1992; Barth et al., 2015). However, only a few studies have identified the visual parameters used by
104 larvae to recognize conspecifics or predators (e.g. Leis & Carson-Ewart, 1999; Booth, 1992; Huijbers
105 et al., 2012; Lecchini et al., 2014). To test how post-larval fish (i.e., larvae having recruited onto a

106 habitat, with metamorphosis still on-going; see Besson et al., 2020) interpret a range of sensory cues,
107 behavioural experiments can reproduce and control a large variety of combinations of visual cues
108 (Barth et al., 2015). VR is potentially an excellent method to test such behaviours as visual factors
109 such as size, colour patterns, and the behaviour of other individuals can be tightly controlled
110 (Stowers et al., 2017; Brookes et al., 2020). Here, we experimentally validate a new and fully
111 immersive VR setup for fish by testing several presentation scenarios, named trials, in three
112 experiments on post-larval fish during recruitment.

113 We used an innovative immersive VR setup to understand how post-larval convict
114 surgeonfish (*Acanthurus triostegus*) visually recognize a suitable habitat, adult conspecifics and one
115 of their predators (bluefin jacks, *Caranx melampygus*). Our first objective was to experimentally
116 validate the use of simulated 3D models of fishes in a VR setup by confirming that they are realistic
117 enough to cause natural reactions in post-larval fish. Three main experiments were carried out to
118 identify the visual cues used by *A. triostegus* post-larvae to recognize adult conspecifics and a
119 predator. Trials included the presentation of virtual habitats and virtual monospecific fish shoals of
120 conspecifics or predators. The trials with virtual fish species were projected either static (moving in
121 place, Experiments 1 and 2) or dynamic (swimming past on one side of the aquarium, Experiments 2
122 and 3) and a coral reef habitat on all other sides, aimed to virtually reproduce previous studies in
123 which the reaction to either static fish in the corners of the aquarium (Katzir, 1981) or to real fish
124 swimming in a separate adjacent aquarium (Roux et al., 2016) had been studied. We also tested
125 post-larval behavioural responses to two virtual fish shoals swimming past on either side of the
126 aquarium, each with different species, inducing a forced choice (Experiment 3). Furthermore, this
127 experiment was designed to test whether post-larvae can discriminate between the size and species
128 of virtual fish, *i.e.*, if post-larval fish consider larger virtual fish as threats irrespective of species. The
129 advantage of our virtual presentation compared to using live stimuli, is that we were able to measure
130 post-larval fish behaviour rapidly in response to different trials. Our second objective was to validate
131 the automation of post-larval fish position tracking within the test aquarium at high temporal
132 resolution, enabling detailed characterization of their behavioural responses to each scenario.

133 The Immersive VR Setup

134 The experimental setup was composed of three connected modules: the focal aquarium in
135 which post-larvae could swim freely suspended inside the test aquarium, the rendering module
136 which projected the interactive 3D virtual environments depicting a subaquatic natural scene with
137 fishes and corals, and the tracking module which recorded post-larval behaviour in real-time. The
138 software was developed in the lab and the hardware was assembled by Immersion™.

139 Test and focal aquaria

140 The test aquarium was a rectangular prism made of 10 mm-thick Plexiglas plates, with a
141 50×50-cm square bottom and 35 cm-high lateral sides. The external faces of the bottom and lateral
142 sides were covered by a retro projection translucent, but not transparent, film, as such post-larval
143 fish inside the aquarium could not see the room surrounding the setup, except for the ceiling. The
144 aquarium was filled with 78 litres of sea water so that the water surface was aligned with the upper
145 limit of the video projection. The entire setup was mounted on a structure made of 4-cm squared-
146 section aluminium bars (**Figure 1A**). A smaller focal aquarium (dimensions 20×20×20 cm) in which the
147 post-larvae were placed, was attached to the structure using chains and positioned inside the test
148 aquarium (**Figure 1B**). This smaller focal aquarium limited post-larval movement maintaining them
149 within the range where geometrical projection distortion and image corrections were minimal and
150 would not affect post-larval behaviour (see Video-based tracking section).

151 Virtual scene rendering

152 Five video projectors ensured an immersive full-field rendering of the virtual scenes on five
153 sides of the test aquarium (Optoma ML1050ST+, running at 60Hz with a resolution of 1280×800 for
154 the side views and 800×800 for the bottom view). The visible range of post-larval *A. triostegus* likely
155 falls within the human visible range, enabling the use of these video projectors for visual stimulation
156 (Losey et al., 2003). The baseline 3D virtual environment, which was projected on all five aquarium
157 sides at all times, consisted of a sandy bottom at 2 m, with simulated surface ripples and caustics
158 projected on the ground. The virtual viewpoint (position of rendering cameras), which defines the
159 physical-to-virtual relationship, was placed at a depth of 0.75 cm and corresponded to the centre of
160 the test aquarium. Different scenarios (trials) consisting of coral pinnacles (healthy or bleached) as
161 well as animated fish of various species were added to the baseline 3D environment, depending on
162 the simulation, on the left or right side of the aquarium, at a distance of 50 cm. Simulated shoals of
163 five fish could either swim in place on either side or follow a tangent trajectory at a given speed. We
164 used Epic Games Unreal™ Engine 4.23 to render the virtual fish and scene according to the desired

165 test conditions, and to manage the sequencing of trial executions. **Figure 2A** shows how three live
166 post-larvae view a virtual scene of corals and adult surgeonfish from within their focal aquarium.

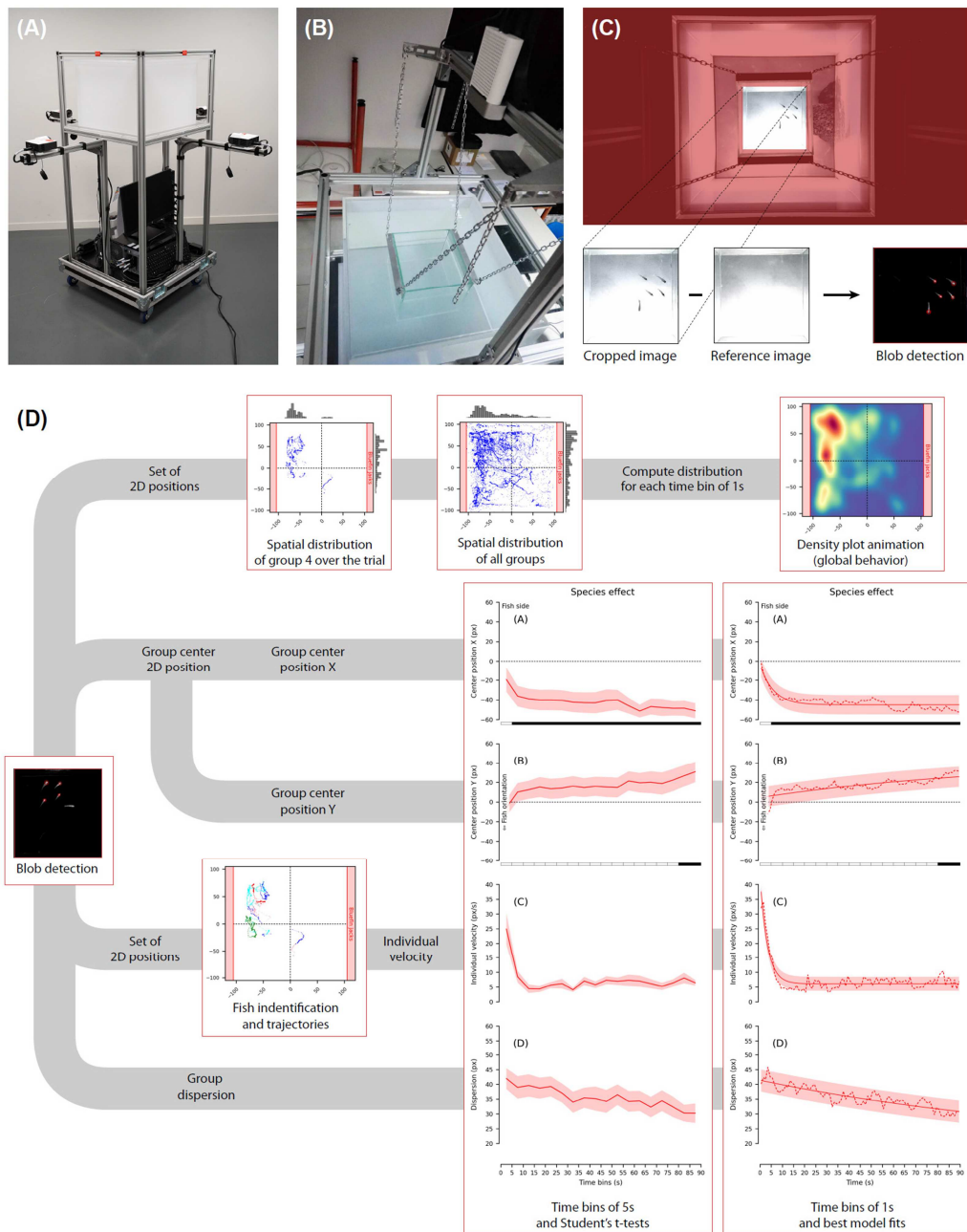
167 **Video-based tracking**

168 A Microsoft™ Kinect Azure depth camera was placed 50 cm above the water surface to
169 continuously monitor post-larval fish behaviour in the focal aquarium (**Figure 1B**). For each trial, a
170 top-view colour video was recorded and processed in real-time at a frequency of 5Hz to compute the
171 2D location of each post-larval fish. In the original design of the setup, we planned to track real-time
172 3D positions of fish with the infra-red (IR) sensor of the Microsoft Azure Kinect depth camera.
173 However, this technology revealed not suitable for underwater tracking due to the large hot-spot
174 created on the surface of the water by the IR grid-spot. For this reason, we switched to the color
175 sensor and could not adjust in real-time the rendering viewpoint according to fish position in the
176 aquarium. The detection pipeline used both the OpenCV image processing library and the Kinect
177 Azure SDK (**Figure 1C**). Images were extracted from the colour video stream and cropped. The
178 constant background was then removed by subtracting a reference image captured before placing
179 fish in the aquarium. The 2D location of each fish was detected using the OpenCV blob detector with
180 parameters adjusted appropriately. The computer performing the virtual rendering also executed
181 this processing pipeline in real-time. The processing had no impact on the frame rate of the visual
182 scene. Tracking performance is provided in Appendix **Fig A1**.

183 **Tracking post-processing**

184 The 2D positions of each post-larval fish in groups of 5 were detected at a sampling rate of
185 5Hz. This automated process is not error-free: in some frames, fewer than five blobs were detected
186 (low signal for smaller juveniles swimming at greater depth or due to overlaps) or more than five
187 blobs (fish reflecting on the Plexiglas when swimming close to the aquarium sides). The tracking post-
188 processing pipeline using Python scripts involved seven steps (**Figure 1D**). First, frames for which only
189 one or two fish were detected were removed to reduce group mean value noise. Second, reflection
190 biases mentioned above were limited by removing the outermost blob when a pair of fish and wall-
191 reflected fish was potentially detected (*i.e.*, when two vertically- or horizontally-aligned blobs were
192 very close to each other and to the edge of the aquarium). Third, as the automated detection cannot
193 identify and track individual fish from one frame to the next (identification problem), a minimal
194 heuristic distance was used to track the fish. This distance was only used when the position change
195 of a blob from one frame to the next was minimal (with five individuals, there were 120 possible
196 combinations across each pair of frames). This provided the (partial) trajectories and instantaneous
197 velocities of each individual. Transiently missing or extra blobs could produce artificial jumps to
198 distant locations; above a given distance threshold between frames (corresponding to 15cm/s) these

199 jumps were ignored in the computation of individual instantaneous velocities. The trajectory
200 reconstructions are plotted with coloured lines for each tracked fish in a (X, Y) square graph
201 representing the aquarium. Fourth, for each validated frame, the X and Y position of the centre of
202 the group, the dispersion relative to the centre (mean distance to the centre), and average individual
203 velocities were computed. To account for the fact that the stimulations were presented either on the
204 right or the left, the sign of the X coordinates was inverted when the stimulation was presented on
205 the left. Fifth, in order to visualize the raw results for each experiment and each tested condition, 2D
206 scatterplots with all valid fish positions from all groups, and normalized X- and Y-position distribution
207 histograms (with 5-pixel large bins) are used (representative scatterplot from Experiment 1 is in **Fig**
208 **A3**). Sixth, in order to visualize the average behavioural responses across time for each experiment
209 and tested condition, heatmaps of fish position density in the aquarium were plotted in successive 1-
210 second intervals. Lastly, the time-series for each of the four behavioural measures (group centre X-
211 and Y-position, dispersion and individual velocities) were binned into 5-second intervals for the
212 statistical tests, and into 1-second intervals to find the best behavioural model fit.



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Figure 1. (A) View of the experimental setup as delivered by Immersion™ with the test aquarium (dimensions 50×50×35 cm) and 5 video projectors (four lateral sides plus bottom). (B) View of the smaller focal aquarium (20×20×20 cm) placed inside the test aquarium in order to limit the displacement range of fish, and of the Microsoft™ Kinect Azure camera recording the behavioural responses. (C) Illustration of the detection pipeline starting from the camera view to blob detection executed after cropping and subtracting the reference image, followed by the tracking post-processing pipeline (D). Detected 2D positions were used to characterize post-larvae behavioural responses. For each condition, the overall behaviour obtained combining data from all tested groups is visible in the animation of the 2D density heat maps generated every second. The X and Y positions of the centre of the groups, the individual velocities and the dispersions were averaged across time-bins of 5s to perform statistical comparisons (Student's t-tests), and across time-bins of 1s to fit the behavioural models (regressions). Individual velocities are extracted from the reconstruction of the trajectories, which was based on the identification of each fish from one frame to another (see text).

227 **Experimental Validation**

228 Three main experiments were carried out to understand how post-larval convict surgeonfish
229 (*Acanthurus triostegus*) visually recognize a suitable habitat, adult conspecifics and one of their
230 predators (bluefin jacks, *Caranx melampygus*). Our first objective was to experimentally validate the
231 use of simulated 3D models of fishes in a VR setup by confirming that they are realistic enough to
232 cause natural reactions in post-larval fish.

233 **Methods**

234 **Specimen collection**

235 Over 200 post-larval *Acanthurus triostegus* (TL = 2.55-2.75 cm) were captured using hand
236 nets at night, shortly after entering the north-eastern reef crest of Moorea, French Polynesia
237 (17°29'52.19"S, 149°45'13.55"W). Individual *A. triostegus* had not yet acquired skin stripes which
238 only form after recruitment, therefore they were still undergoing metamorphosis, and were
239 considered 'post-larvae' (Besson et al., 2020).

240 **Ethical note**

241 Ethical approval for the study was granted from The Animal Ethics Committee, Centre
242 National de la Recherche Scientifique (permit number 006725). This study also complies with the
243 rules defined by the Direction de l'Environnement de la Polynésie Française (DIREN) regarding
244 experiments on coral fish in aquaria. After captured, post-larval fish were placed in acclimatization
245 aquaria at CRIOBE for 36 hours, in groups of 40 maximum, filled with UV-sterilized and filtered (10-
246 µm filter) seawater maintained at 28.5 °C, under a 12:12 LD cycle. Stress was minimized during
247 transport using occluded small aquaria. Once the experiment was over, animals were returned to
248 their natural habitat.

249 **Experimental protocol**

250 The behavioural response to the multiple trials – different habitats or fish shoals – was
251 assessed for groups of five post-larval fish placed together in the aquarium. A neutral, baseline 3D
252 environment (sandy bottom with animated caustics) was displayed throughout the experimental
253 sessions on all five sides of the aquarium. Virtual fish or coral pinnacles appeared and disappeared at
254 specific times and in specific virtual locations depending on the trial. Each experiment started with a
255 4 min habituation period in the baseline environment, followed by trials each lasting 90 seconds
256 (experiment 1 and 2) or 60 seconds (experiment 3). To minimize interference, the baseline
257 environment was also displayed for 2 min between trials. For each experiment, the presentation
258 order of trials was randomised and balanced to avoid order effects and allow for statistical
259 comparisons between pairs of conditions. To exclude possible side biases from the random sand

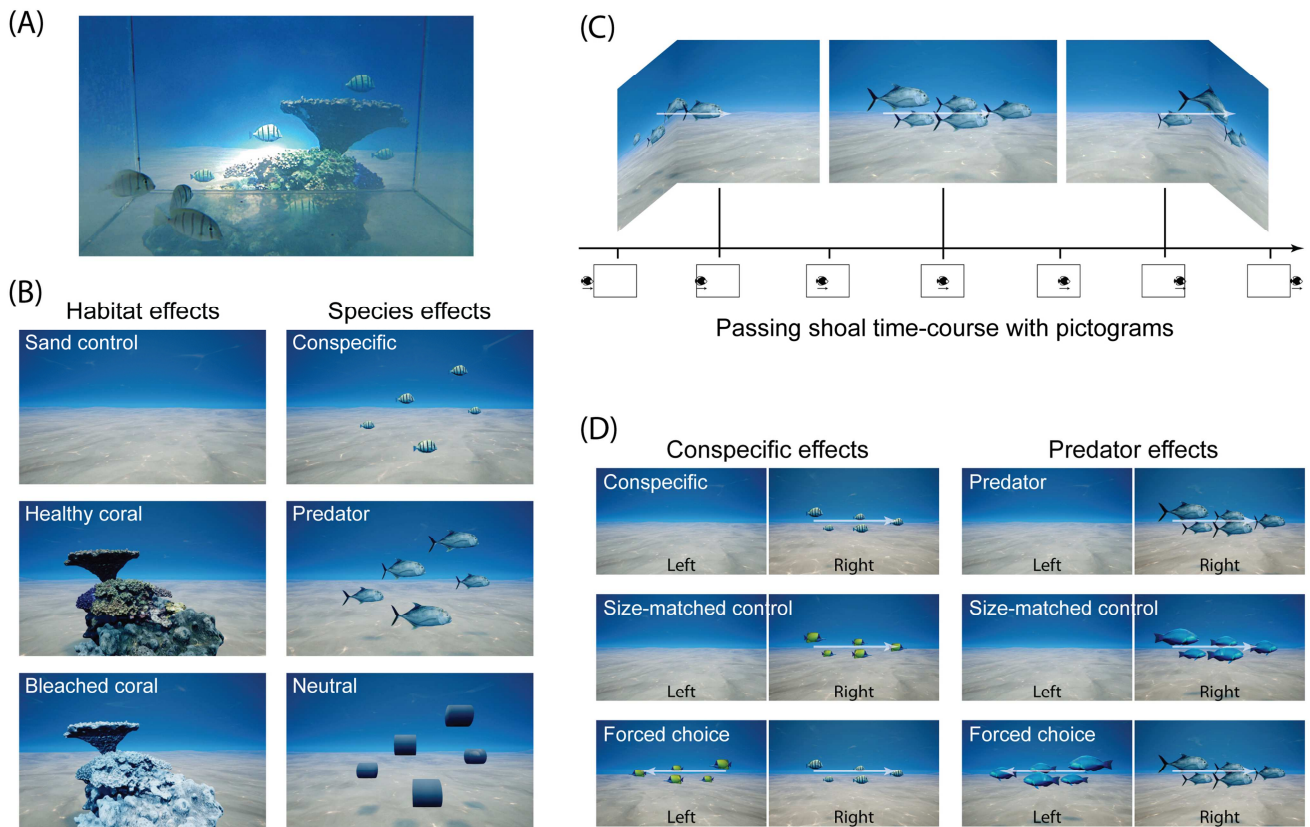
260 texture pattern or from the room's ceiling and lightning, the stimulation side was randomly balanced
261 between the left and right of the aquarium. Lastly, after every half-day the aquarium was emptied,
262 washed with freshwater, and refilled and the focal aquarium was oxygenated between replicates.

263 **Experiment 1. Effects of static presentation of habitat and fish**

264 Groups of five post-larval fish were presented with six trials: three virtual habitats and three
265 virtual fish or neutral shapes on only one side of the aquarium (randomised) each for 90 seconds
266 (**Figure 2B**). Virtual simulations rendered the post-larval fish at a depth of 1.25 m. The three virtual
267 habitats tested were: Sand control (only the sandy baseline environment); Healthy coral (a pinnacle
268 with healthy tabular and branched corals); and Bleached coral (the same pinnacle, but all corals were
269 bleached). The three virtual fish species consisted of shoals of five virtual fish swimming in place in
270 the same pattern and position: Conspecifics (five adult convict surgeonfish, *Acanthurus triostegus*);
271 Predators (five bluefin jacks, *Caranx melampygus*); and Neutral (five large untextured cylinders). The
272 individual positions of five post-larval fish were constantly tracked during the six successive trials
273 presented in the following order: Sand control (1st); Healthy or Bleached coral (randomly 2nd or 3rd);
274 Conspecific, Predator or Neutral (randomly 4th, 5th, or 6th). Sixty post-larval fish were tested in 12
275 groups of 5 fish, and for each the total experimental duration was approximately 22 minutes.

276 **Experiment 2. Effects of static versus dynamic presentation of fish**

277 In Experiment 2, with 8 trials, behavioural responses to the static presentation of shoals of
278 five virtual fish was compared with behavioural responses to a more realistic dynamic situation in
279 which shoals of five fish appeared, swam past the post-larval fish in a non-aggressive manner and
280 disappeared. Three virtual fish shoal trials were projected: one static shoal swimming in place (as in
281 Experiment 1) for 90 seconds; one shoal swimming by during the first 30 seconds, then disappearing,
282 followed by 60 seconds of the baseline sand environment; or three successive shoals of five fish
283 swimming nearby and disappearing, each over 30 seconds. In each, virtual shoals swam for 30
284 seconds at 15 cm/s (slow pace) along a virtual line placed 115 cm from the centre of the test
285 aquarium, covering a total distance of 4.5 m (**Figure 2C**). The virtual fish shoals were either
286 surgeonfish conspecifics or bluefin jack predators and were presented in 8 successive trials in the
287 following order: Sand control (1st); Conspecific/Predator static, 1 pass, or 3 passes (randomly
288 presented in 2nd, 3rd, or 4th position); sand control (5th); Conspecific/Predator static, 1 pass, or 3
289 passes (randomly presented in 6th, 7th, or 8th position). The order in which the fish shoal was
290 presented (conspecifics or predators first) was varied and the side of the aquarium on which the
291 virtual fish were presented was randomised. Sixty post-larval fish were tested in 12 groups of 5 post-
292 larvae, and for each the total experimental duration was approximately 26 minutes.



293
 294 **Figure 2.** Illustration of the stimuli used in the experiments. **(A)** Rendered scene with virtual corals and
 295 adult surgeonfish as seen from inside the focal aquarium by real larvae. **(B)** Experiment 1. Habitat
 296 effects (left): Sand control, Healthy or Bleached coral (pinnacles). Species effects (right): Conspecific
 297 (adult surgeonfish, *Acanthurus triostegus*), Predator (bluefin jacks, *Caranx melampygus*), Neutral
 298 (untextured cylinders). The fish stimuli were presented in shoals of five individuals swimming in place,
 299 centred at a virtual location corresponding to 25 cm behind one of the sides of the test aquarium. **(C)**
 300 Dynamic presentation of a virtual fish shoal swimming past the post-larval fish (either 1 pass or 3 passes
 301 for Experiment 2 and only 1 pass for Experiment 3). The virtual fish species were either conspecifics or
 302 predators (as shown here). Shoals swam along a 4.5-m straight line for 30 seconds at 15 cm/s (slow
 303 pace). **(D)** Experiment 3. Three conditions were tested to examine the response of the post-larval fish to
 304 conspecifics (left): Conspecific alone, Conspecific-sized control alone (butterflyfish, *Forcipiger*
 305 *longirostris*), and Forced choice of conspecific vs. conspecific-sized control. Three conditions were also
 306 tested to examine the response of the post-larval fish to predators (right): Predator alone, predator-
 307 sized Control alone (parrotfish, *Scarus psittacus*), and Forced choice of predator vs. predator-sized
 308 control. Similarly to Experiment 2, the virtual shoals swam along a 6-m straight line for 40 seconds at
 309 15 cm/s (slow pace).

310 **Experiment 3. Effect of size-controlled dynamic presentation of fish**

311 In Experiment 3, behavioural responses to a dynamically swimming shoal of conspecifics or
 312 predators, was compared with behavioural responses to size-matched heterospecifics. Six virtual fish
 313 shoal trials were projected (**Figure 2D**): surgeonfish conspecifics on one side; conspecific-sized
 314 control fish on one side (butterflyfish, *Forcipiger longirostris*); conspecifics and conspecific-sized

315 controls on opposite sides (two-alternative choice); bluefin jack predators on one side; predator-
316 sized control fish on one side (parrotfish, *Scarus psittacus*); predators and predator-sized controls on
317 opposite sides (two-alternative choice). Changes in post-larval fish positions from before to after a
318 virtual shoal swam by were identified. All virtual fish swam at 15 cm/s (slow pace) for 40 seconds
319 along a virtual line placed at 115 cm from the centre of the aquarium (total distance travelled: 6 m).
320 The order in which fish shoal types was presented, conspecifics or predators, was balanced between
321 groups, however, the two-alternative choice was always presented after the single-choice trials. The
322 order of single-choice trials was also balanced. In all conditions, the stimulus was presented over 40
323 seconds, and post-larval position recording started 10 seconds before and ended 10 seconds after
324 the stimulus (total duration of 60 seconds). Eighty post-larval fish were tested in 16 groups of 5 fish,
325 and for each the total experimental duration was approximately 26 minutes.

326 **Data analysis**

327 For all experiments, tracking data was sampled at 5Hz, during 90-second trials for Experiment
328 1 and 2, and 60-second trials for Experiment 3 (see **Figure 1C**). Representative trajectories of post-
329 larval groups are available in Appendix **Fig A2**, **Fig A7** and **Fig A11** for Experiment 1, 2 and 3,
330 respectively. Animated heatmaps of fish position density in the aquarium for all conditions are
331 available in Appendix **Video A4**, **Video A8** and **Video A12** for Experiment 1, 2 and 3, respectively.
332 Note that for all 2D plots, data is organized so that the simulation is always presented on the right
333 side, except for the forced-choice conditions of Experiment 3, for which the stimulation is presented
334 on both left and right sides. General repeated-measures ANOVAs with trial and 5-second time-bin as
335 main factors were generated using the four behavioural measures (group centre X- and Y-position,
336 dispersion and individual velocity). Comparisons between relevant trials (paired Student t-tests) and
337 the deviation from zero of the group's X and Y positions (Student t-tests against a single value of 0)
338 were conducted for each time bin. The alpha value for significance was adjusted using Bonferroni's
339 correction for multiple comparisons on a single dataset and visualized in the plots using different
340 grey levels (white for $P > 0.05$ and from light grey for $P < 0.05/1$ to black for $P < 0.05/n_{\text{Tests}}$ with $n_{\text{Tests}} = 3$
341 for either the 3 paired comparisons or the 3 single-value comparisons). Plots displaying the
342 timeseries of the four behavioural measures (binned in 5-second intervals) and the results from the
343 statistical comparisons are provided in the Appendix **Fig A5**, **Fig A9** and **Fig A13** for Experiment 1, 2
344 and 3, respectively.

345 **Behavioural model fit**

346 To characterize the temporal aspect of post-larval fish behavioural responses, we designed
347 several models taking into account the distance of the post-larval fish to the simulated shoals. The
348 position of the virtual simulations was sustained and constant throughout the trials in Experiment 1
349 and in the static trials of Experiment 2, but the virtual shoals moved in the dynamic conditions of

350 Experiments 2 and 3. Because of this major difference between the static and dynamic conditions,
351 we used a different set of possible behavioural models for either type of trial to measure behaviour
352 (centre position, individual velocity and dispersion). For the static conditions, we tested three simple
353 ecologically relevant models (linear, quadratic and exponential) and for dynamic conditions, we
354 added a periodic component to capture the cyclic variations of the stimulation (**Table 1**). The average
355 behavioural measures obtained for the tested groups (n=12 or n=16), binned in 1-second intervals,
356 were fitted using each of the three models. The fit quality was estimated with the root mean square
357 error (RMSE) between the average data points and the model predictions. In order to avoid data
358 over-fitting, we used a limited number of models and selected the best model based on the trade-off
359 between fit quality (RMSE) and the number of parameters. Linear models (for static conditions) and
360 linear periodic models (for dynamic conditions) have one parameter fewer than the quadratic and
361 exponential models. They were favoured when the RMSE difference with the other models was
362 below 5% (e.g., if the linear and quadratic fits had RMSEs of 10 and 9.6 resp., the linear model was
363 selected). Lastly, in order to check the validity of each fit, the obtained RMSE was compared to the
364 RMSE distribution obtained by applying, for the given condition and measure, the same fitting
365 procedure but with scrambled time-bins a thousand times. Two quality criteria were used: if the
366 obtained RMSE was lower than 0.8 times the mean RMSE distribution, and below the lower 1%
367 confidence interval bound of the distribution, the fit was considered valid (good signal-to-ratio level).
368 The results are summarized in plots displaying, for each condition, the time-series of each measure in
369 bins of 1 second, the best behavioural model fit, and the outcome of the statistical comparisons. For
370 each condition, the selected model and adjusted parameters are detailed in Appendix **Table A6**,
371 **Table A10** and **Table A14** for Experiment 1, 2 and 3.

	Name	Equation	Description and meaning of the parameters
Static conditions	Linear	$a \cdot t + b$	Linear models capture responses that change constantly in time. These drifts can be observed in slow responses to a potential danger. a : linear term (drift speed) b : offset (start position)
	Quadratic	$a \cdot t^2 + b \cdot t + c$	Quadratic models capture responses that change constantly in time (1 st order) and that have a reversal (2 nd order). Reversal are observed of after a loss of interest when attracted by the stimulus ($a < 0$ if in right side) or after habituation to a fearful stimulus ($a > 0$). a : quadratic term (reversal) b : linear term (drift speed) c : offset (start position)
	Exponential	$l - (l - s) \cdot \exp(-k \cdot t)$	Exponential attenuation models capture natural decaying phenomena within a limited spatial or temporal range. These can be observed in quick escape response to a fearful stimulation. l : limit of the attenuation (reached when $t \rightarrow \infty$) s : offset (starting position) k : attenuation factor (positive value, higher for faster decays)
Dynamic conditions	Linear periodic	$a \cdot t + b + \frac{1}{2}A \cdot \cos(2\pi \cdot (t - t_0) / T)$	Linear models combined with a periodic component capture linear drift responses to a cyclic stimulation. Periodic component parameters: A : amplitude of the oscillations (positive value) t_0 : phase of the oscillations (peak time, within [0 s, T]) T : period (fixed parameter set to 30 s and 40 s for Experiment 2 and 3)
	Quadratic periodic	$a \cdot t^2 + b \cdot t + c + \frac{1}{2}A \cdot \cos(2\pi \cdot (t - t_0) / T)$	Quadratic models combined with a periodic component capture 1 st and 2 nd order responses to a cyclic stimulation.
	Exponential periodic	$l - (l - s) \cdot \exp(-k \cdot t) + \frac{1}{2}A \cdot \cos(2\pi \cdot (t - t_0) / T)$	Exponential attenuation models combined with a periodic component capture naturally decaying periodic responses to a cyclic stimulation.

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Table 1. The sets of behavioural models fitted for the static and dynamic trials. For each model, the equation, the description of the behaviour that it captures, and the meaning of its parameters are detailed.

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Results

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Experiment 1. Effects of static presentation of habitat and fish

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The behavioural responses to the trials were assessed for groups of five post-larval *A. triostegus* placed together in the aquarium. All trials were tested on all groups of post-larval fish. Typical individual trajectories of post-larval fish in response to each of the six trials are shown in Appendix **Fig A2**, scatterplots with all positions occupied by all post-larval fish in Appendix **Fig A3**, and animated heatmaps with the presence density at each successive 1-second intervals in Appendix **Video A4**.

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Habitat effect

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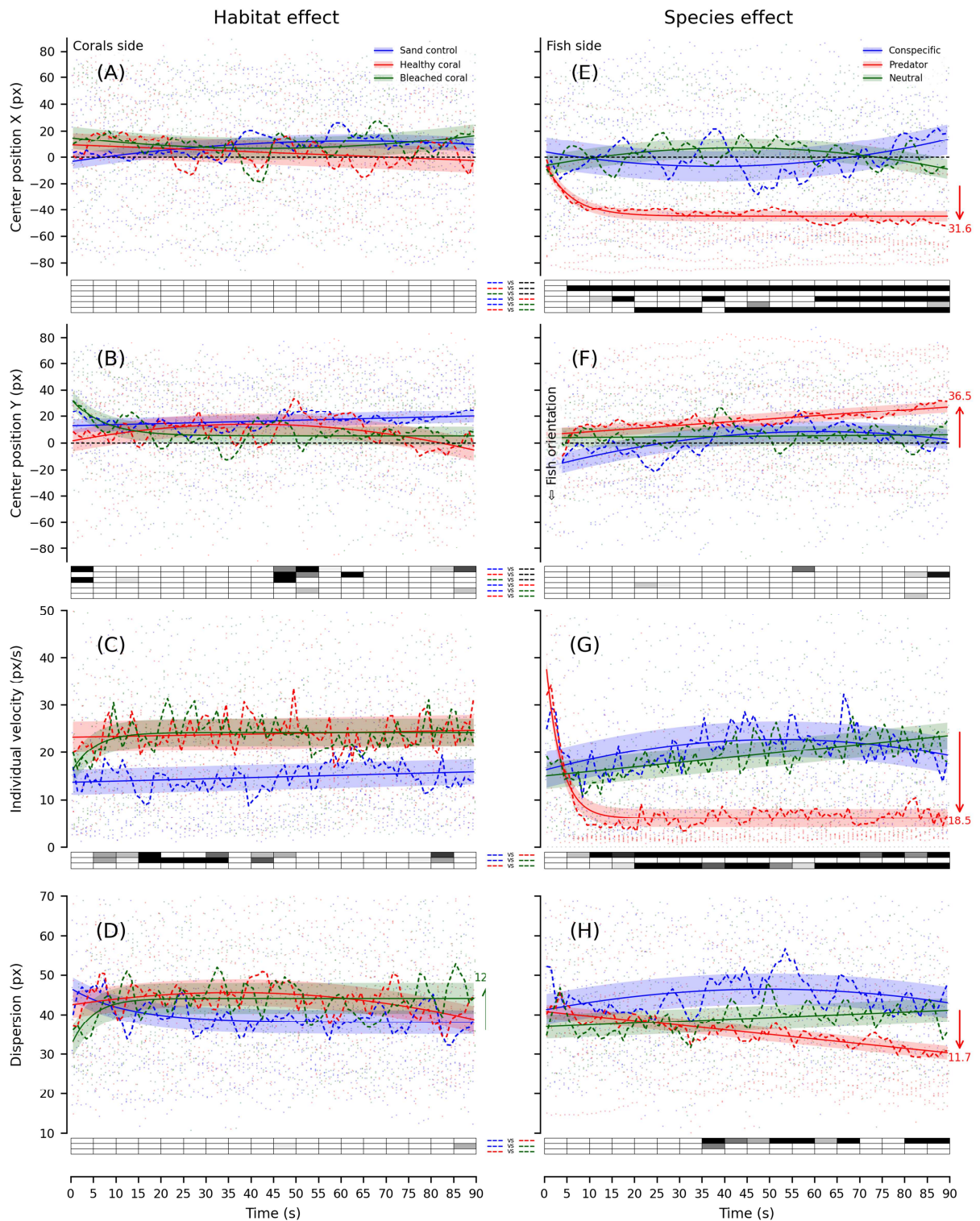
Post-larval fish reactions were similar across habitat types (Sand control, Healthy and Bleached coral). Post-larval fish group centre's X- and Y-positions were not significantly different between trials either over the whole test period or for most 5-second intervals (lack of significance in boxes below plots in **Figure 3A** and **B**). Apart from a small positive bias (16.5 px) in the Y-position for the Sand control (entire range: $t(11)=2.39$, $P<0.04$), possibly related to the initial location in which fish were placed in the aquarium, the X- and Y-positions were not biased to either side of the

391 aquarium (**Figure 3A and B**). The level of noise did not allow for good quality fits of linear, quadratic
392 or exponential models (**Table A6**), but the best fit models largely overlapped, confirming non-
393 significant differences in response behaviours across habitat types. In contrast, there was a
394 significant effect of habitat on individual velocities ($F_{2,22}=5.5$, $P<0.015$, $\eta_G^2=0.12$), with post-larval fish
395 moving faster (more activity) when presented with Healthy (23.8 px/s, $t(11)=2.26$, $P<0.05$) and
396 Bleached (23.7 px/s, $t(11)=3.11$, $P<0.01$) corals compared to no corals in the Sand control (14.7 px/s)
397 over the whole time range and during some of the 5-second intervals (shaded boxes below plot in
398 **Figure 3C**). No significant difference in group dispersion – either with statistics or model fitting – was
399 observed across habitat types (**Figure 3D**).

400 **Species effect**

401 Post-larval fish reactions did not differ when presented with static shoals of conspecifics
402 (surgeonfish) and neutral cylinders, but were significantly different when presented with predators
403 (jack fish). Type of fish shoal impacted the post-larval fish group centre's X-position (main effect,
404 $F_{2,22}=8.3$, $P<0.002$, $\eta_G^2=0.27$), which was significantly lower with Predators (–42.3 px) than with
405 Conspecifics (–1.5 px, $t(11)=3.14$, $P<0.01$) or Neutral cylinders (1.8 px, $t(11)=3.7$, $P<0.005$) across the
406 entire time-range and for most 5-second intervals (shaded boxes below plot in **Figure 3E**). Post-larval
407 fish swam away from the virtual predators: they moved 31.6 px from the first to the last time-bin
408 ($t(11)=2.83$, $P<0.02$), mostly at the beginning of the trial (exponential model with k parameter of
409 0.17 s^{-1}). On the other hand, with virtual conspecifics post-larval fish hit the sides of the aquarium
410 near the conspecifics more often compared to neutral cylinders or sand control. There was no global
411 effect of the type of virtual species on post-larval fish Y-positions across the entire time-range and for
412 any interval (**Figure 3F**). However, post-larval fish moved slowly toward the upper-left quadrant of
413 the aquarium – the opposite side to the stimulation – and moved to the back of the virtual shoal
414 (linear model with a slope of $a=0.238\text{ px/s}$) moving 36.5 px ($t(11)=3.09$, $P<0.01$) behind the virtual
415 predators, reducing dispersion (Appendix **Video A4**). Type of fish shoal had a significant effect on
416 individual velocities ($F(2,22)=11.08$, $P<0.001$, $\eta_G^2=0.20$), with lower speeds with Predators (7.3 px/s)
417 than with Conspecifics (20.9 px/s, $t(11)=4.09$, $P<0.002$) and Neutral cylinders (19.3 px/s, $t(11)=3.23$,
418 $P<0.01$) over the entire time range, and most 5-second intervals (**Figure 3G**). Furthermore, with
419 predators individual velocities rapidly decreased by 18.5 px/s over the first ten seconds ($t(11)=3.47$,
420 $P<0.005$), remaining at 6.1 px/s until the end of the trial (exponential model with a very high k
421 parameter value of 0.30 s^{-1}). Movement, as well as space occupied in the aquarium, were similar
422 when presented with surgeonfish, cylinders or only sand (Appendix **Fig A3**). The effect of fish shoal
423 type on group dispersion was nearly significant ($F(2,22)=2.89$, $P=0.077$, $\eta_G^2=0.071$), due to less
424 dispersion with Predators (35.5 px) than with Conspecifics (44.9 px) across the entire time range
425 ($t(11)=3.37$, $P<0.007$), and in most intervals after 35 seconds (**Figure 3H**). With predators, group
426 dispersion decreased slowly and progressively during the trial (linear model with $a=-0.12\text{ px/s}$), by

427 11.7 px ($t(11)=2.90$, $P<0.015$): post-larval fish tended to gather after detecting a threat. The best-
428 fitting models of the behavioural reactions to virtual bluefin jacks highlight natural repulsion from a
429 fear-invoking stimulation: the exponential models captured quick responses in a limited space/time
430 range (X-position and individual velocity), whereas the linear models captured slow drifting
431 responses (Y-position and dispersion). In general, the four different behavioural indicators (X and Y
432 position, individual velocity, and dispersion) were not different between conspecifics and neutral
433 cylinders, and despite high variability between individuals resulting in poor quality fits, models also
434 mostly overlapped (**Table A6**). However, when presented with predators, the quality of model fitting
435 for all behavioural measures was excellent i.e. post-larvae showed homogeneous behaviours within
436 each group as well as across groups.



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Figure 3. Experiment 1 group time series, with best model fits and statistics. X- and Y-positions of the group centre, individual velocity, and group dispersion for Habitat (A-D) and Species trials (E-H). Coloured dashed lines show the average at each time-point of n=12 groups (Habitat trials: Sand control in blue, Healthy coral in red, Bleached coral in green; Species trials: Conspecific in blue, Predator in red, Neutral in green). Coloured lines show the best fitting model for the corresponding trials and error stripes show the RMSE. For each 5-second time-bin, average performances were compared either between each trial or to zero with paired and single-value Student t-tests. Significance levels are

445 provided in the boxes below the plots (ranging from light grey for $P < 0.05/1$ to black for $P < 0.05/n_{\text{Tests}}$
446 using Bonferroni's correction for $n_{\text{Tests}}=3$; white for $P > 0.05$).

447 Experiment 2. Effects of static versus dynamic presentation of fish

448 Typical individual trajectories of post-larval *A. triostegus* in response to each of the eight
449 trials are shown in Appendix **Fig A7**, and animated heatmaps with the presence density at each
450 successive 1-second interval in Appendix **Video A8**. Sand control trials were presented before the
451 conspecific and predator trials to provide acclimatization periods. Since no qualitative or statistical
452 differences in behaviour were observed, these results are not reported here.

453 Conspecific effect

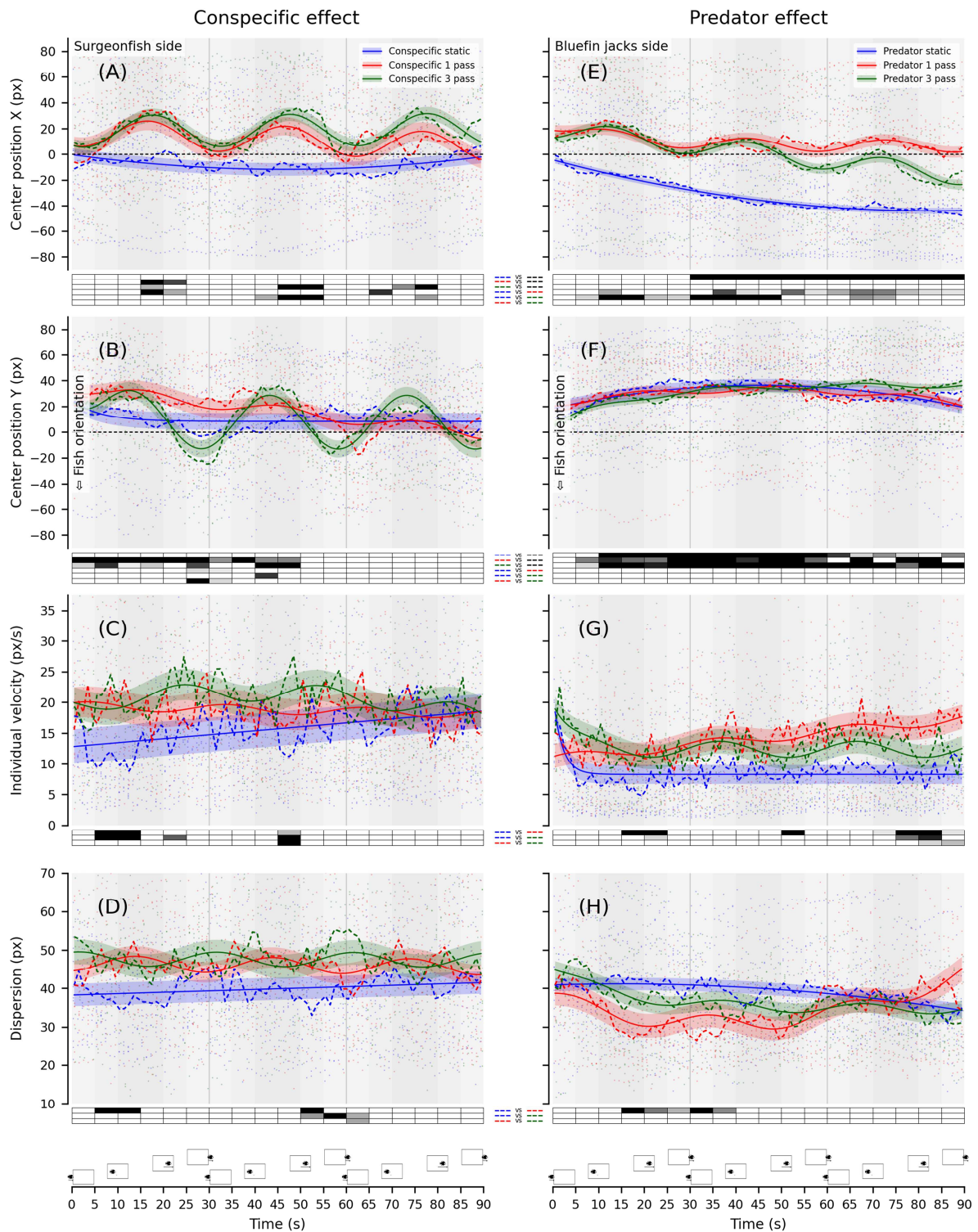
454 Post-larval fish reactions were different when presented with either static (swimming in
455 place) or dynamic (1- or 3-passes) surgeonfish. In the static trial, groups came close to the
456 stimulation (hitting the aquarium side), similarly to Experiment 1, and the group centre's X-position
457 did not deviate significantly from zero. The best-fitting model quadratic only of average fit but its
458 positive a parameter suggested an habituation to an initially slightly fear-invoking stimulus (**Figure**
459 **4A** and **Table A10**). In contrast, in the dynamic 1- or 3-pass trials, post-larval fish joined and moved
460 with the virtual surgeonfish shoal as it travelled along the aquarium side (oscillating X- and Y-
461 positions, **Figure 4A** and **B**). When the shoal passed 3 times, post-larval fish kept repeating the same
462 behaviour periodically, without any noticeable attenuation indicating no loss of interest (**Video A8**).
463 The linear periodic model was the best fit, as it captured the post-larval fish cyclic response, with a
464 phase t_0 of approximately 17 s for both 1- and 3-pass tests, and an amplitude A of 21.6 px (1 pass)
465 and 24.7 px (3 passes). The linear component had an offset b of about 14 px for both pass types and
466 a slope a of -0.13 px/s (1 pass) and null (3 passes). Oscillations in the X-position showed a significant
467 deviation from zero toward the stimulation side when the shoal was passing (from 15 to 25 s for both
468 1- and 3-passes, and from 45 to 55 and 75 to 85 s for 3-passes). For the static trial the group centre's
469 Y-position did not significantly deviate from zero and the best fit was exponential, but of poor quality
470 (**Figure 4B**). For the 1-pass trial, the linear periodic model had the best fit, with a similar phase
471 ($t_0=15.6$ s) but with a much smaller amplitude ($A=8.5$ px) than for the X-position. The number of post-
472 larval fish that followed the single shoal passage (**Video A8**) was too limited to produce a clear
473 trough, resulting in less vertical motion. Moreover, the linear decay and loss of synchronization in the
474 model after the first cycle was due to the absence of stimulation after 30 seconds. For the 3-pass
475 trial, the best fitting model was exponential and periodic, with a strong attenuation factor of
476 $k=0.177$ s $^{-1}$, rapid oscillations around $l=7.7$ px, a peak at $t_0=13.2$ s, and large amplitudes ($A=41.8$ px)
477 (**Figure 4B**). The first two oscillations showed significant deviations from zero on peaks and troughs.
478 The X- and Y-oscillations were synchronized and phase-locked with the three virtual surgeonfish
479 shoal passes: vertical motion widely overlapped with the shoal's linear displacement along the

480 aquarium side, and the horizontal motion corresponded to the movement of post-larvae closer to
481 the shoal at each pass. The swimming kinematic analyses of swimming provide evidence of cohesive
482 group behaviour, in which larvae naturally recognize and follow their conspecifics. Individual
483 velocities were rapidly significantly lower (at 12 px/s then increased linearly, with a fitted slope of
484 0.064 px/s) with static compared to dynamic virtual conspecifics (**Figure 4C**). This is consistent with
485 an initially slightly fearful response. In dynamic trials, individual velocities oscillated around 20 px/s
486 and decreased to similar values as in static trials towards the end of the trial, but noise was too high
487 for the periodic models to reach a good fit (**Table A10**). Group dispersion tended to be lower for the
488 static than dynamic trials, and fit was poor (**Figure 4D**).

489 **Predator effect**

490 Post-larval fish reacted very differently in response to static compared to dynamic bluefin
491 jack shoals. Static presentation produced the same reaction as in Experiment 1: movement to the
492 opposing side and to the back of the virtual shoal with reduced group dispersion. In the 1- or 3-pass
493 dynamic tests, the density patterns of heatmaps were bimodal, indicative of two reaction types: one
494 similar to that of the static trial, while the second was an asynchronized back-and-forth movement in
495 the X-dimension with the post-larvae moving behind the virtual predators after each pass. The
496 balance between these two reactions varied, with the proportion of static-like behaviour increasing
497 through time. In the static condition, the gradual decrease in X-position (significant deviation from
498 zero in all time-bins beyond 30 seconds) was best fitted with a quadratic model ($a=0.006 \text{ px/s}^2$, $b=-$
499 0.96 px/s and $c=-4.3 \text{ px}$), with parameters for the climax and reversal outside of the trial time-range
500 (**Figure 4E** and **Table A10**). The best fitting model for the 3-pass condition was linear periodic, with
501 peaks occurring earlier than with conspecifics ($t_0=12.8 \text{ s}$), a limited amplitude ($A=14.6 \text{ px}$), and a
502 linear drift (slope $a=-0.404$) of post-larval fish that gradually moved to the opposing aquarium side.
503 For the 1-pass trial, the response to the first pass overlapped with that of the 3-pass ($t_0=12.7 \text{ s}$ and
504 $A=9.5 \text{ px}$) and after this pass, oscillations and drifting were exponentially attenuated. None of the X-
505 positions in the dynamic scenario deviated significantly from zero, but post-larval fish were
506 significantly closer to the stimulation side than in the static trial over multiple time bins. The Y-
507 position was very similar in the three trials (**Figure 4F**): post-larvae moved rapidly behind bluefin jack
508 shoals whether swimming in place (quadratic model) or passing nearby once (quadratic periodic) or
509 three times (exponential periodic). Despite good quality fits, these models largely overlapped and
510 deviated significantly from the midline zero in almost every time bin after the first 5 seconds. At the
511 end of the trials, post-larvae tended to move back towards the midline in the static condition
512 (habituation) and in the 1-pass condition (no more stimulation). Consistent with Experiment 1,
513 individual velocity in the static trial decreased rapidly from 21.3 px/s ($k=0.50 \text{ s}^{-1}$) to 8.2 px/s, which
514 matched an exponential attenuation model (**Figure 4G**). In the 3-pass condition, post-larval velocities
515 fit an exponential periodic model, decreasing rapidly from 19.4 px/s ($k=0.27 \text{ s}^{-1}$) to oscillate around

516 12.4 px/s at small amplitude ($A=2.6$ px). Furthermore, velocities decreased when post-larval fish
517 noticed predators at each new pass (peak phases locked to $t_0=6.5$ s). In the 1-pass trial, oscillations
518 had a lower amplitude ($A=1.5$ px), and individual velocities increased linearly after the shoal pass,
519 with underlying oscillations starting once speeds exceeded 10.9 px/s. The only difference between
520 trials occurred towards the end of the static and 1-pass trials, when post-larval fish swam faster in
521 the absence of stimulation. Group dispersion was lower for the dynamic 1-pass than for static trials
522 (15-40 seconds; **Figure 4H**), increasing again in the absence of stimulation (quadratic periodic model).
523 In the static trial, dispersion decreased slowly (quadratic model) but less than in Experiment 1,
524 possibly due to the influence of dynamic trials used in the design. In the 3-pass trial, dispersion was
525 exponentially attenuated, starting at 45 px dropping almost linearly to 34 px (low k factor of 0.06 s⁻¹).



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Figure 4. Experiment 2 group time series, with best model fits and statistics. X- and Y-positions of the group centre, individual velocity, and group dispersion for Conspecific (A-D) and Predator trials (E-H). Coloured dashed-lines show the average at each time-point of n=12 groups (Conspecifics and Predator trials: static in blue, 1 pass in red, 3 pass in green). Coloured lines show the best fitting model for the corresponding trials and error stripes show the RMSE. For each 5-second time-bin, average performances were compared either between each trial or to zero with paired and single-value Student t-tests. Significance level is provided in the boxes below the plots (ranging from light grey for $P < 0.05/1$ to black for $P < 0.05/n_{\text{Tests}}$ using Bonferroni's correction for $n_{\text{Tests}}=3$; white for $P > 0.05$). Shaded areas

535 highlight the stimuli critical periods based on the on-going distance of the virtual shoals in the 3-pass
536 conditions (30-second periodicity) illustrated by the fish positions relative to the side screen shown
537 above the time axis.

538 **Experiment 3. Effect of size-controlled dynamic presentation of fish**

539 Typical individual trajectories of post-larval *A. triostegus* in response to each of the six trials
540 are shown in Appendix **Fig A11**, and animated heatmaps with the presence density at each
541 successive 1-second interval in Appendix **Video A12**. Contrary to previous experiments, model fitting
542 was limited to the interval of 10-50 s, during which the stimulus was visible: the cycle duration of the
543 periodic component was set to 40 s.

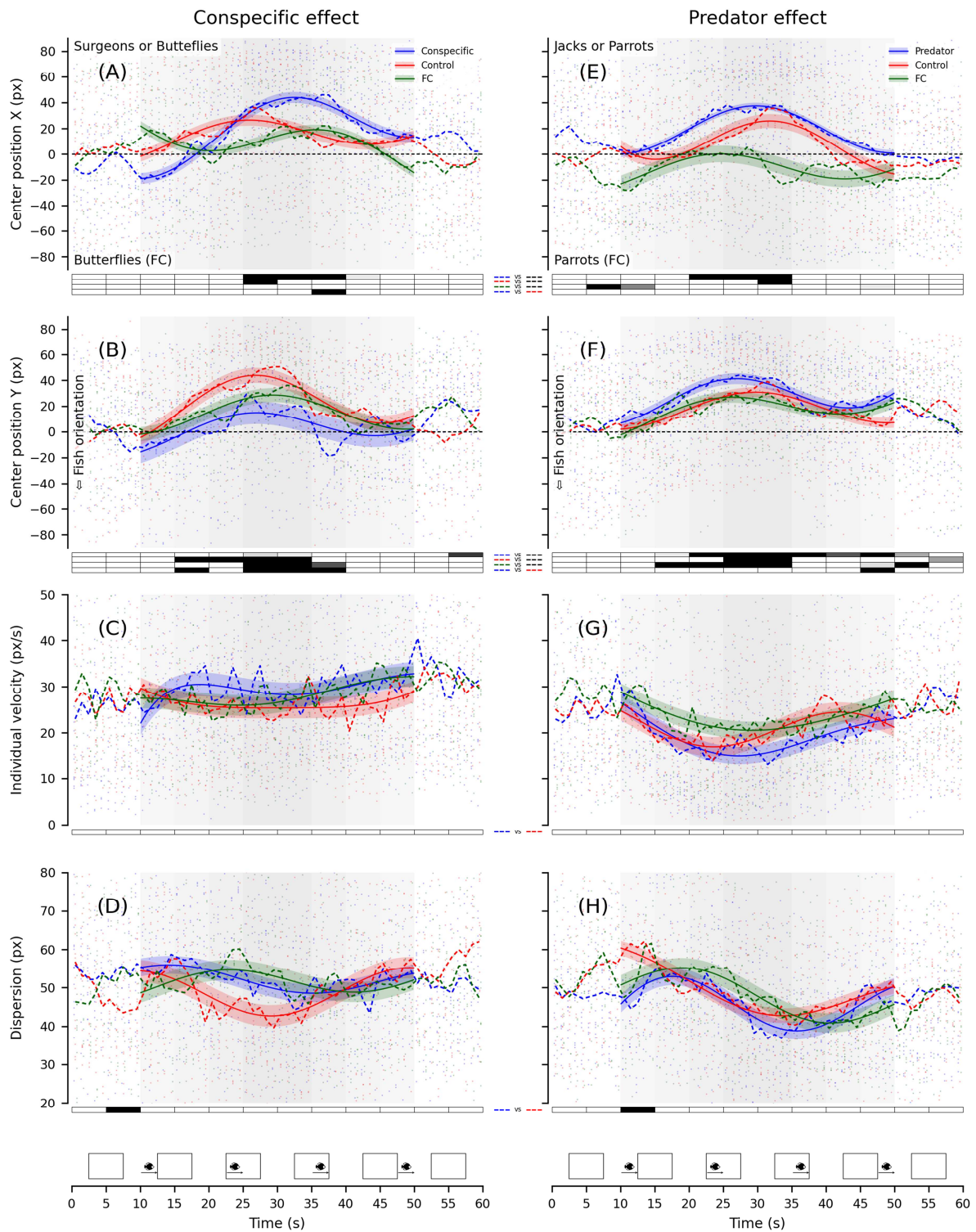
544 **Conspecific effect**

545 Post-larval fish reactions to virtual conspecific surgeonfish or control size-matched
546 butterflyfish passing nearby were mostly similar, yet with subtle differences. Post-larval fish showed
547 the same behaviour as in Experiment 2: they moved to the upper-right quadrant, but fewer post-
548 larval fish followed the virtual shoal of non-aggressive size-matched controls along the Y-axis
549 compared to conspecifics. Furthermore, dispersion increased faster in the Control compared to
550 Conspecific trials. When both conspecifics and controls were presented (Forced choice), most post-
551 larval fish gathered on the conspecifics side and followed the shoal. The best model for all conditions
552 was linear periodic, except when stated otherwise (**Table A14**). The linear components of the change
553 in X-positions in the Conspecific and Control trials were positive, suggesting an increase in interest
554 over the trial (**Figure 5A**). However, the oscillation during the single pass had a larger amplitude and
555 peaked a few seconds later for surgeonfish ($A=45.7$ px and $t_0=31$ s) than control butterflyfish
556 ($A=26.3$ px and $t_0=24$ s). The time interval during which the X-position deviated significantly from the
557 midline was wider for conspecifics (more than three time-bins compared to a single one) and there
558 was a significant difference in X-position between the Control and Conspecific trials during the 35-
559 40 s interval. The linear component of the Y-position in both the Conspecific and Control trials had
560 the same positive slope ($a\approx 0.4$ px/s) and oscillation phase ($t_0\approx 26$ s), but the amplitude in the
561 Conspecific trial was smaller ($A=26$ px against 46 px, **Figure 5B**). The Y-position did not deviate from
562 the midline, and was significantly higher in the Control trial. When both fish shoals were presented
563 (Forced choice), post-larval fish responses were less intense than when presented with only one
564 stimulation but still showed the same two reaction types, *i.e.*, following the conspecifics or remaining
565 in the upper-left quadrant. The X-position was positive and peaked at $t_0=38$ s, but deviation from
566 midline was not significant due to a strong linear decrease ($a=-0.92$). Altogether, these results
567 indicate that post-larval fish were more attracted to and followed conspecific surgeonfish, whilst
568 spending more time in the upper-right quadrant with similarly-sized butterflyfish. Individual velocity
569 measurements were noisy but remained relatively constant throughout the three trials (at 30 px/s),

570 with fits that mostly overlapped (**Figure 5C**). The linear component of the good quality fit group
571 dispersion model was similar in all three trials, with a nearly null slope (constant) at $b \approx 50$ px (**Figure**
572 **5D**). There were differences in the periodic component, with oscillations of smaller amplitude and
573 troughing 6s later with Conspecifics ($A=6.6$) compared to Controls ($A=12.2$ px), confirming that
574 dispersion increased faster after the butterflyfish shoal passed than after the conspecifics.

575 **Predator effect**

576 Heatmaps of post-larval fish position density highlight subtle differences in their reaction to
577 dynamic virtual predators versus control parrotfish. With predators, post-larval fish rapidly moved to
578 the upper-right quadrant and remained at the back of the shoal. In contrast, with non-aggressive
579 size-matched controls, post-larval fish also gathered in the upper-right quadrant, but some rapidly
580 started to follow the shoal: group dispersion increased more and earlier than with predators. When
581 both predators and non-aggressive controls were presented (Forced choice), most post-larval fish
582 gathered in the upper-left quadrant with controls, with only a few staying on the predator side or
583 following either predators or controls. The periodic component of the X-position for the Predator
584 trial was smaller and peaked earlier ($A=36.9$ px and $t_0=29.7$ s) than for Controls ($A=41.6$ px and
585 $t_0=32$ s, **Table A14**): with bluefin jacks, post-larval fish moved more to the stimulation side and earlier
586 (20-35 s) than with parrotfish (30-35 s, **Figure 5E**). When both fish shoals were presented (Forced
587 choice), X-position of post-larval fish fluctuated around the midline ($b=-19.4$ px and $A=25.1$ px), with
588 a non-significant deviation towards parrotfish. The periodic component of the Y-position peaked for
589 all conditions at similar times ($t_0=24.2-29.6$ s), with post-larval fish moving rapidly behind fish shoals
590 (**Figure 5F**), especially with predators. However, the combined periodic amplitude and quadratic
591 component in the predator trial, and the linear component in the forced choice task, indicated that,
592 while post-larval fish tended to follow parrotfish and were distributed centrally along the Y-axis at
593 the end of the stimulation (deviating from midline only in the 25-35 s interval), they remained in the
594 upper-half of the aquarium behind virtual predators (Y-position always positive throughout the
595 stimulation). Across the three trials, individual velocities decreased rapidly to about 20 px/s, with
596 troughs at similar times ($t_0=22.8-25.8$ s). However, model fit of velocity had a quadratic component
597 highlighting that velocity decreased over a longer period of time in the Predator compared to Control
598 trial before increasing again (**Figure 5G; Table A14**). Dispersion decreased earlier than velocities and
599 increased slower with Predators (trough at 35.5 s) than Controls (trough at 31.9 s) although not
600 statistically significant (**Figure 5H**).



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Figure 5. Experiment 3 group time series, with best model fits and statistics. X- and Y-positions of the group centre, individual velocity, and group dispersion for Conspecific (**A-D**) and Predator trials (**E-H**). Coloured dashed-lines show the average at each time-point of $n=16$ groups (Conspecific trials: Conspecific in blue, Control in red, Forced-choice in green; Predator trials: Predator in blue, Coloured lines show the best fitting model for the corresponding trials and error stripes show the RMSE. For each 5-second time-bin, average performances were compared either between each trial or to zero with paired and single-value Student t-tests. Significance level is provided in the boxes below the plots (ranging from light grey for $P<0.05/1$ to black for $P<0.05/n_{\text{Tests}}$ using Bonferroni's correction for $n_{\text{Tests}}=3$;

610 white for $P > 0.05$). Shaded areas highlight the stimuli critical period based on the on-going distance of
611 the passing virtual shoals illustrated by the fish positions relative to the side screen shown above the
612 time axis.

613 Discussion

614 Virtual corals – healthy or bleached – displayed on one aquarium side had a significant effect
615 on post-larval fish behaviour (**Figure 3A-D**). Post-larval fish swimming speed increased and the time
616 spent close to the aquarium side displaying the simulated corals increased compared to sand
617 controls with no corals. Interestingly, both healthy and bleached corals attracted post-larval fish. As
618 herbivores, corals are not part of the diet of *A. triostegus*, so this attraction may be due to
619 anfractuositities in the coral framework, potentially providing shelter and/or a hiding place (Leis &
620 McCormick, 2002). In addition, displays of virtual fish highlighted clear and distinct behavioural
621 responses in post-larval *A. triostegus* (**Figure 3E-H**). When presented with five virtual 3D adult
622 conspecifics, post-larval fish were attracted to them within ten to twenty seconds. In contrast, when
623 presented with their natural predators, five virtual bluefin jacks (*C. melampygus*) (Siu et al., 2017),
624 post-larval fish moved to the opposite side of the aquarium with a rapidly decreasing velocity, and
625 then slowly gathered behind the virtual predator shoal. These contrasting responses highlight the
626 ability of these post-larval coral reef fish to visually identify virtual conspecifics and predators and
627 respond differently, with either attraction (conspecifics) or repulsion and/or avoidance (predators),
628 consistent with expectations from natural behaviours. Furthermore, the movement of post-larval fish
629 behind the predator shoal not only confirms their recognition of the virtual predator but also of
630 virtual body features (distinguishing head from tail and positioning themselves accordingly).

631 The presentation of static or dynamically moving conspecifics led to contrasted reactions in
632 post-larval fish (**Figure 4A-D**). The sudden appearance of a static conspecific shoal startled post-larval
633 fish, causing them to move away or remain stationary, but then they showed attraction, even hitting
634 the side of the aquarium where conspecifics were displayed. However, when the virtual conspecific
635 shoals appeared 2.5 meters away and slowly got closer, no startle responses were observed, rather
636 post-larval fish followed the virtual shoals along the side of the aquarium, even after three identical
637 passes. This dynamic scenario is particularly interesting as it highlights the post-larval fish's natural
638 cohesive group behaviour, even with virtual conspecifics. Such shoaling and cohesive behaviours
639 with virtual conspecifics have previously been observed in other experiments (e.g., in adult zebrafish
640 *Danio rerio*, Saverino & Gerlai, 2008). In contrast, when static predators suddenly appeared on one
641 aquarium side, post-larval fish slowly moved to the opposite side of the aquarium and gathered
642 behind the virtual predator shoal (**Figure 4E-H**). When virtual bluefin jacks swam by, the reaction of
643 the post-larval fish was less clear across individuals, but mostly consisted of an overall decrease in
644 swimming speed and/or synchronized movements to hide behind the moving virtual predators.

645 When virtual bluefin jacks passed three times, the static-like behaviour became more frequent and
646 individual swimming speeds decreased with each new pass.

647 We then tested whether these post-larval fish responses were simply due to the size of
648 virtual fish – repulsion to larger fish and attraction to smaller fish – or whether post-larval fish are
649 able to differentiate between virtual fish species. We found subtle yet noticeable differences in post-
650 larval fish reactions to virtual shoals of same-sized non-aggressive controls, butterflyfish *Forcipiger*
651 *longirostris* and parrotfish *Scarus psittacus*, compared to conspecifics (surgeonfish) and predators
652 (bluefin jacks) respectively. Post-larval fish showed stronger and longer-lasting attraction towards
653 conspecifics with clear shoal-following behaviour and less dispersion, compared to butterflyfish and a
654 preference for conspecifics when both were presented simultaneously (forced choice, **Figure 5A-D**).
655 When presented with predators, the post-larval fish's back-and-forth escape reaction was triggered
656 earlier, post-larval fish gathered behind the virtual fish for longer and displayed periods with reduced
657 velocity and dispersion at each shoal passage compared to parrotfish (**Figure 5E-H**). In the forced
658 choice task, post-larval fish had a tendency to prefer size-matched parrotfish rather than predators.
659 Altogether, these findings provide evidence that, based on visual cues alone, post-larval fish can
660 distinguish a conspecific from an equally small but non-aggressive fish species, and a predator from
661 an equally large but non-aggressive fish species, suggesting that post-larval surgeonfish respond
662 behaviourally not only to size, but also to the shape and colour pattern of virtual fish.

663 Over the last two decades, a range of studies have demonstrated that post-larval fish possess
664 developed behavioural and sensory abilities, rejecting the traditional paradigm that they are passive
665 plankton (Leis, 2015; Beldade et al., 2012, 2016). In particular, post-larval fish survival depends on
666 their ability to correctly evaluate sensory cues and select appropriate behavioural responses, *e.g.*,
667 move toward conspecifics or flee predators (Barth et al., 2015). Among the sensory cues used by
668 coral reef post-larval fish, visual cues are the most discussed, but their importance is the least
669 understood (Lecchini et al., 2014). The visual abilities of post-larval fish increase during their pelagic
670 life to reach a maximum near the onset of metamorphosis (Lara, 2001). Our experiments yield
671 convincing and quantified behavioural results highlighting the role of visual cues in post-larval fishes
672 at a stage in their development during which they seek a suitable recruitment habitat. In particular,
673 post-larval *A. triostegus* showed marked attraction towards corals, potentially due to the complex 3D
674 structures with which they are associated. Furthermore, post-larval fish used visual cues to
675 discriminate between conspecifics and predators and tailor their movement and behaviour to either
676 follow their conspecifics (in ecological settings, this could be used to find a suitable settlement
677 habitat) or avoid predation.

678 **VR for Behavioural Studies**

679 **Proof of concept**

680 The experimental program shows that coral reef post-larval convict surgeonfish (*Acanthurus*
681 *triostegus*) visually recognize possible hiding places, adult conspecifics, and bluefin jack (*Caranx*
682 *melampygus*) predators, presented virtually. These results provide a successful proof of concept of
683 our innovative virtual reality setup with automated tracking of fish responses to simulated 3D models
684 of habitats and fish shoals. We overcame two technical challenges: we simulated 3D models of fishes
685 and habitats that were realistic enough to elicit natural reactions in post-larval coral reef fish, and we
686 detected their individual positions in the aquarium in real-time during the trials using a video-based
687 tracking system. The detailed behavioural reactions of *A. triostegus* post-larvae to conspecifics and
688 predators through several relevant kinematic measures such as their position in the aquarium,
689 individual swimming speed, and group dispersion and the multi-factorial analysis of these measures
690 enabled us to disentangle responses that could yield very similar results in less controlled
691 experimental designs (sand vs. corals, parrotfish vs. bluefin jacks, butterflyfish vs. surgeonfish).

692 **Current limitations and solutions**

693 In this project, we relied on the Microsoft Kinect Azure depth camera, an IR-based
694 technology that proved unsuitable for underwater tracking, forcing us to only use its embedded
695 colour camera. Tracking with a single camera limits the detection of fish in the aquarium to 2D
696 horizontal positions, so we blocked the real-time updating of the viewpoint (see Video-based
697 tracking section). Rendering of the animated scene was however geometrically correct at the centre,
698 and distortions remained limited within the focal aquaria. We believe the behavioural response to
699 the presentation of virtual coral reef habitats would be stronger with the real-time updating of the
700 viewpoint, by enhancing the fish sensation of physically reaching the virtual anfractuositities. To
701 overcome these limitations in our future projects, we recently updated the VR setup to include a pair
702 of high-resolution colour cameras, and developed a complex underwater calibration procedure.
703 Today we can triangulate the detected fish positions from each view, in order to compute in real-
704 time a fish's 3D position and update the rendering viewpoint in the virtual scene accordingly.
705 Coupling tracking data with the simulation also allows placing the desired test stimulus in the line of
706 sight of focal individuals.

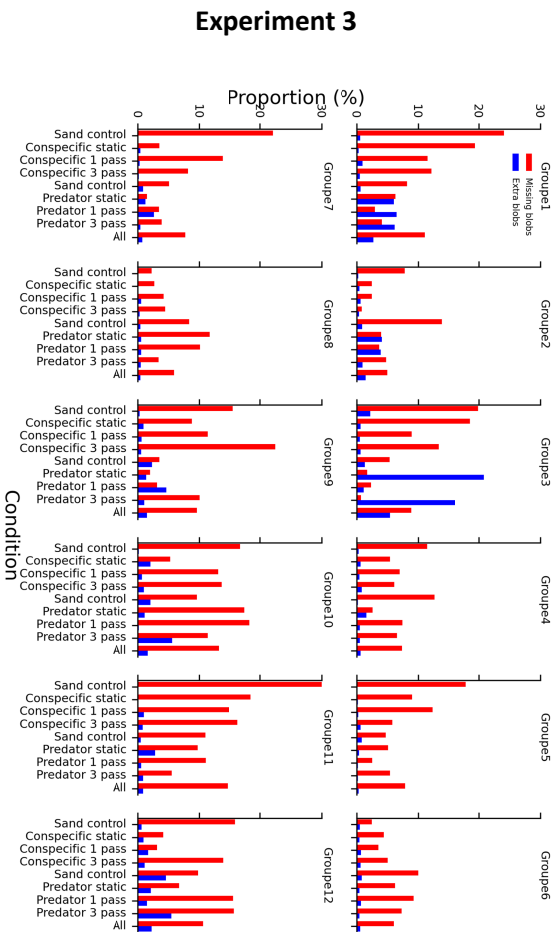
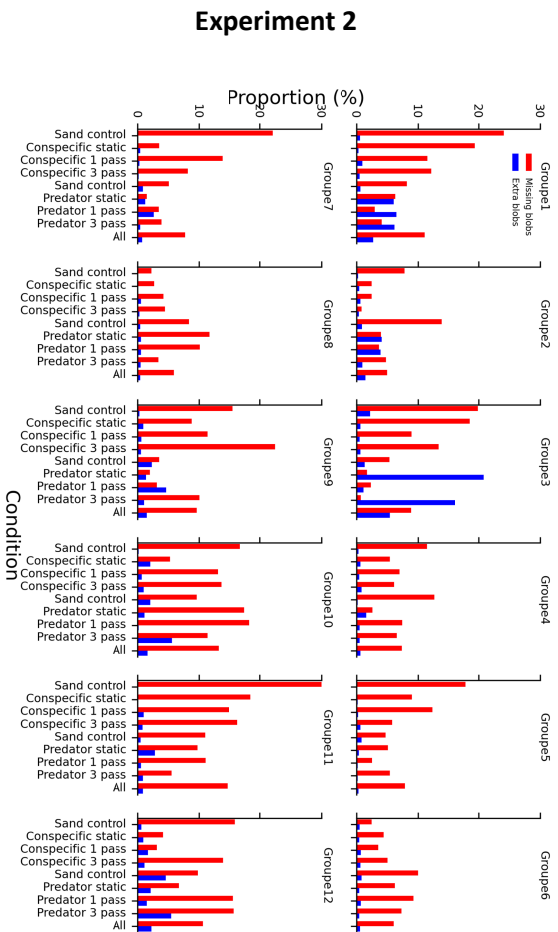
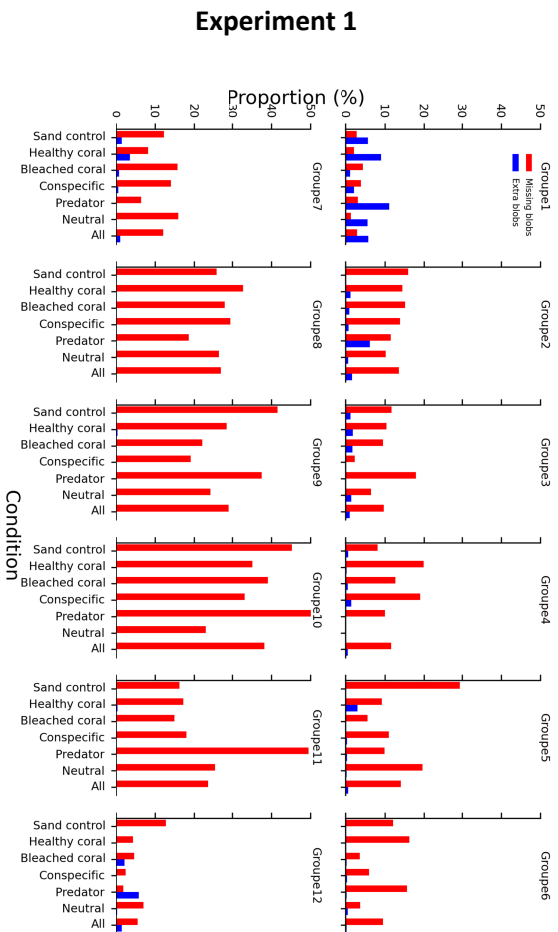
707 **Perspectives**

708 Numerous other simulations could be used with such experimental setup to test a wide
709 variety of parameters on coral reef fishes at different stages of development, or test coral reef fishes

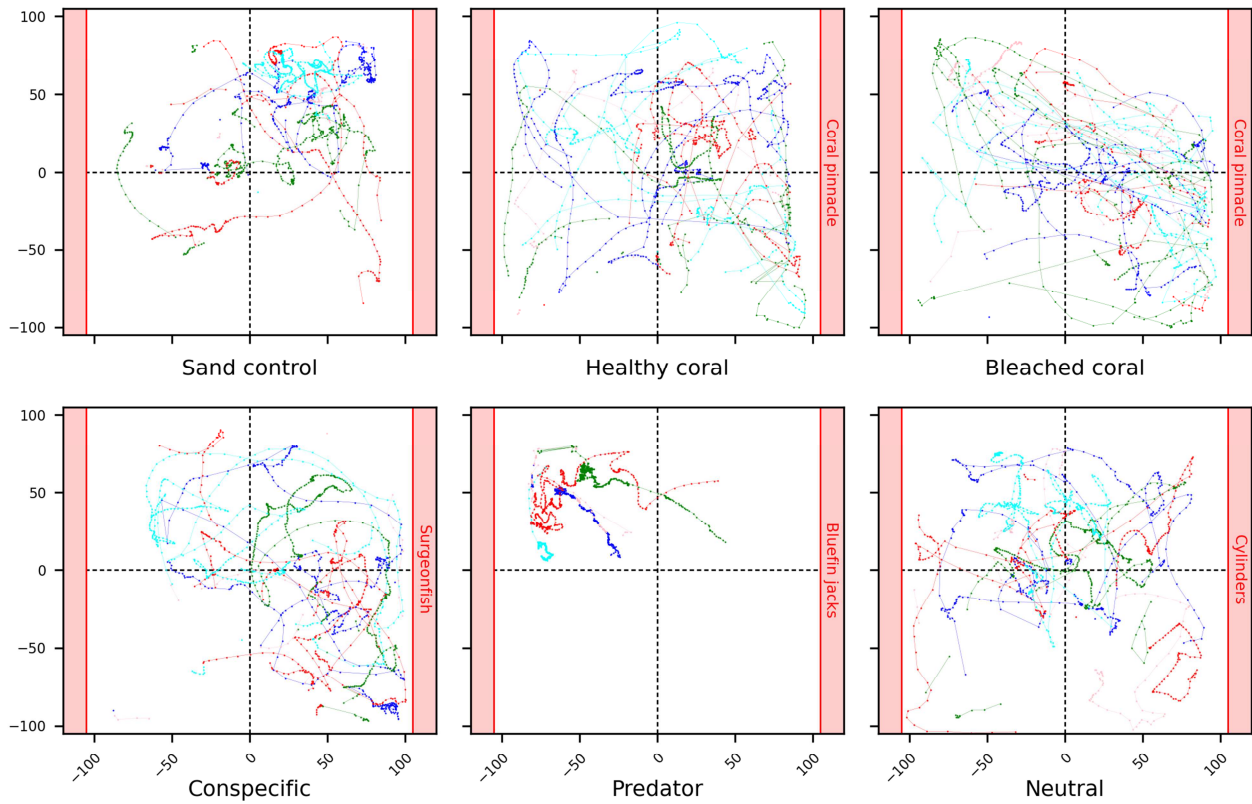
710 reared in different conditions or exposed to different stresses prior to the visual cue experiment, and
711 quantitatively characterize the impact on their behaviour. The use of VR offers countless new
712 research opportunities including to better understand behaviours of coral reef fish in response to
713 local and global changes (Beldade et al., 2017; Mills et al., 2020; Nedelec et al., 2017; Schligler et al.,
714 2021) and how they impact the role of vision in habitat selection at recruitment. To understand the
715 mechanisms involved in the visual recognition of conspecifics or predators, our VR set-up can be
716 used to manipulate as many visual factors as needed, including size, colour patterns, fin
717 arrangements, but also the behaviour of other individuals (aggressive, curious, social, fleeing). Even if
718 some experimental protocols in aquaria partially control for these factors and facilitate observations
719 compared to *in situ* protocols (e.g. Katzir, 1981; Roux et al., 2016; Besson et al., 2017), the
720 acclimation time required to perform experiments limits the range of possible manipulations. In
721 addition, when real fish are used as stimuli (e.g. Lecchini et al., 2014), the experiment cannot be
722 reproduced multiple times in a reliably comparable manner, as the movement of the stimulus fish
723 cannot be controlled. This study demonstrates that VR, even with a static rendering viewpoint,
724 provides an excellent methodology to apply perfectly-controlled virtual stimuli, which post-larval fish
725 are able to correctly identify. VR can reproduce fairly realistic natural situations that can yield robust
726 statistical results and allow for highly-precise quantifications of post-larval behaviour in response to
727 highly diverse scenarios. In addition, video-based modern tracking technologies have recently
728 emerged in the field of animal behaviour and neuroscience, offering the possibility to conduct fine-
729 grained kinematic analyses of the reactions of animals to specific situations (Portugues & Engert,
730 2009; Harvey et al., 2009; Barker & Baier, 2015; Dunn et al., 2016; Stowers et al., 2017; Larsch &
731 Baier, 2018; Drew, 2019; Harpaz et al., 2021).

732 In conclusion, we show proof of concept of our new VR visual stimulation set-up combined
733 with an automated tracking system in an aquarium. The benefits and disadvantages of studying fish
734 behaviour with such technology are listed here. Benefits include shorter habituation phases in the
735 aquarium (approximately 30 seconds, compared to 5-7 minutes when experimenting with non-VR
736 methods, see Nanninga et al., 2017), which we attributed to the non-aggressive immersive baseline
737 environment; a perfect control of visual stimulation (timing and content) allowing for the same
738 stimulations to be repeated within or between individuals several times; the possibility to test the
739 same individuals in multiple successive trials, and in a controlled order; and, as mentioned earlier,
740 the setup enabled us to automatically collect numerous parameters about the kinematics of fish
741 reactions in real-time, which contributed to a precise and objective characterization of their
742 behaviour. Disadvantages include testing the behaviour of fish in a laboratory setting out of their
743 natural milieu, with a visual rendering that cannot reach the quality of a real environment; and the
744 technical skills in computer science required to prepare experiments and to process the large
745 amount of data. However, this successful proof of concept of our new VR setup and automated

746 tracking system on relatively fragile and small post-larval coral reef fish in response to both habitats
747 and different fish species holds significant promise for the field of fish behavioural ecology across all
748 life-stages and fish species in response to multiple biotic and abiotic conditions. The experimental
749 set-up can be scaled up or down as a function of the size of the focal fish and, as such, our VR set-up
750 holds tremendous promise for the future of the study of teleost behaviour.



753 **Fig A1.** Tracking performance for each experiment and tested fish group in all conditions. The average
754 proportions of missing/extra blobs are given in red/blue. Tracking accuracy was evaluated by computing
755 the average proportion of detected blobs relative to the number of expected blobs (5 per frame) across
756 all recorded frames of the trial. In Experiment 1, on average, 16.3% blobs were missing, and 1.0% extra
757 blobs were detected. The tracking was rather poor for 4 groups (8, 9, 10 and 11), for which there were
758 on average 29.3% missing blobs. However, these groups remained in the analyses as the sampling rate
759 of 5Hz was high enough for the overall data to be valid. In order to improve the tracking performance,
760 we reduced the luminosity variations by using opaque black curtains for the experimental room's
761 windows. In Experiment 2 and 3, 8.9% and 10.9% of blobs were missing, and 1.5% and 1.3% extra blobs
762 were detected on average respectively.



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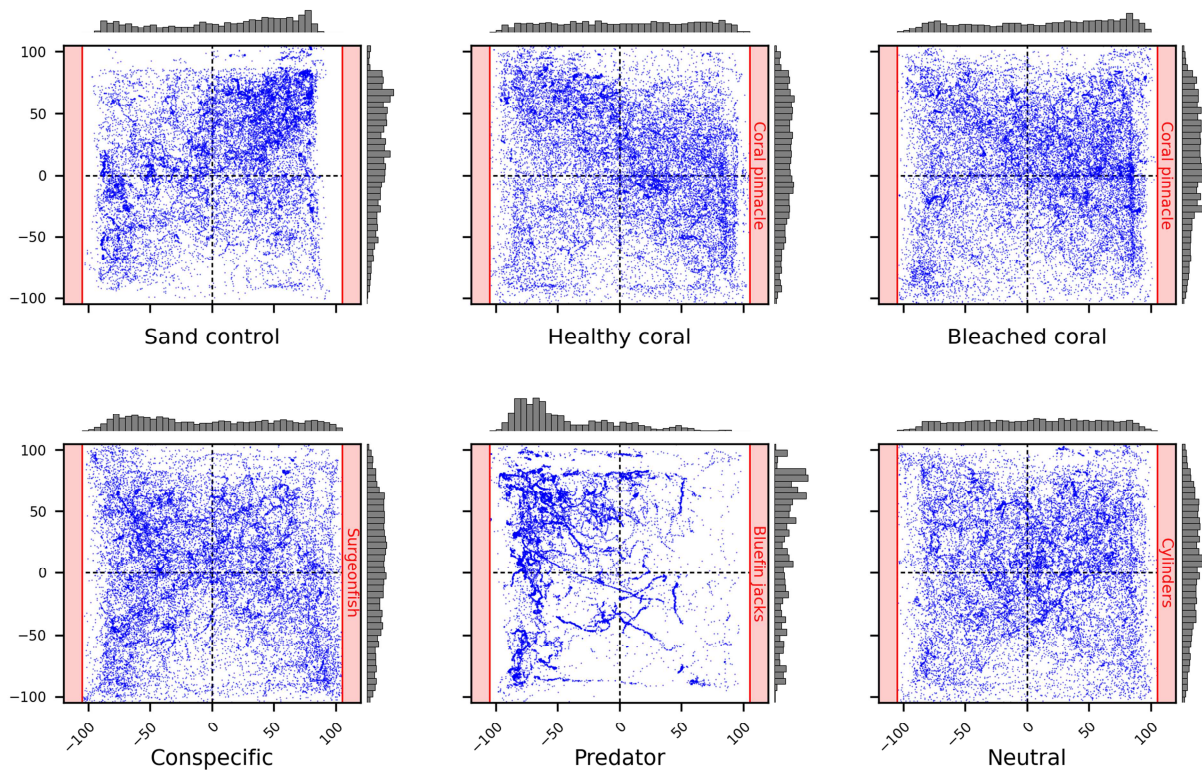
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Fig A2. Example of individual trajectories reconstruction in Experiment 1. Individual fish trajectories illustrating behavioural reactions of a typical group to the 6 stimuli presented on the aquarium sides (red shaded areas) of Experiment 1. The validated positions of fish are connected by coloured lines using a minimal distance heuristic to track individual fish in successive frames.



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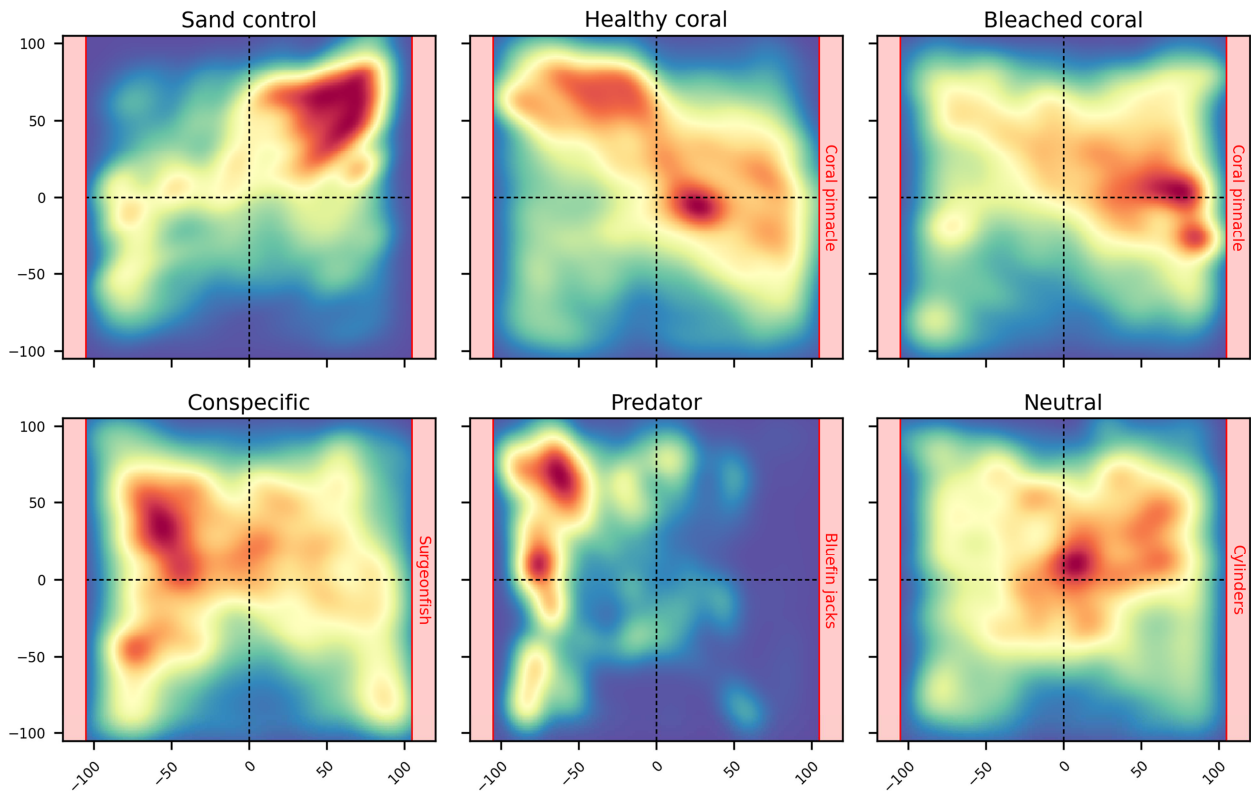
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Fig A3. Experiment 1 raw results visualization. 2D positions of all fish and all groups (n=12), in the 6 tested conditions: Sand control, Healthy coral, Bleached coral, Conspecific, Predator, and Neutral. For each condition, the stimulation was presented on the right side. All valid positions detected at each frame throughout trial durations are presented. Normalized X- and Y-position distribution histograms are provided above and to the right of each plot.



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Video A4. Animation showing the average behavioural responses of Experiment 1. Heatmaps for each condition where fish position density in the aquarium is plotted in successive intervals of 1 second. Density ranges from low (dark blue) to high (dark red).

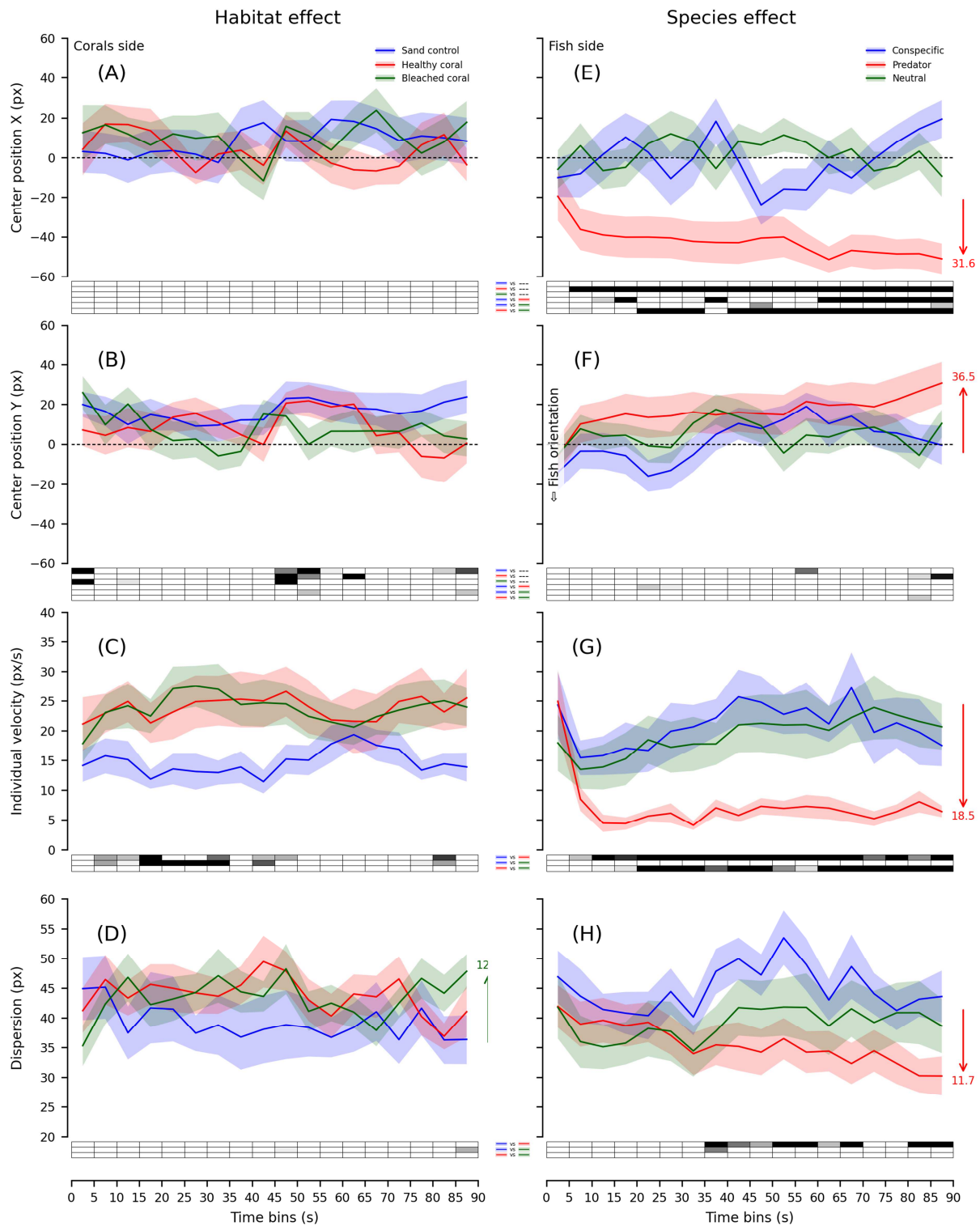
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778 **Links to videos:**

779 ×3 (30s): <https://amubox.univ-amu.fr/s/C5KfGMSD7mcWfR9>

780 ×6 (15s): <https://amubox.univ-amu.fr/s/oiaKRwAAqRdeWXw>



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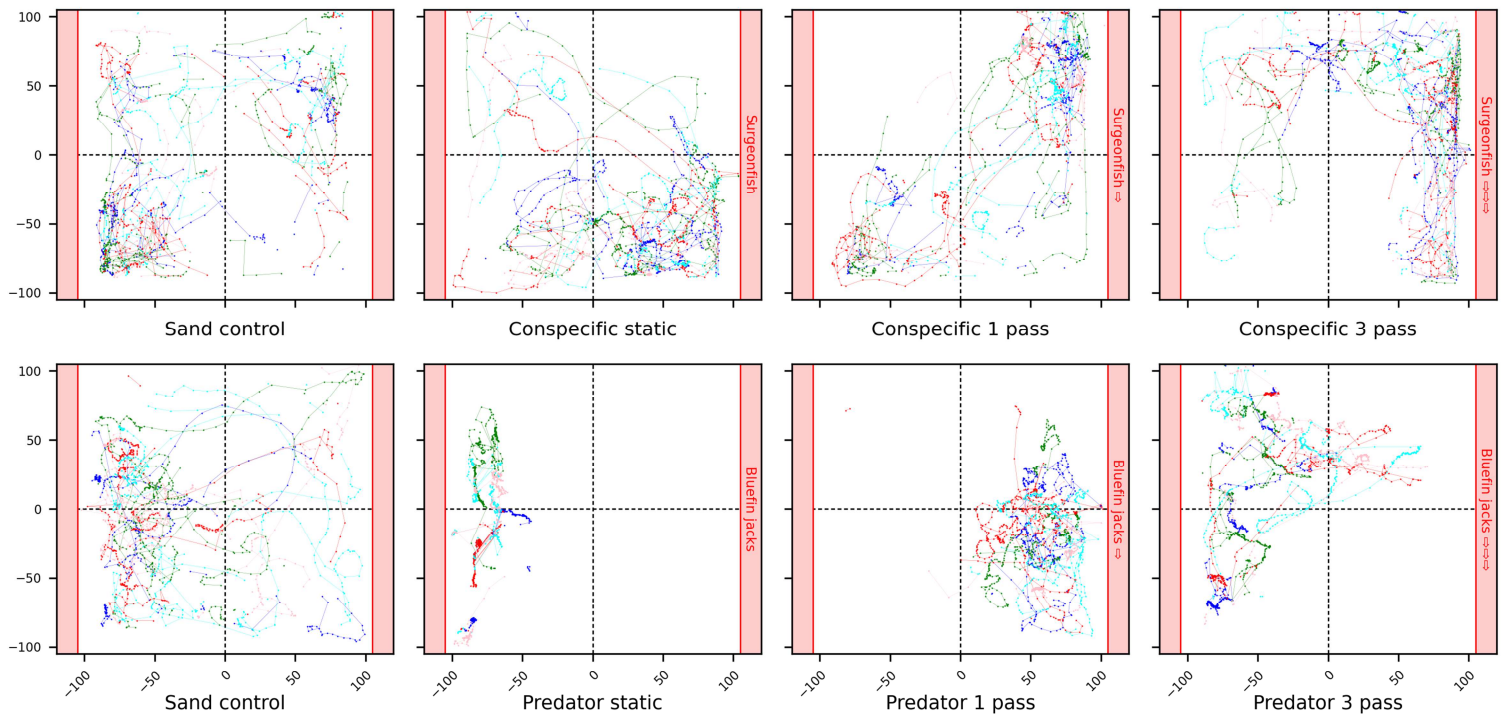
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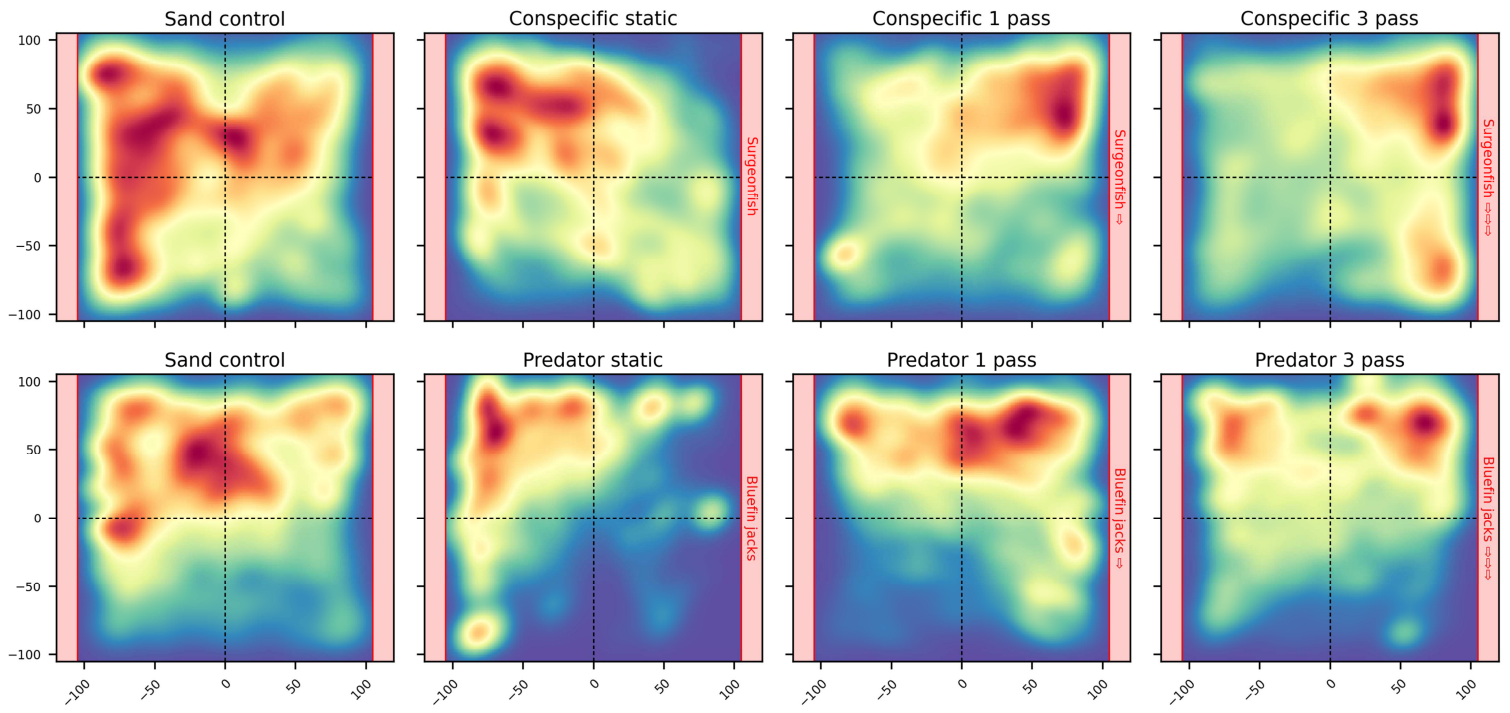
Fig A5. Experiment 1 group time series and statistics. X- and Y-positions of the group centre, individual velocity, and group dispersion plotted in time-bins of 5 seconds for the Habitat effect (A-D) and the Species effect (E-H). For each 5-second time-bin, average performance in the conditions was compared to each other or to zero with paired and single-value Student t-tests. Significance level is provided in the boxes below the plots (ranging from light grey for $P < 0.05/1$ to black for $P < 0.05/n_{\text{Tests}}$ using Bonferroni's correction for $n_{\text{Tests}}=3$; white for $P > 0.05$).

	Habitat effect				Species effect			
	Condition	Best model	Parameters	RMSE	Condition	Best model	Parameters	RMSE
X-position	Sand control	Quadratic	$a=-0.004$ $b=0.489$ $c=-3.445$	5.59	Conspecific	Quadratic	$a=0.008$ $b=-0.581$ $c=4.006$	11.42
	Healthy coral	Linear	$a=-0.132$ $b=9.209$	9.13	Predator	Exponential	$s=-3.506$ $l=-45.064$ $k=0.172$	3.93
	Bleached coral	Quadratic	$a=0.005$ $b=-0.389$ $c=14.843$	8.51	Neutral	Quadratic	$a=-0.007$ $b=0.627$ $c=-6.574$	7.40
Y-position	Sand control	Linear	$a=0.081$ $b=12.659$	4.78	Conspecific	Quadratic	$a=-0.007$ $b=0.881$ $c=-18.470$	7.41
	Healthy coral	Quadratic	$a=-0.008$ $b=0.627$ $c=1.228$	7.99	Predator	Linear	$a=0.238$ $b=5.842$	4.57
	Bleached coral	Exponential	$s=33.413$ $l=5.127$ $k=0.142$	6.84	Neutral	Linear	$a=0.025$ $b=3.738$	7.20
Velocity	Sand control	Linear	$a=0.025$ $b=13.637$	2.66	Conspecific	Quadratic	$a=-0.002$ $b=0.252$ $c=16.156$	3.78
	Healthy coral	Linear	$a=0.016$ $b=23.210$	3.24	Predator	Exponential	$s=42.560$ $l=6.096$ $k=0.301$	1.94
	Bleached coral	Exponential	$s=15.511$ $l=24.201$ $k=0.203$	2.88	Neutral	Linear	$a=0.095$ $b=14.962$	2.77
Dispersion	Sand control	Exponential	$s=46.648$ $l=38.060$ $k=0.080$	3.01	Conspecific	Quadratic	$a=-0.002$ $b=0.212$ $c=41.197$	3.91
	Healthy coral	Quadratic	$a=-0.002$ $b=0.173$ $c=42.461$	3.33	Predator	Linear	$a=-0.117$ $b=40.813$	1.72
	Bleached coral	Exponential	$s=31.970$ $l=44.103$ $k=0.217$	3.90	Neutral	Linear	$a=0.047$ $b=36.909$	3.02

788 **Table A6.** Experiment 1 best behavioural model fitting. The best model, parameters and corrected RMSE
789 for each of the 6 conditions and each of the 4 behavioural measures. For each fit, we assessed the
790 quality of fits computing a RMSE distribution by fitting 1000 times data with shuffled time-bins. Two
791 quality criteria were used: $RMSE < dCI(1\%)$ and $RMSE+20\% < RMSE_{mean}$. Fits highlighted in red didn't
792 meet the quality criteria.



793 **Fig A7.** Example of individual trajectories reconstruction in Experiment 2. Individual fish trajectories
 794 illustrating behavioural reactions of a typical group to the 8 stimuli presented on the aquarium sides
 795 (red shaded areas) of Experiment 2. The validated positions of fish are connected by coloured lines using
 796 a minimal distance heuristic to track individual fish in successive frames.

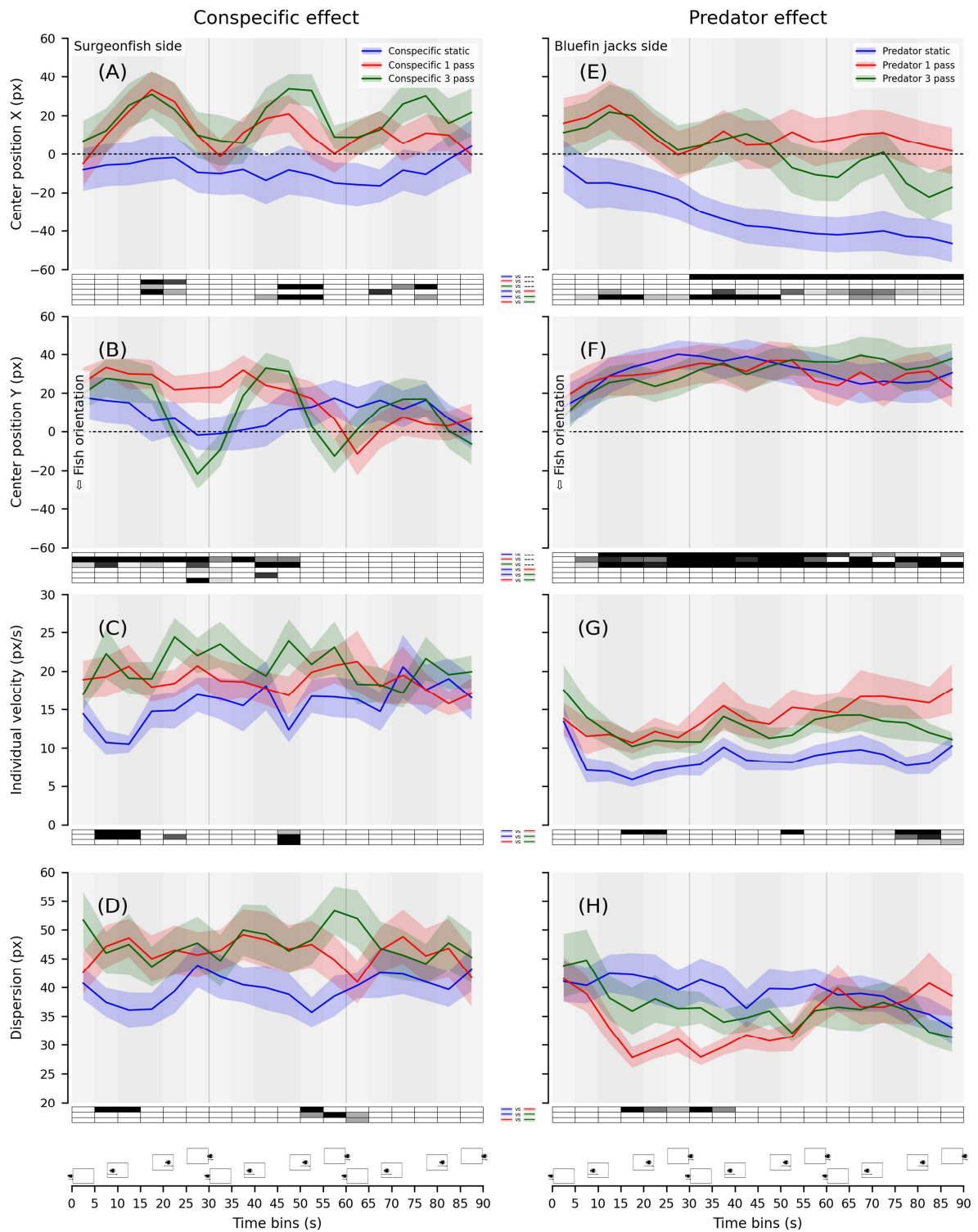


797 **Video A8.** Animation showing the average behavioural responses of Experiment 2. Heatmaps for each
 798 condition where fish position density in the aquarium is plotted in successive intervals of 1 second.
 799 Density ranges from low (dark blue) to high (dark red).

800 **Links to videos:**

801 ×3 (30s): <https://amubox.univ-amu.fr/s/F3TWSJoK2PYmP8y>

802 ×6 (15s): <https://amubox.univ-amu.fr/s/oNojasWfoS6j8K5>

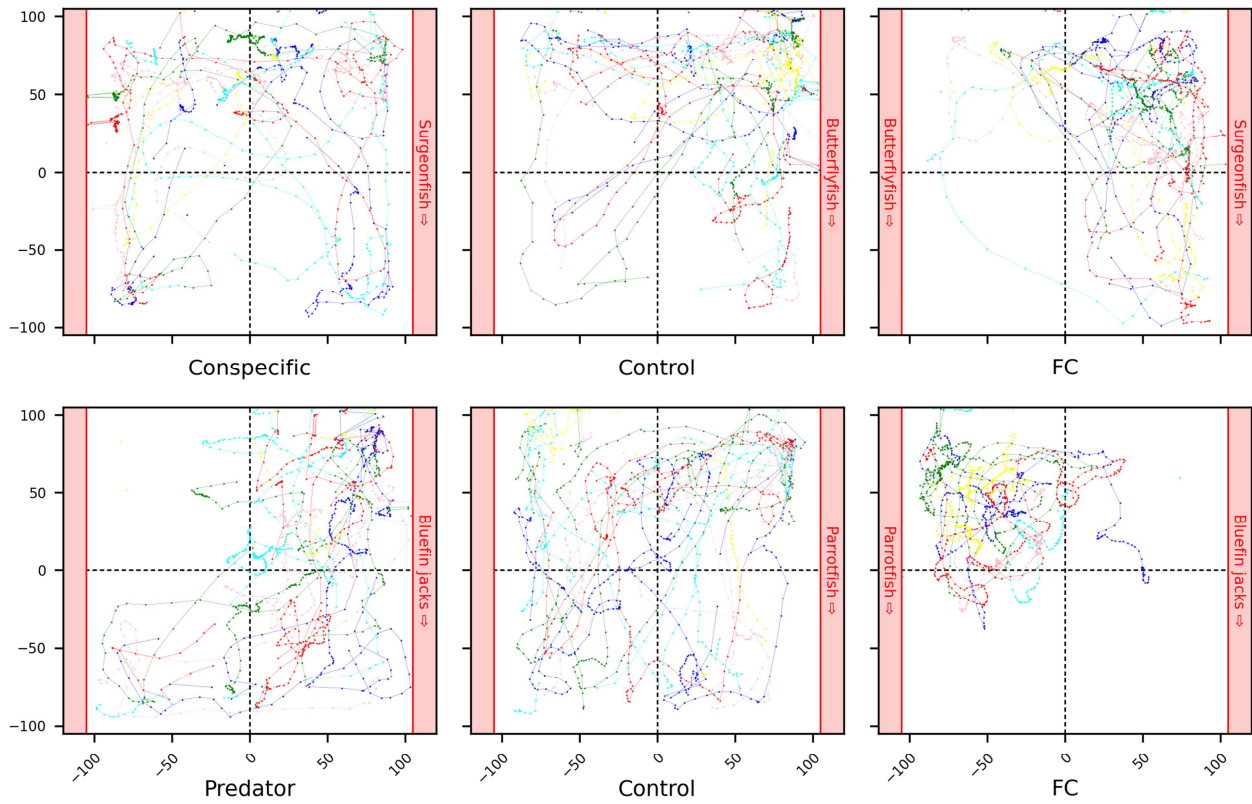


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Fig A9. Experiment 2 group time series and statistics. X- and Y-positions of the group centre, individual velocity, and group dispersion plotted in time-bins of 5 seconds for the Conspecific effect (A-D) and the Predator effect (E-H). For each 5-second time-bin, average performance in the conditions was compared to each other or to zero with paired and single-value Student t-tests. Significance level is provided in the boxes below the plots (ranging from light grey for $P < 0.05/1$ to black for $P < 0.05/n_{\text{Tests}}$ using Bonferroni's correction for $n_{\text{Tests}}=3$; white for $P > 0.05$). Shaded areas highlight the stimuli critical periods based on the on-going distance of the passing virtual shoals in the 3-pass conditions (30 s periodicity).

	Conspecific effect				Predator effect			
	Condition	Best model	Parameters	RMSE	Condition	Best model	Parameters	RMSE
X-position	Conspecific static	Quadratic	$a=0.005$ $b=-0.492$ $c=-0.336$	4.94	Predator static	Quadratic	$a=0.006$ $b=-0.957$ $c=-4.335$	2.42
	Conspecific 1 pass	Linear periodic	$a=-0.131$ $b=17.240$ $A=10.792$ $t_0=13.197$	7.05	Predator 1 pass	Exponential periodic	$s=22.873$ $l=6.347$ $k=0.057$ $A=4.743$ $t_0=17.323$	4.37
	Conspecific 3 pass	Linear periodic	$a=0.020$ $b=17.755$ $A=12.337$ $t_0=12.368$	5.20	Predator 3 pass	Linear periodic	$a=-0.404$ $b=19.353$ $A=7.300$ $t_0=17.241$	3.93
Y-position	Conspecific static	Exponential	$s=24.604$ $l=8.093$ $k=0.159$	6.57	Predator static	Quadratic	$a=-0.009$ $b=0.792$ $c=18.338$	5.00
	Conspecific 1 pass	Linear periodic	$a=-0.399$ $b=34.457$ $A=4.243$ $t_0=14.416$	7.08	Predator 1 pass	Quadratic periodic	$a=-0.006$ $b=0.515$ $c=21.644$ $A=2.372$ $t_0=10.932$	3.88
	Conspecific 3 pass	Exponential periodic	$s=44.447$ $l=7.666$ $k=0.177$ $A=20.884$ $t_0=16.758$	6.63	Predator 3 pass	Exponential periodic	$s=6.331$ $l=36.362$ $k=0.058$ $A=0.000$ $t_0=0.000$	3.28
Velocity	Conspecific static	Linear	$a=-0.064$ $b=12.814$	2.79	Predator static	Exponential	$s=21.299$ $l=8.257$ $k=0.497$	1.56
	Conspecific 1 pass	Linear periodic	$a=-0.015$ $b=19.439$ $A=0.704$ $t_0=26.117$	2.51	Predator 1 pass	Linear periodic	$a=0.073$ $b=10.845$ $A=0.752$ $t_0=24.805$	1.98
	Conspecific 3 pass	Quadratic periodic	$a=-0.002$ $b=0.115$ $c=19.511$ $A=1.497$ $t_0=5.857$	2.53	Predator 3 pass	Exponential periodic	$s=19.405$ $l=12.386$ $k=0.269$ $A=1.302$ $t_0=23.524$	2.00
Dispersion	Conspecific static	Linear	$a=0.036$ $b=38.272$	2.90	Predator static	Quadratic	$a=-0.001$ $b=0.038$ $c=40.885$	1.63
	Conspecific 1 pass	Linear periodic	$a=-0.011$ $b=46.519$ $A=2.033$ $t_0=16.082$	2.66	Predator 1 pass	Quadratic periodic	$a=0.005$ $b=-0.350$ $c=37.443$ $A=2.112$ $t_0=25.626$	3.20
	Conspecific 3 pass	Linear periodic	$a=-0.003$ $b=47.685$ $A=1.871$ $t_0=28.369$	3.22	Predator 3 pass	Exponential periodic	$s=45.052$ $l=34.639$ $k=0.064$ $A=1.280$ $t_0=22.249$	2.34

811 **Table A10.** Experiment 2 best behavioural model fitting. The best model, parameters and corrected
812 RMSE for each of the 6 retained conditions and each of the 4 behavioural measures. For each fit, we
813 assessed the quality of fits computing a RMSE distribution by fitting 1000 times data with shuffled time-
814 bins. Two quality criteria were used: $RMSE < dCI(1\%)$ and $RMSE+20\% < RMSE_{mean}$. Fits highlighted in
815 red didn't meet the quality criteria.



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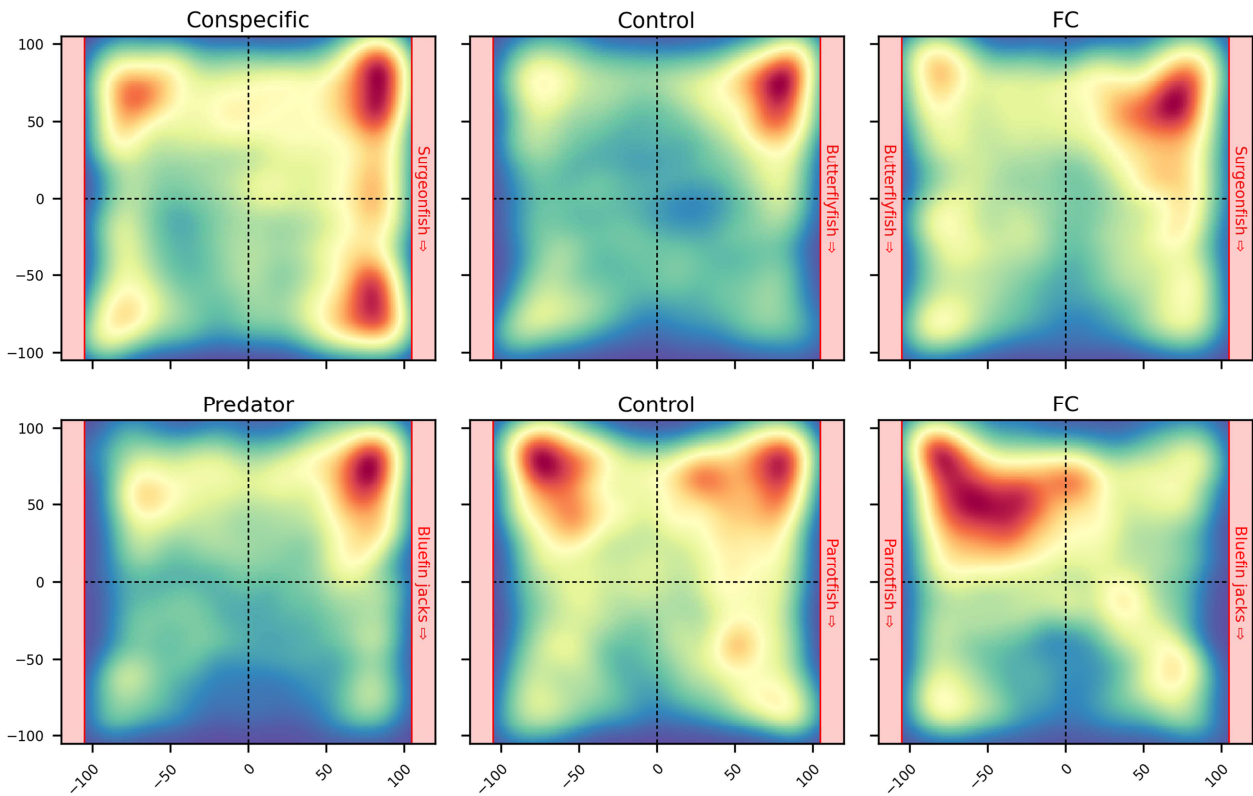
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Fig A11. Example of individual trajectories reconstruction in Experiment 3. Individual fish trajectories illustrating behavioural reactions of a typical group to the 6 stimuli presented on the aquarium sides (red shaded areas) of Experiment 3. The validated positions of fish are connected by coloured lines using a minimal distance heuristic to track individual fish in successive frames.



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Video A12. Animation showing the average behavioural responses of Experiment 3. Heatmaps for each
 823 condition where fish position density in the aquarium is plotted in successive intervals of 1 second.
 824 Density ranges from low (dark blue) to high (dark red).

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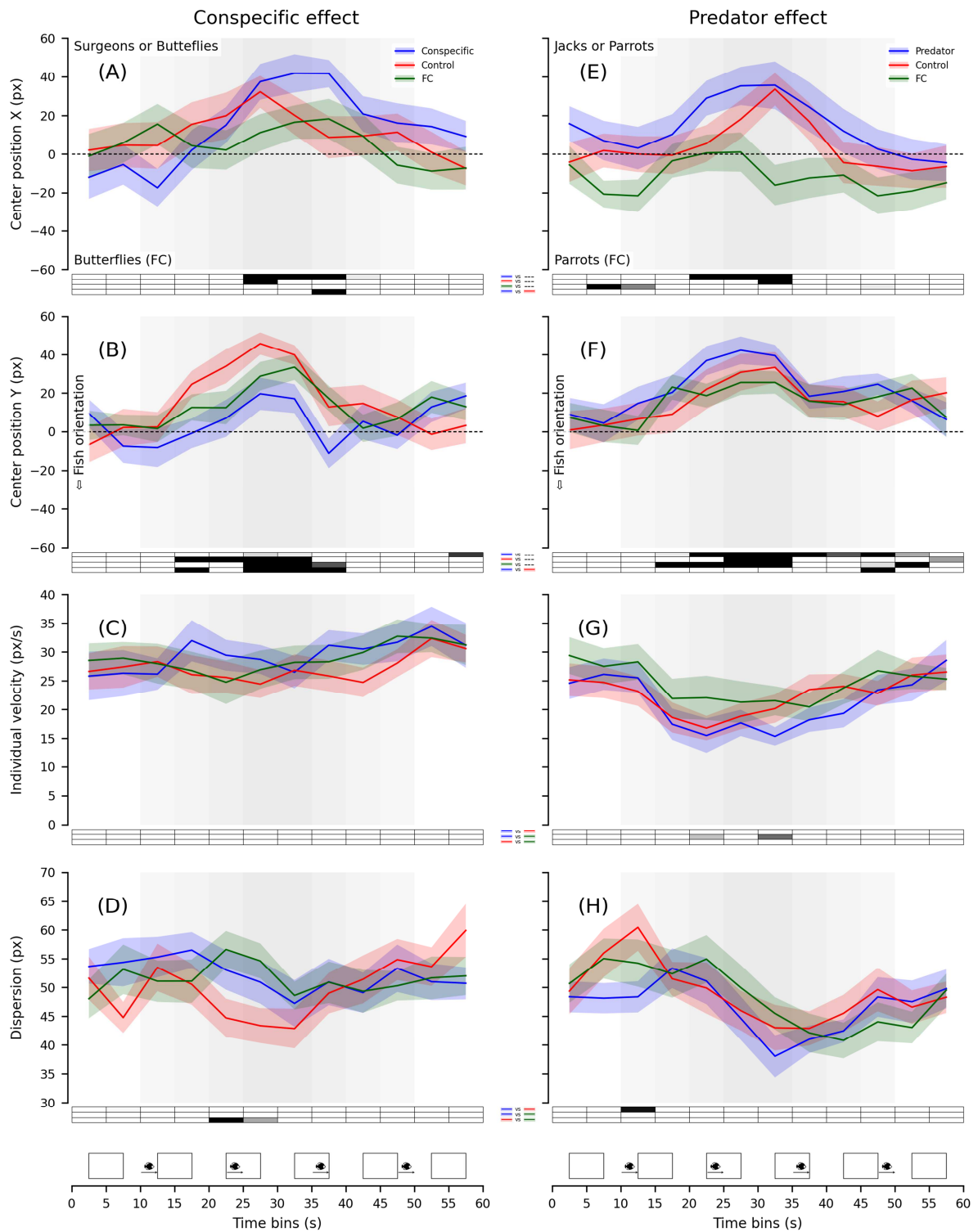
Links to videos:

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×3 (20s): <https://amubox.univ-amu.fr/s/32EN2ox6temcaeM>

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×6 (10s): <https://amubox.univ-amu.fr/s/6Zq322jsciYDwka>



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Fig A13. Experiment 3 group time series and statistics. X- and Y-positions of the group centre, individual velocity, and group dispersion plotted in time-bins of 5 seconds for the Conspecific effect (A-D) and the Predator effect (E-H). For each 5-second time-bin, average performance in the conditions was compared to each other or to zero with paired and single-value Student t-tests. Significance level is provided in the boxes below the plots (ranging from light grey for $P < 0.05/1$ to black for $P < 0.05/n_{\text{Tests}}$ using Bonferroni's correction for $n_{\text{Tests}}=3$; white for $P > 0.05$). Shaded areas highlight the stimuli critical period based on the on-going distance of the passing virtual shoals.

	Conspecific effect				Predator effect			
	Condition	Best model	Parameters	RMSE	Condition	Best model	Parameters	RMSE
X-position	Conspecific	Linear periodic	$a=0.808$ $b=-4.566$ $A=22.852$ $t_0=8.962$	4.26	Predator	Linear periodic	$a=0.019$ $b=18.456$ $A=18.453$ $t_0=10.294$	2.41
	Control	Linear periodic	$a=0.407$ $b=3.059$ $A=13.168$ $t_0=15.348$	4.29	Control	Linear periodic	$a=-0.440$ $b=21.053$ $A=18.382$ $t_0=6.960$	5.71
	FC	Linear periodic	$a=-0.916$ $b=36.477$ $A=16.326$ $t_0=2.082$	3.88	FC	Linear periodic	$a=0.292$ $b=-19.370$ $A=12.545$ $t_0=16.313$	6.54
Y-position	Conspecific	Linear periodic	$a=0.445$ $b=-10.035$ $A=13.061$ $t_0=14.388$	8.80	Predator	Quadratic periodic	$a=0.026$ $b=-1.000$ $c=29.847$ $A=19.427$ $t_0=13.941$	4.29
	Control	Linear periodic	$a=0.426$ $b=9.874$ $A=22.756$ $t_0=13.900$	5.88	Control	Linear periodic	$a=0.128$ $b=13.793$ $A=13.220$ $t_0=10.641$	4.01
	FC	Linear periodic	$a=0.099$ $b=11.310$ $A=14.318$ $t_0=10.886$	5.47	FC	Linear periodic	$a=0.633$ $b=-1.310$ $A=12.229$ $t_0=15.789$	4.36
Velocity	Conspecific	Exponential periodic	$s=-100.0$ $l=30.610$ $k=0.248$ $A=2.306$ $t_0=27.964$	2.60	Predator	Quadratic periodic	$a=0.015$ $b=-1.000$ $c=33.344$ $A=2.584$ $t_0=35.378$	1.95
	Control	Quadratic periodic	$a=0.016$ $b=-1.000$ $c=39.365$ $A=1.311$ $t_0=9.304$	2.30	Control	Linear periodic	$a=-0.091$ $b=23.697$ $A=4.623$ $t_0=37.174$	2.12
	FC	Linear periodic	$a=0.117$ $b=24.698$ $A=1.798$ $t_0=32.692$	2.25	FC	Quadratic periodic	$a=0.016$ $b=-1.000$ $c=36.671$ $A=0.752$ $t_0=34.227$	1.87
Dispersion	Conspecific	Linear periodic	$a=-0.039$ $b=53.221$ $A=3.296$ $t_0=25.351$	2.35	Predator	Quadratic periodic	$a=-0.015$ $b=1.000$ $c=31.155$ $A=9.249$ $t_0=24.507$	2.27
	Control	Linear periodic	$a=0.020$ $b=48.171$ $A=6.079$ $t_0=30.805$	2.80	Control	Quadratic periodic	$a=0.012$ $b=-1.000$ $c=65.723$ $A=3.597$ $t_0=28.083$	1.90
	FC	Linear periodic	$a=0.085$ $b=49.016$ $A=3.720$ $t_0=17.870$	2.61	FC	Linear periodic	$a=-0.123$ $b=51.698$ $A=5.906$ $t_0=20.223$	2.54

836 **Table A14.** Experiment 3 best behavioural model fitting. The best model, parameters and corrected
837 RMSE for each of the 6 conditions and each of the 4 behavioural measures. For each fit, we assessed the
838 quality of fits computing a RMSE distribution by fitting 1000 times data with shuffled time-bins. Two
839 quality criteria were used: $RMSE < dCI(1\%)$ and $RMSE+20\% < RMSE_{mean}$. Fits highlighted in red didn't
840 meet the quality criteria.

841 Data availability

842 Raw data files, global plots (all groups), and individual plots (trajectories, position scatter
843 plots, animated position density maps, grouped bin data) and the Python code used for data
844 processing for each experiment can be found in the following public repository:

845 <https://amubox.univ-amu.fr/s/WyYRBirn3pmz0qT>

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