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## **Probiotic modulation of the microbiota-gut-brain axis and behaviour in zebrafish**

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## Supplementary Methods

**Viability test.** The viability of the lyophilized *Lactobacillus rhamnosus* IMC 501 (Synbiotec s.r.l., Camerino, Italy) was performed through plate count. For estimation of the growth rate, 1 g of the lyophilized probiotic was inoculated in 100 ml of de Man Rogosa Sharpe (MRS) broth and, after mixing, was incubated at 37°C for 24 hours. Viable cells were counted by plating serial dilutions of bacterial culture on MRS/agar followed by anaerobic incubation at 37°C for 24 hours, resulting in  $1.65 \times 10^{10}$  CFU/g.

**Aseptic dissection procedure.** Each zebrafish was placed in a sterile Petri dish and its surface was disinfected with 100% Ethanol under a laminar flow hood.

At first, the head was removed with a sterile blade removing the soft tissue from the ventral side of the skull with sterile forceps. Eyes were removed using small spring scissors. Once the skull was opened, the bone was removed from the ventral side of the brain and, at the end, the skin and skull bones were removed from the dorsal side of the brain that was put into a sterile tube containing 1 ml of RNA Later solution and stored at -80°C until use.

The skin and underlying muscle along the belly from the anal fin to the operculum region were cut. Then, the skin and underlying muscle beginning from above the operculum region posteriorly along the side of the fish and down to the anal fin were cut. The skin and underlying muscle from the side of the fish were removed. Once identified, the whole intestine was separate from the rest of the organs and it was put into a sterile tube containing 1 ml of RNA Later solution and stored at -80°C until use. All the operations were performed with sterile instruments (scissors, forceps and surgical blades).

## **Video legend**

**Video S1** Difference between the swimming behaviour of probiotic fed (left) and control (right) zebrafish after four weeks of treatment.

**Table S1** Results of ANOVA analysis. DF, degrees of freedom; ns, not significant; \*\*\*,  $p < 0.001$ , \*\*,  $p < 0.01$ , \*,  $p < 0.05$ .

| Average distance (AD)<br>ANOVA Table                        |                     |           |                 |
|---|---------------------|-----------|-----------------|
| Source of Variation   | F (DFn, DFd)        | p value   | p value summary |
| Time  | F (1, 88) = 55.20   | < 0.0001  | ***             |
| Treatment   | F (1, 88) = 2.239   | 0.1382    | ns              |
| Interaction   | F (1, 88) = 0.08536 | 0.7708    | ns              |
| <b>Sidak's multiple comparisons test: Control - Treated</b> |                     |           |                 |
|   | <b>t</b>            | <b>DF</b> |                 |
| T0  | 0.8514              | 88        | ns              |
| T4  | 1.265               | 88        | ns              |

| Distance variance (DV)<br>ANOVA Table                       |                   |           |                 |
|---|-------------------|-----------|-----------------|
| Source of Variation   | F (DFn, DFd)      | P value   | P value summary |
| Time  | F (1, 88) = 71.66 | < 0.0001  | ***             |
| Treatment   | F (1, 88) = 9.042 | 0.0034    | **              |
| Interaction   | F (1, 88) = 17.79 | < 0.0001  | ***             |
| <b>Sidak's multiple comparisons test: Control - Treated</b> |                   |           |                 |
|   | <b>t</b>          | <b>DF</b> |                 |
| T0  | 0.8558            | 88        | ns              |
| T4  | 5.108             | 88        | ***             |

| Nearest distance (ND)<br>ANOVA Table                        |                   |           |                 |
|---|-------------------|-----------|-----------------|
| Source of Variation   | F (DFn, DFd)      | P value   | P value summary |
| Time  | F (1, 88) = 2.531 | 0.1152    | ns              |
| Treatment   | F (1, 88) = 7.948 | 0.0059    | **              |
| Interaction   | F (1, 88) = 3.110 | 0.0813    | ns              |
| <b>Sidak's multiple comparisons test: Control - Treated</b> |                   |           |                 |
|   | <b>t</b>          | <b>DF</b> |                 |
| T0  | 0.7466            | 88        | ns              |
| T4  | 3.240             | 88        | **              |

| Shoal size area (OA) |
|----------------------|
|----------------------|

| ANOVA Table   |                    |         |                 |
|---|--------------------|---------|-----------------|
| Source of Variation   | F (DFn, DFd)       | P value | P value summary |
| Time  | F (1, 20) = 0.5958 | 0.4492  | ns              |
| Treatment   | F (1, 20) = 6.609  | 0.0182  | *               |
| Interaction   | F (1, 20) = 4.423  | 0.0483  | *               |
| <b>Sidak's multiple comparisons test: Control - Treated</b> |                    |         |                 |
|   | t                  | DF      | P value summary |
| T0  | 0.3308             | 20      | ns              |
| T4  | 3.305              | 20      | **              |

| Column position (CP)<br>ANOVA Table                         |                   |          |                 |
|---|-------------------|----------|-----------------|
| Source of Variation   | F (DFn, DFd)      | P value  | P value summary |
| Time  | F (1, 20) = 522.0 | < 0.0001 | ***             |
| Treatment   | F (1, 20) = 302.4 | < 0.0001 | ***             |
| Interaction   | F (1, 20) = 377.8 | < 0.0001 | ***             |
| <b>Sidak's multiple comparisons test: Control - Treated</b> |                   |          |                 |
|   | t                 | DF       | P value summary |
| T0  | 1.447             | 20       | ns              |
| T4  | 26.04             | 20       | ***             |

| Shuttling Frequency (SF)<br>ANOVA Table                     |                   |          |                 |
|---|-------------------|----------|-----------------|
| Source of Variation   | F (DFn, DFd)      | P value  | P value summary |
| Time  | F (1, 20) = 27.64 | < 0.0001 | ***             |
| Treatment   | F (1, 20) = 26.73 | < 0.0001 | ***             |
| Interaction   | F (1, 20) = 7.862 | 0.0127   | *               |
| <b>Sidak's multiple comparisons test: Control - Treated</b> |                   |          |                 |
|   | t                 | DF       | P value summary |
| T0  | 1.673             | 16       | ns              |
| T4  | 5.638             | 16       | ***             |

**Table S2** Differences in microbiota composition between the control (CTRL) and probiotic fed (PROBIO) group of zebrafish. The last column reports the t-test p value. Significant p values (<0.05) are in bold.

| Phylum              | CTRL1        | CTRL1        | CTRL1        | CTRL1        | CTRL2        | CTRL2        | CTRL2        | CTRL3        | CTRL3        | CTRL3        | PROBIO1        | PROBIO1        | PROBIO1        | PROBIO1        | PROBIO2        | PROBIO2        | PROBIO3        | PROBIO3        | PROBIO3        | PROBIO3        | p t-test        |
|---------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|
| Actinobacteria      | 0            | 0            | 0            | 0            | 2            | 4            | 7948         | 1080         | 34           | 106          | 0              | 0              | 0              | 0              | 6              | 12             | 0              | 0              | 0              | 0              | 0,260639        |
| Bacteroidetes       | 0            | 0            | 0            | 0            | 0            | 0            | 0            | 0            | 0            | 0            | 0              | 0              | 0              | 0              | 0              | 0              | 1              | 2403           | 19             | 1889           | 0,152152        |
| Cyanobacteria       | 152          | 218          | 121          | 101          | 118          | 54           | 2347         | 218          | 1317         | 162          | 106            | 154            | 131            | 114            | 101            | 146            | 0              | 74             | 83             | 152            | 0,134198        |
| Firmicutes          | 6496         | 3261         | 295          | 240          | 301          | 160          | 8427         | 272          | 423          | 358          | 562            | 967            | 37965          | 38696          | 362            | 954            | 46173          | 27526          | 310            | 4567           | <b>0,038644</b> |
| Fusobacteria        | 46610        | 39970        | 50575        | 54677        | 66026        | 23673        | 12779        | 33037        | 1897         | 45062        | 44630          | 58523          | 756            | 3058           | 53502          | 674            | 48881          | 56091          | 40913          | 29370          | 0,702074        |
| Proteobacteria      | 4342         | 40433        | 7494         | 669          | 4742         | 419          | 10908        | 4742         | 419          | 10908        | 2004           | 4674           | 176            | 279            | 856            | 22153          | 4119           | 9260           | 1087           | 4945           | 0,420368        |
| Verrucomicrobia     | 0            | 0            | 0            | 0            | 0            | 0            | 0            | 0            | 2            | 0            | 0              | 0              | 0              | 0              | 0              | 0              | 0              | 315            | 0              | 104            | 0,209731        |
| <b>Class</b>        | <b>CTRL1</b> | <b>CTRL1</b> | <b>CTRL1</b> | <b>CTRL1</b> | <b>CTRL2</b> | <b>CTRL2</b> | <b>CTRL2</b> | <b>CTRL3</b> | <b>CTRL3</b> | <b>CTRL3</b> | <b>PROBIO1</b> | <b>PROBIO1</b> | <b>PROBIO1</b> | <b>PROBIO1</b> | <b>PROBIO2</b> | <b>PROBIO2</b> | <b>PROBIO3</b> | <b>PROBIO3</b> | <b>PROBIO3</b> | <b>PROBIO3</b> | <b>p t-test</b> |
| Bacilli             | 6496         | 3261         | 295          | 240          | 278          | 152          | 4052         | 221          | 369          | 304          | 562            | 967            | 37965          | 38696          | 334            | 913            | 46046          | 27444          | 298            | 4542           | 0,032628        |
| Fusobacteriia       | 46610        | 39970        | 50575        | 54677        | 66026        | 23673        | 12779        | 33037        | 1897         | 45062        | 44630          | 58523          | 756            | 3058           | 53502          | 674            | 48881          | 56091          | 40913          | 29370          | 0,702074        |
| Alphaproteobacteria | 1582         | 191          | 79           | 50           | 40           | 15           | 3733         | 351          | 1789         | 130          | 150            | 218            | 63             | 79             | 60             | 59             | 18             | 3841           | 97             | 1404           | 0,721783        |
| Betaproteobacteria  | 460          | 315          | 46           | 22           | 30           | 9            | 6852         | 38           | 304          | 33           | 83             | 71             | 49             | 97             | 79             | 16361          | 5              | 1803           | 25             | 1396           | 0,505191        |
| Deltaproteobacteria | 78           | 482          | 0            | 0            | 0            | 0            | 0            | 0            | 0            | 0            | 1              | 0              | 0              | 1              | 0              | 0              | 0              | 0              | 0              | 0              | 0,25987         |
| Gammaproteobacteria | 2222         | 39445        | 7369         | 597          | 4672         | 395          | 323          | 5084         | 406          | 3366         | 1770           | 4385           | 64             | 102            | 717            | 5733           | 4096           | 3616           | 965            | 2145           | 0,30359         |
| Clostridia          | 0            | 0            | 0            | 0            | 23           | 8            | 4375         | 51           | 54           | 54           | 0              | 0              | 0              | 0              | 28             | 41             | 127            | 82             | 12             | 25             | 0,342225        |
| Flavobacteriia      | 0            | 0            | 0            | 0            | 0            | 0            | 0            | 1080         | 34           | 106          | 0              | 0              | 0              | 0              | 0              | 0              | 1              | 2403           | 19             | 1889           | 0,32807         |
| Verrucomicrobiae    | 0            | 0            | 0            | 0            | 0            | 0            | 0            | 0            | 2            | 0            | 0              | 0              | 0              | 0              | 0              | 0              | 0              | 315            | 0              | 104            | 0,209731        |

**Table S2** (continued)

| Order              | CTRL1 | CTRL1 | CTRL1 | CTRL1 | CTRL2 | CTRL2 | CTRL2 | CTRL3 | CTRL3 | CTRL3 | PROBIO1 | PROBIO1 | PROBIO1 | PROBIO1 | PROBIO2 | PROBIO2 | PROBIO3 | PROBIO3 | PROBIO3 | PROBIO3 | p t-test        |
|--------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-----------------|
| Streptophyta       | 152   | 218   | 121   | 101   | 118   | 54    | 2347  | 132   | 200   | 147   | 106     | 154     | 131     | 114     | 101     | 146     | 0       | 7       | 83      | 132     | 0,254067        |
| Bacillales         | 83    | 145   | 42    | 30    | 44    | 17    | 26    | 0     | 0     | 0     | 41      | 49      | 108     | 119     | 37      | 334     | 0       | 0       | 0       | 0       | 0,408801        |
| Lactobacillales    | 6413  | 3116  | 253   | 210   | 229   | 133   | 909   | 221   | 369   | 304   | 521     | 918     | 37857   | 38577   | 295     | 555     | 46046   | 27444   | 298     | 4542    | <b>0,029665</b> |
| Fusobacteriales    | 46610 | 39970 | 50575 | 54677 | 66026 | 23673 | 12779 | 33037 | 1897  | 45062 | 44630   | 58523   | 756     | 3058    | 53502   | 674     | 48881   | 56091   | 40913   | 29370   | 0,702074        |
| Rhizobiales        | 1292  | 147   | 52    | 30    | 40    | 15    | 3733  | 208   | 690   | 76    | 124     | 198     | 48      | 55      | 60      | 59      | 8       | 3189    | 64      | 793     | 0,731049        |
| Burkholderiales    | 460   | 315   | 46    | 22    | 30    | 9     | 6852  | 38    | 304   | 33    | 83      | 71      | 49      | 97      | 79      | 16361   | 5       | 1803    | 25      | 1396    | 0,505191        |
| Desulfovibrionales | 78    | 482   | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1       | 0       | 0       | 1       | 0       | 0       | 0       | 0       | 0       | 0       | 0,25987         |
| Aeromonadales      | 807   | 302   | 7031  | 408   | 4669  | 390   | 14    | 4862  | 244   | 3295  | 1658    | 4257    | 35      | 58      | 712     | 36      | 4006    | 2215    | 893     | 1684    | 0,501784        |
| Enterobacteriales  | 1221  | 687   | 112   | 34    | 0     | 0     | 0     | 146   | 21    | 29    | 41      | 47      | 19      | 20      | 0       | 0       | 83      | 86      | 61      | 10      | 0,162198        |
| Pseudomonadales    | 144   | 47    | 126   | 140   | 3     | 5     | 309   | 76    | 141   | 42    | 45      | 42      | 1       | 4       | 5       | 5697    | 7       | 1315    | 11      | 451     | 0,261791        |
| Vibrionales        | 50    | 38409 | 100   | 15    | 0     | 0     | 0     | 0     | 0     | 0     | 26      | 39      | 9       | 20      | 0       | 0       | 0       | 0       | 0       | 0       | 0,329473        |
| Actinomycetales    | 0     | 0     | 0     | 0     | 2     | 4     | 7948  | 0     | 0     | 0     | 0       | 0       | 0       | 0       | 6       | 12      | 0       | 0       | 0       | 0       | 0,331237        |
| Clostridiales      | 0     | 0     | 0     | 0     | 23    | 8     | 4375  | 51    | 54    | 54    | 0       | 0       | 0       | 0       | 28      | 41      | 127     | 82      | 12      | 25      | 0,342225        |
| Flavobacteriales   | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1080  | 34    | 106   | 0       | 0       | 0       | 0       | 0       | 0       | 1       | 2403    | 19      | 1889    | 0,32807         |
| Stramenopiles      | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 86    | 1117  | 15    | 0       | 0       | 0       | 0       | 0       | 0       | 0       | 67      | 0       | 20      | 0,322208        |
| Rhodobacterales    | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 13    | 2     | 0       | 0       | 0       | 0       | 0       | 0       | 9       | 493     | 6       | 521     | 0,1502          |
| Rhodospirillales   | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 142   | 1086  | 52    | 0       | 0       | 0       | 0       | 0       | 0       | 1       | 159     | 27      | 90      | 0,368674        |
| Verrucomicrobiales | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 2     | 0     | 0       | 0       | 0       | 0       | 0       | 0       | 0       | 315     | 0       | 104     | 0,209731        |
| Other              | 290   | 44    | 27    | 20    | 5     | 2     | 3117  | 0     | 0     | 0     | 26      | 20      | 15      | 24      | 2       | 24      | 0       | 0       | 0       | 0       | 0,286033        |



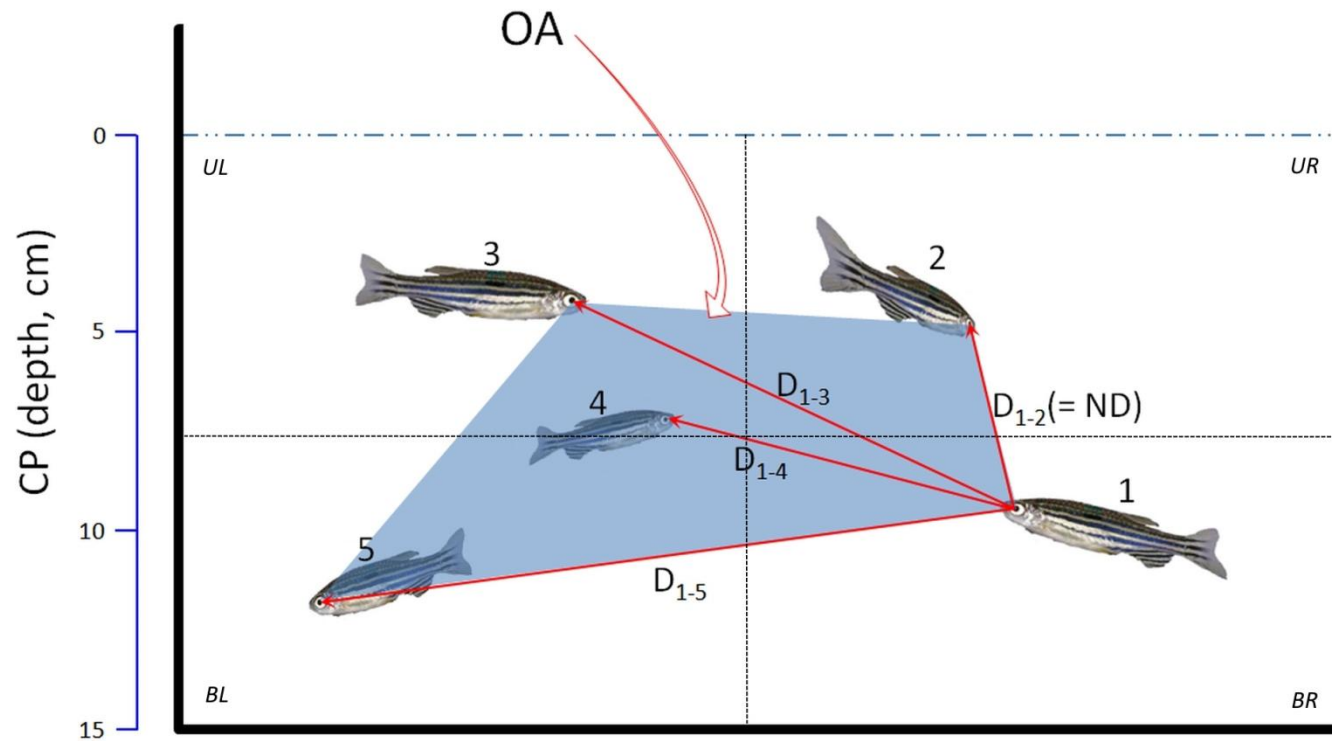
**Table S2** (continued)

| Genus             | CTRL1 | CTRL1 | CTRL1 | CTRL1 | CTRL2 | CTRL2 | CTRL2 | CTRL3 | CTRL3 | CTRL3 | PROBIO1 | PROBIO1 | PROBIO1 | PROBIO1 | PROBIO2 | PROBIO2 | PROBIO3 | PROBIO3 | PROBIO3 | PROBIO3 | p t-test        |
|-------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-----------------|
| Bacillus          | 83    | 145   | 42    | 30    | 0     | 0     | 0     | 0     | 0     | 0     | 41      | 49      | 108     | 119     | 0       | 0       | 0       | 0       | 0       | 0       | 0,937725        |
| Lactobacillus     | 4590  | 2186  | 102   | 99    | 98    | 59    | 38    | 84    | 134   | 97    | 190     | 132     | 20299   | 18926   | 121     | 90      | 5060    | 715     | 62      | 291     | 0,156305        |
| Leuconostoc       | 150   | 220   | 112   | 73    | 107   | 65    | 40    | 100   | 178   | 153   | 116     | 279     | 170     | 186     | 99      | 153     | 1       | 9       | 94      | 136     | 0,887507        |
| Streptococcus     | 303   | 140   | 9     | 5     | 0     | 0     | 0     | 3     | 6     | 11    | 37      | 15      | 3759    | 4368    | 0       | 0       | 4792    | 2519    | 19      | 643     | <b>0,024902</b> |
| Cetobacterium     | 46588 | 39960 | 50554 | 54662 | 66005 | 23663 | 12779 | 33037 | 1897  | 45062 | 44617   | 58505   | 756     | 3057    | 53486   | 674     | 48881   | 56091   | 40913   | 29370   | 0,702385        |
| Bradyrhizobium    | 811   | 3     | 0     | 1     | 0     | 0     | 0     | 0     | 0     | 0     | 31      | 42      | 3       | 1       | 0       | 0       | 0       | 0       | 0       | 0       | 0,375445        |
| Lawsonia          | 78    | 482   | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1       | 0       | 0       | 1       | 0       | 0       | 0       | 0       | 0       | 0       | 0,25987         |
| Plesiomonas       | 1221  | 687   | 112   | 34    | 0     | 0     | 0     | 146   | 21    | 29    | 41      | 47      | 19      | 20      | 0       | 0       | 83      | 86      | 61      | 10      | 0,162198        |
| Pseudomonas       | 144   | 45    | 124   | 125   | 1     | 4     | 2     | 76    | 141   | 42    | 45      | 42      | 1       | 4       | 4       | 4413    | 7       | 1315    | 11      | 451     | 0,220921        |
| Vibrio            | 49    | 37103 | 94    | 15    | 0     | 0     | 0     | 0     | 0     | 0     | 26      | 37      | 9       | 20      | 0       | 0       | 0       | 0       | 0       | 0       | 0,329507        |
| Micrococcus       | 0     | 0     | 0     | 0     | 0     | 0     | 1147  | 0     | 0     | 0     | 0       | 0       | 0       | 0       | 1       | 4       | 0       | 0       | 0       | 0       | 0,332625        |
| Mycobacterium     | 0     | 0     | 0     | 0     | 1     | 1     | 2827  | 0     | 0     | 0     | 0       | 0       | 0       | 0       | 3       | 1       | 0       | 0       | 0       | 0       | 0,330861        |
| Propionibacterium | 0     | 0     | 0     | 0     | 1     | 3     | 3974  | 0     | 0     | 0     | 0       | 0       | 0       | 0       | 2       | 7       | 0       | 0       | 0       | 0       | 0,331106        |
| Staphylococcus    | 0     | 0     | 0     | 0     | 0     | 1     | 0     | 0     | 0     | 0     | 0       | 0       | 0       | 0       | 0       | 270     | 0       | 0       | 0       | 0       | 0,332315        |
| Enterococcus      | 0     | 0     | 0     | 0     | 0     | 0     | 823   | 0     | 0     | 0     | 0       | 0       | 0       | 0       | 0       | 1       | 0       | 0       | 0       | 0       | 0,331138        |
| Pediococcus       | 0     | 0     | 0     | 0     | 1     | 0     | 0     | 0     | 0     | 0     | 0       | 0       | 0       | 0       | 2       | 293     | 0       | 0       | 0       | 0       | 0,328617        |
| Acidaminococcus   | 0     | 0     | 0     | 0     | 2     | 0     | 4365  | 0     | 0     | 0     | 0       | 0       | 0       | 0       | 6       | 5       | 0       | 0       | 0       | 0       | 0,331513        |
| Cupriavidus       | 0     | 0     | 0     | 0     | 15    | 4     | 9     | 0     | 0     | 0     | 0       | 0       | 0       | 0       | 12      | 16315   | 0       | 0       | 0       | 0       | 0,330989        |
| Polynucleobacter  | 0     | 0     | 0     | 0     | 5     | 0     | 6834  | 0     | 0     | 0     | 0       | 0       | 0       | 0       | 1       | 3       | 0       | 0       | 0       | 0       | 0,330458        |
| Acinetobacter     | 0     | 0     | 0     | 0     | 2     | 1     | 307   | 0     | 0     | 0     | 0       | 0       | 0       | 0       | 1       | 1284    | 0       | 0       | 0       | 0       | 0,469653        |
| Flavobacterium    | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1080  | 34    | 106   | 0       | 0       | 0       | 0       | 0       | 0       | 1       | 2403    | 19      | 1889    | 0,32807         |
| Mesorhizobium     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 13    | 222   | 10    | 0       | 0       | 0       | 0       | 0       | 0       | 7       | 644     | 25      | 356     | 0,29503         |
| Agrobacterium     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 18    | 105   | 32    | 0       | 0       | 0       | 0       | 0       | 0       | 1       | 2404    | 24      | 301     | 0,295262        |
| Rhodobacter       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 11    | 2     | 0       | 0       | 0       | 0       | 0       | 0       | 8       | 202     | 4       | 212     | 0,150636        |
| Hydrogenophaga    | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 15    | 123   | 8     | 0       | 0       | 0       | 0       | 0       | 0       | 2       | 1051    | 14      | 1003    | 0,178005        |
| Prostheobacter    | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 2     | 0     | 0       | 0       | 0       | 0       | 0       | 0       | 0       | 315     | 0       | 104     | 0,209731        |
| Other             | 2978  | 2667  | 7205  | 559   | 4801  | 446   | 9188  | 5292  | 2879  | 3590  | 1954    | 4625    | 13800   | 15316   | 817     | 207     | 40305   | 27691   | 1122    | 6069    | 0,113137        |

**Table S3** Sequence (5'-3') of the primers used.

| Gene                   | GenBank accession number | Forward  | Reverse                 |
|------------------------|--------------------------|--|-------------------------|
| <i>β-actin</i>         | NM_131031                | CACAGATCATGTTTCGAGACC                              | GGTCAGGATCTTCATCAGGT    |
| <i>bdnf</i>            | U42489                   | GCTCAGTCATGGGAGTCC                                 | ATAGTAACGAACAGGATGG     |
| <i>tph1a</i>           | AF548566                 | CAGTTCAGTCAGGAGATTGG                               | GACAGTGCGTGCTTCAG       |
| <i>tph1b</i>           | NM_001001843             | TTATATTATTATCTGCCTTGCTG                            | AGTGCTCTGTGGTATTTGG     |
| <i>tph2</i>            | NM_214795                | CAAGAGACAACAGCAACTATG                              | AAGCCCAACAGGTGATTTAG    |
| <i>htr1aa</i>          | EH441641                 | AGAGCAGCGAGGTGAC                                   | GAGCCGATGATTTGGTAAC     |
| <i>slc6a4a</i>         | DQ285098                 | AGTGGACCTGGGCAATG                                  | AGAAGATACGGCAAGAGAAG    |
| <i>mao</i>             | NM_212827                | GCAGTCAGAGCCCGAATC                                 | CACACCCATAAACTTGAGGAATC |
| 16S rRNA (V3 region)   |                          | CGCCCGCCGCGCGCGGGCGGGGCGGGGACGGGGGCTACGGGAGGCAGCAG | ATTACCGCGGCTGCTGG       |
| 16S rRNA (V1-2 region) |                          | AGAGTTTGATCMTGGCTCAG                               | GCWGCCTCCCGTAGGAGT      |
| 16S rRNA (V1-2 region) |                          | AGAGTTTGATCMTGGCTCAG                               | GCWGCCACCCGTAGGTGT      |

**Figure 1 Schematic representation of behavioural indexes utilized in the present study.** Position of each animal in the tank (frontal projection) is defined by the coordinates (in cm) of the fish eye in a x,y plan whose origin is in the left-bottom corner.  $D$  = inter-individual distance;  $AD$  = average inter-individual distance;  $ND$  = nearest distance;  $OA$  = shoal size area;  $CP$  = column position, i.e. the vertical distance (in cm) from water surface. During the tracking analysis (with Tracker 4.87), the front plane of the tank was digitally divided into four quadrants: Upper Left (UL), Bottom Left (BL), Upper Right (UR) and Bottom Right (BR). The counting of the total number per minute of the times the fish entered the left, upper, right and left halves of the tank measured the shuttling frequency (SF) of animals and represented the general locomotor activity of the shoal.



$$AD_1 = \frac{(D_{1-2} + D_{1-3} + D_{1-4} + D_{1-5})}{4}$$

$DV_1$  = standard deviation of  $AD_1$