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SHORT COMMUNICATION

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Response of rainbow trout (*Oncorhynchus mykiss)* to mirror images

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Abstract

The response of cultured rainbow trout to their mirrored image was investigated. Thirty fish were placed individually in two novel aquariums consecutively for 10 min each. Walls in one aquarium were covered with mirrors on all four sides, whereas the walls of the other aquarium were non-transparent black. Because all four walls were covered with mirrors, the mirrored image of the fish was reproduced multiple times such that 'a group' of fish was created surrounding the individual. Half of the fish started in the aquarium with the mirrors, whereas the other half started in the mirrorless aquarium. Fish swim faster in the aquarium with mirrors than in the mirrorless aquarium (2.95 *vs*. 2.40 cm/s; $p < 0.01$), indicating a positive behavioural response towards their mirrored images. Fish did not show aggressive interactions towards their mirrored images. Being confronted with 'a group' of fish and not just one 'opponent' may have inhibited aggressive behavior, or individuals may not have considered the images to be fellow individuals. Fish that swam faster in the mirrorless aquarium also did so in the aquarium with mirrors ($r = 0.73$; $p < 0.0001$), indicating a persistent behavioural coping response (boldness) in response to the two novel environments. Mirrors may be used to influence social behaviour of fish in aquaculture; further research is needed to investigate the influence of mirror placement in tanks of group housed trout on growth and behaviour.

Additional key words: activity; behaviour; fish; novel environment; speed.

Abbreviations used: BW (body weight).

Authors' contributions: Analysis of data and writing the manuscript: WMR. Conception and design of the experiment: LGR. Performed the experiments: WMR, LGR, LAGC, MV. Critically drafting and revising the work and approval of final version: LGR, LAGC, MV. Obtaining funding: WMR, LAGC, MV, LGR.

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Introduction

In aquaculture, fish to fish interaction is influenced by stocking density, however, the densities at which welfare becomes compromised remains ambiguous (Laursen *et al*., 2015). Maintaining fish at a density and group size that is too low may result in a poor feeding response, and inter-individual competition and spontaneous aggressive behaviour may result from the formation of dominance hierarchies (Ellis *et al*., 2002). Fish in lower ranking suffer more social stress and reduced appetite resulting from a cortisol-mediated increase in plasma glucose (Gregory & Wood, 1999). In addition, their lower social status results in receiving a smaller share of the group meal and therefore a reduction and greater day to day variability in food intake (McCarthy *et al*., 1992). At high stocking densities in large groups, the formation and maintenance of hierarchies becomes more difficult, however, welfare may be negatively affected because fish are exposed to crowding resulting in potential decreases in oxygen levels and water quality and increased chances of abrasion (Bagley *et al*., 1994; North *et al*., 2006; Laursen *et al*., 2015).

Not investigated to date is the effect of optically changing group size without affecting the tank size, stocking density or water quality, by using fish tank walls that are covered with mirrors. Mirrors may allow for virtual group sizes that are large enough to prevent the fish from forming strong dominance hierarchies, but that, in reality, are small enough to prevent negative side effects of deterioration of water quality and aggressive and non-aggressive behavioural interactions. To investigate this possibility we first need to establish the response of cultured fish to their mirrored image. The present study investigated the behavioural response of individual trout to their mirrored image as measured during ten min in a novel aquarium covered with mirrors *vs* covered with black sidings.

Material and methods

A test was carried out with 30 rainbow trout *Oncorhynchus mykiss*, weighing between 11 and 26 g (mean=18.9 g; SD=6.05 g), and with a length between 9 and 13.8 cm (mean=12.2 cm; SD=1.33 cm). Fish were housed in four cylindrical tanks measuring 120 L in groups of 6-10 animals. The tanks were located in a greenhouse where natural light entered from all four sides of the building. Fish were individually challenged two times consecutively in an aquarium measuring 50 × 24 × 30 cm^3 for 10 min each between 9:00 and 13:00h. Two aquariums were placed a meter distance from each other on a shelf, adjacent to the home tanks. Water temperature was around 14°C with O_2 = 7.2 ppm and pH = 7.6. In one aquarium, all walls were covered with mirrors, whereas the second aquarium had black siding (light entered both aquaria from above). In this set up fish could not see beyond their own aquarium walls. Randomly, with a small net, half of the fish were placed in the mirrorless aquarium first, and 10 min after in the aquarium with mirrors; the other half were placed in the aquarium with mirrors first and then in the mirrorless aquarium. Behaviour was recorded for each aquarium with a Canon PowerShot SX50 camera at Full HD 1080p hanging overhead, which was placed through a hole in a Styrofoam board a meter above each aquarium. No people remained in the room during the test. The work described has been approved by the Animal Ethics Committee of the Instituto Tecnológico Agrario, Junta de Castilla y León (Nr CEH-3-14).

Activity measured in both aquariums was analysed individually with the software Smart © version 3.0 (Panlab Harvard Apparatus ®) which estimated the total distance travelled, and from this the average speed. Movement was analysed for 10 min, starting 5 s after the fish was released into the aquarium to prevent artefacts resulting from water reflection. In order to filter body movements when the fish were not actually moving, the anti-vibrations filter was set to ≤ 2 cm/s. In order to filter movements due to artefacts, the artefact rejection filter was set to ≥ 25 cm/s. The program automatically corrects those artefacts by a linear interpolation of the subject's position. In addition, a 'locally weighted scatterplot smoothing' was applied that uses a modern regression algorithm that is designed to generate a more smoothed and thus realistic trajectory of the subject. Right after the second test, body weight (BW) and body length were recorded.

The SAS program was used for the statistical analysis of all traits. A repeated measures ANOVA in the procedure GLM was used to test the response to the mirror as measured on the same subjects:

$$Y_{ij} = \mu + MIRROR_i + b_1 \times BW_j + e_{ij}, \qquad [1]$$

where μ = overall mean, $MIRROR_i$ = effect of aquarium i (mirror, mirrorless; within-subject measurement on the same individual), $b_1 \times BW_j$ = the effect of body weight j (covariate), and e_{ij} = error term of animal j in aquarium i, with body weight j, e_{ij}~NID(0, δ^2_e). Speed tested by this model was denoted by Y_{ij}. Initially, the effect of tank of origin, and the order in which animals were tested was also included in the analysis but because these effects were not significant they were removed for further analysis. Results were considered statistically significant when p < 0.05; means, standard deviation (SD), lower (LCL), and upper (UCL) 95% confidence levels of the mean are given. The phenotypic partial correlation between speed in the mirrorless aquarium with that in the aquarium with mirrors was estimated with the procedure CORR (Pearson's correlation coefficient), after adjustment for the effect of body weight.

Results and discussion

An example of the track record of a fish with a particularly high speed and that of a fish with a particularly low speed is given in Figures 1a and 1b, respectively. Fish weighted on average 18.9 g (6.05 SD) and were 12.2 cm (1.33 SD) long. Shorter (r = -0.37; p < 0.05) and lighter (r = -0.39; p < 0.05) animals swam faster than longer and heavier fish. Therefore, BW was included as a covariate in model [1] and speed was corrected for BW in the estimation of the correlation. Overall, fish swam faster in the aquarium with mirrors (2.95 cm/s mean, 2.02 SD; 2.20 LCL, 3.70 UCL) than in the aquarium without mirrors (2.40 cm/s mean, 1.66 SD; 1.78 LCL, 3.02 UCL; DF = 1; F-value = 12.47; p = 0.0015). Fish that swam faster in the mirrorless aquarium also did so in the aquarium with mirrors (r = 0.73; p < 0.0001; the partial correlation after correction for BW is given in Figure 2).

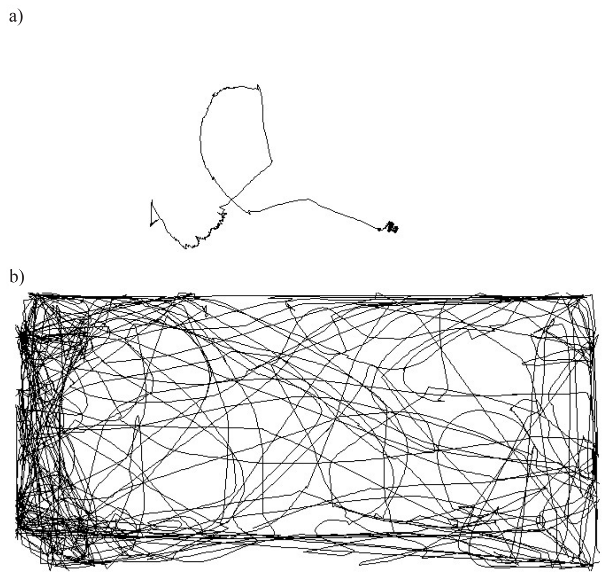

Figure 1. Examples of the track records of fish with a very low (a) and with a very high (b) speed in the 10-min test.

Since both aquariums represent novel environments, both can be expected to elicit a stress response independent of mirror placement. The stress response and variety in coping styles to a novel environment in animals has been extensively discussed by Koolhaas *et al*. (1999). An animal copes when it modifies its behaviour and physiology in an effort to master the situation; however, these responses are characterized by large individual variation. Generally, as described in a wide variety of animal species, as opposed to reactive (passive, shy) animals, proactive (active, bold) animals attack or flee from an opponent, are aggressive, fast exploring, impulsive, actively manipulate events, score high in frustration tests, and are risk takers and novelty seekers (Coppens *et al*., 2010). A novel environment can be used to measure activity as an indirect measure of shyness-boldness, including in fish species (Toms *et al*., 2010). Sneddon (2003) showed that rainbow trout could be categorized according to boldness *vs*. shyness by time spent under cover, swimming activity and the speed of learning a conditioning tasks; the frequency of swimming was relatively higher in bold fish. The present experiment showed variation in the activity response to a novel environment. The positive correlation between observations in the aquarium with mirrors and the mirrorless aquarium indicates a persistent behavioural coping response (boldness) in response to the two distinct novel environments.

In the present experiment, the response to a mirrored image was investigated in an environment that also introduced stress resulting from novelty, *i.e*., fish were taken from a large, round, group-housed tank to a small, square, individual aquarium. Animals reacted more strongly to the novel environment in which mirrors were placed. Because all four walls were covered with mirrors, the mirrored image of the fish was reproduced multiple times such that 'a group' of fish was created surrounding the individual. Virtually increasing group size in aquaculture to a level that is large enough to prevent the formation of strong dominance hierarchies, while maintaining actual group size at a level that is small enough to maintain water quality is a measure that has not been investigated to date. Bégout Anras & Lagardère (2004) showed that stocking density and concomitant social interactions affects swimming activity of rainbow trout as measured with an acoustic positioning telemetry system. Vice versa, Adams *et al*. (1995) indicate that swimming activity inhibits aggressive interactions. As extensively reviewed by Liao (2007), swimming patterns of fish in a group are closely related to energy expenditure. Therefore, the use of mirrors in aquaculture may be an interesting avenue of modifying fish behaviour to improve feed efficiency and fish welfare in commercial production systems.

The mirror-test is a classic test that is used to test an animal's self-awareness. For a long time, only humans and great apes passed, and not even all of those (Van der Waal, 2016). However, as this author indicates, it is hard to image that any species remains completely unaware of itself since it is has to be able to set its body apart from its surroundings and thus to make a distinction between self and non-self. If a fish is not able to recognize itself in the mirror, the next question is whether it will consider the mirrored images to be fellow individuals. Small fighting fish may react to their mirror image by courting or attacking it (Ros *et al*., 2006; Van der Waal, 2016); however, Desjardins & Fernald (2010) indicated that brain gene expression levels between African cichlid fish fighting their mirror

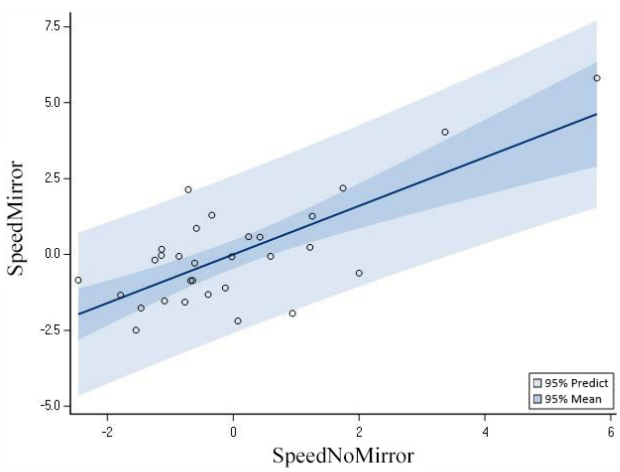

Figure 2. Correlation between speed in the mirrorless aquarium (SpeedNoMirror) and that in the aquarium with the mirrors (SpeedMirror), after correction for body weight.

image *vs*. fighting a real opponent were different such that they concluded that "clearly, the fish recognize something unusual about the mirror image and the differential brain response may reflect a cognitive distinction". In the study of Balzarini *et al*. (2014), one of three sympatric cichlid species (*Neolamprologus pulcher*) showed a correlated response between a mirror test and a live opponent test, whereas in two others (*Telmatochromis vittatus* and *Lepidiolamprologus elongatus*) there was no such relationship. In juvenile brown trout, Peterssona & Järvi (2000) observed aggressive behavior as indicated by "swimming against the mirror, frequently including biting attempts" and "swimming parallel and adjusting to its mirror image, sometimes including biting attempts and darting". In the present study, none of the fish displayed such behaviour towards their mirrored images in the two 10-min tests. Fish from a different origin, tested in a different experimental setting may account for the observed differences. In addition, our study differed in that the image was reproduced multiple times through the placement of mirrors covering all walls such that the individual was confronted with 'a group' of fish and not just one 'opponent' which may have inhibited aggressive behavior. Fish swam significantly faster in the test with the mirrors than in the test without, indicating a positive response to their mirrored images, however, it was not possible to determine whether they distinguished mirror images from real fish. In the study by Pitcher (1979), bream appeared to ignore a mirror image since it did not turn away at the individual distance. This author concluded that some sense other than vision is normally involved in addition, and that this sense is the lateral line. Vision is the dominant sense of many fishes, and visual signals are a key factor in social interactions (Rowland, 1999); however, the acoustico-lateralis sense and olfaction give fish additional information about stimuli (Hemmings, 1966). The lateral line is a system of sense organs that detect vortices and vibrations in the motion of nearby fish and prey, environmental clues that are not transmitted by mirror images. Indeed, Hemmings (1966) concluded that although most work on the sensory basis of schooling in fish (swimming together), as opposed to shoaling (staying together), indicates that vision is the primary sense involved, a mirror image was not as effective as a free swimming real fish (Hemmings, 1966). Instead he suggested that the school structure, in addition to vision, is initiated and maintained by olfaction and lateral line sense. Our results indicate a positive behavioural response in rainbow trout towards their mirrored images, however, it remains to be investigated how a group of fish will respond and their group behaviour altered when all walls are replaced by mirrors. Further research will be needed to determine which will be the optimal group size in mirrored tanks, and which will be the costs associated with the design.

References

Adams CE, Huntingford FA, Krpal J, Jobling M, Burnett SJ, 1995. Exercise, agonistic behaviour and food acquisition in Arctic charr, Salvelinus alpinus. Environ Biol Fish 43: 213-218. https://doi.org/10.1007/BF00002494

Bagley MJ, Bentley B, Gall GAE, 1994. A genetic evaluation of the influence of stocking density on the early growth of rainbow trout (Oncorhynchus mykiss). Aquaculture 121: 313-326. https://doi.org/10.1016/0044-8486(94)90267-4

Balzarini V, Taborsky M, Wanner S, Koch F, Frommen JG, 2014. Mirror, mirror on the wall: the predictive value of mirror tests for measuring aggression in fish. Behav Ecol Sociobiol 68 (5): 871-878. https://doi.org/10.1007/s00265-014-1698-7

Bégout Anras ML, Lagardère JP, 2004. Measuring cultured fish swimming behavior: first results on rainbow trout using acoustic telemetry in tanks. Aquaculture 240: 175-186. https://doi.org/10.1016/j.aquaculture.2004.02.019

Coppens CM, De Boer SF, Koolhaas J, 2010. Coping styles and behavioural flexibility: towards underlying mechanisms. Philos T R Soc B 365 (1569): 4021-4028. https://doi.org/10.1098/rstb.2010.0217

Desjardins JK, Fernald RD, 2010. What do fish make of mirror images? Biol Letters 6 (6): 744-747. https://doi.org/10.1098/rsbl.2010.0247

Ellis T, North B, Scott AP, Bromage NR, Porter M, Gadd D, 2002. The relationship between stocking density and welfare in farmed rainbow trout. J Fish Biol 61 (3): 493-531. https://doi.org/10.1111/j.1095-8649.2002.tb00893.x

Gregory TR, Wood CM, 1999. The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. Physiol Biochem Zool 72 (3): 286-295. https://doi.org/10.1086/316673

Hemmings CC, 1966. Olfaction and vision in fish schooling. J Exp Biol 45: 449-464.

Koolhaas JM, Korte SM, De Boer SF, Van der Vegt BJ, Van Reenen CG, Hopster H, De Jong IC, Ruis MA, Blokhuis HJ, 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci Biobehav R 23 (7): 925-935. https://doi.org/10.1016/S0149-7634(99)00026-3

Laursen DC, Silva PIM, Larsen BK, Höglund E, 2015. High oxygen consumption rates and scale loss indicate elevated aggressive behaviour at low rearing density, while elevated brain serotonergic activity suggests chronic stress at high rearing densities in farmed rainbow trout. Physiol Behav 122: 147-154. https://doi.org/10.1016/j.physbeh.2013.08.026

Liao JC, 2007. A review of fish swimming mechanics and behaviour in altered flows. Philos T R Soc B 362 (1487): 1973-1993. https://doi.org/10.1098/rstb.2007.2082

McCarthy ID, Carter CG, Houlihan DF, 1992. The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, Oncorhynchus mykiss (Walbaum). J Fish Biol 41: 257-263. https://doi.org/10.1111/j.1095-8649.1992.tb02655.x

North BP, Turnbull JF, Ellis T, Porter MJ, Migaud H, Bron J, Bromage NR, 2006. The impact of stocking density on the welfare of rainbow trout (Oncorhynchus mykiss). Aquaculture 255: 466-479. https://doi.org/10.1016/j.aquaculture.2006.01.004

Peterssona E, Järvi T, 2000. Both contest and scramble competition affect the growth performance of brown trout, Salmo trutta, Parr of wild and of sea-ranched origins. Environ Biol Fish 59 (2): 211-218. https://doi.org/10.1023/A:1007645411586

Pitcher T, 1979. Sensory information and the organization of behaviour in a shoaling cyprinid fish. Anim Behav 27: 126-149. https://doi.org/10.1016/0003-3472(79)90133-7

Ros AFH, Becker K, Oliveira RF, 2006. Aggressive behavior and energy metabolism in a cichlid fish, Oreochromis mossambicus. Physiol Behav 89 (2): 164-170. https://doi.org/10.1016/j.physbeh.2006.05.043

Rowland WJ, 1999. Studying visual cues in fish behavior: a review of ethological techniques. Environ Biol Fish 56 (3): 285-305. https://doi.org/10.1023/A:1007517720723

Sneddon LU, 2003. The bold and the shy: individual differences in rainbow trout. J Fish Biol 62 (4): 971-975. https://doi.org/10.1046/j.1095-8649.2003.00084.x

Toms CN, Echevarria DJ, Jouandot DJ, 2010. A methodological review of personality-related studies in fish: focus on the shy-bold axis of behavior. Int J Comp Psych 23: 1-25.

Van der Waal F, 2016. Are we smart enough to know how smart animals are? W.W. Norton & Company, NY.