Electronic Supplementary Material belonging to:

**Injury-mediated decrease in locomotor performance increases predation risk in schooling fish**

J. Krause1,2,#,J. E.Herbert-Read3,4,#, F. Seebacher5, P. Domenici6, A. D. M. Wilson1,4, S. Marras6, M. B. S. Svendsen7, D. Strömbom3,8, J. F. Steffensen7, S. Krause9, P. E. Viblanc2, P. Couillaud10, P. Bach11, P. S. Sabarros11, P. Zaslansky12 and R. H. J. M. Kurvers1,13

1 *Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany*

2 *Faculty of Life Sciences Humboldt-Universität zu Berlin, Invalidenstrasse 42, 10115 Berlin*

3 *Department of Mathematics, Uppsala University, Uppsala, Sweden*

*4 Department of Zoology, Stockholm University, Stockholm, Sweden*

5 *School of Life and Environmental Sciences, the University of Sydney, Sydney, NSW 2006, Australia*

6 *IAMC-CNR, Istituto per l’Ambiente Marino Costiero, Consiglio Nazionale delle Ricerche, Località Sa Mardini, 09170, Torregrande, Oristano, Italy*

7 *Marine Biological Section, University of Copenhagen Strandpromenaden 5, DK-3000 Helsingør, Denmark*

8 *Department of Biology, Lafayette College, Easton, 18042, Pennsylvania, USA*

9 *Department of Electrical Engineering and Computer Science, Lübeck University of Applied Sciences, 23562 Lübeck, Germany*

*10 Département de la Licence Sciences et Technologies, Université Pierre et Marie Curie, 4 place Jussieu 75005 Paris, France*

*11 Institut de Recherche pour le Développement, UMR 248 MARBEC, Avenue Jean Monnet,  CS 30171, 34203 Sète Cedex, France*

*12 Julius Wolff Institute for Biomechanics and Musculoskeletal Regeneration, Charité - Universitätsmedizin Berlin, Philippstraße 13, Haus 11, 10115 Berlin*

*13 Center for Adaptive Rationality, Max Planck Institute for Human Development, Lentzeallee 94, 14195, Berlin, Germany*

**Injury analysis of sardines**

To rotate and crop each image, we imported the video stills (n = 39) into ImageJ (v 1.36b) and determined the x, y positions of the school’s front centre, back centre, and two sides at the school’s widest point (Fig. 2a). We marked a polygon around the edge of the school’s members and cleared all pixels from outside this polygon, (effectively setting each of these pixels’ RGB channels to 255). We then adjusted the brightness and contrast of each image so that only the injury marks on the fish were visible. By adjusting the brightness and contrast for each image appropriately (Fig. 2b), we could then binary threshold the images to reveal the pixels in each image where the injuries had occurred (Fig. 2c). Binary thresholding resulted in pixels depicting injuries having an RGB value of 0 across all three channels (black), whilst uninjured pixels having a value of 255 across all three channels (white). We imported these binary images into Microsoft paint, and marked the positions of the front, sides and back of the school as determined earlier. These images were then rotated in Microsoft PowerPoint (2007) so that all schools faced the same direction (vertically orientated with the front of the school at the top of the image, and the rear of the school at the bottom, as shown in Fig. 2a), determined by the *x, y* coordinates of the school’s front and back as previously marked (Fig. 2a). We then cropped the images so that the total height of the image was determined by the school's front and back, and the school’s sides determined the width of the image. We finally imported these images into ImageJ, and resized them so that they were all 450 rows (image height), binary thresholded them again (due to Microsoft Powerpoint unavoidably grey-scaling some pixels during rotation), and then imported these converted images into MATLAB (2012b). Each image was therefore represented by a matrix (450 rows x n columns) where cells equal to 0 (black pixels) were injured parts of the school and cells equal to 255 (white pixels) were uninjured parts of the school. For each shoal we counted how many pixels were equal to 0 in the front (rows 1 – 225) and rear half (rows 226 – 450) of the shoal.

The differences observed in the number of injured pixels between the front and rear half of the schools could have been driven by differences in the total number of pixels (i.e. non-injured and injured pixels combined) in the front and rear half of the schools. To check whether this was the case, we applied the technique described above to the rotated images, but instead of adjusting the colour and contrast of each image to detect the injured parts of the school, we cleared all pixels outside the school that did not represent a fish (by setting all pixels’ RGB channels outside the marked polygon to 0). This resulted in silhouettes of the schools, where any pixel within the silhouette was classified as part of the school. As before, we imported these images (450 pixels x n pixels) into MATLAB and counted the number of pixels not equal to 0 in the front (rows 1-255) and rear (rows 256-450) half of the school in each image. To test whether the total number of pixels representing fish differed between the front and rear half of the school, we performed a paired t-test on the total number of front versus back pixels. There was no statistical difference in the total number of pixels between the front and rear half of the schools representing fish (paired t-test, t = 0.63, df = 38, P = 0.53).

**Swimming charactersistics of sardines**

We carried out an additional test to quantify potential effects of injuries on the positioning behaviour of injured and uninjured fish within schools. We selected an injured and an uninjured fish that were within 2 body lengths of each other (measured perpendicular to the swimming direction of the school) and within 1 body length of the mid-line of the school which could be in the front or rear half of the school (Fig. S1). We picked pairs (of injured and uninjured fish) from the same schools and observed them over the same time period to control for potentially confounding effects such as differences in school size and attack behaviour of the sailfish.

For fish in the front half, we scored whether they successfully maintained their position in the front half (“1”) or whether they fell back into the rear half (“0”). For fish in the rear half, we scored whether they managed to advance to the front half (“1”) or whether they maintained position in the rear half (“0”). In total, we analyzed 21 pairs of injured and uninjured fish over a period of 0.9 ± 0.47 s (mean ± SD). We then used a Wilcoxon signed-rank test to test whether injured and uninjured fish differed in their ability to advance their position from the rear to the front half of schools, or maintain position in the front half of schools.

**Figure S1**. Selection of injured and uninjured sardines relative to each other and the mid-line of the school to test for differences in positioning behaviour.



mid-line

1 BL

1 BL

2 BL

injured fish

uninjured fish

