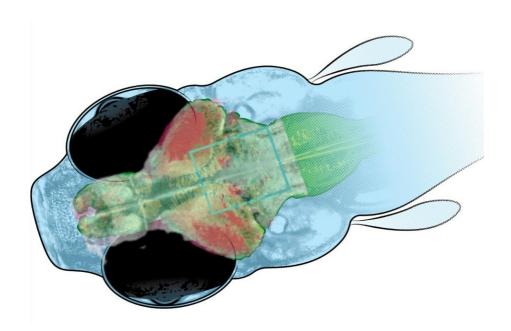








# Licenciatura em Engenharia Biomédica Projeto em Engenharia Biomédica



# Change in heading of preceding movement predicts left-right escape choice in larval zebrafish

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# **Acknowledgements**

It was a pleasure and an honor to be part of the Champalimaud Research community. I want to thank Michael Orger, principal investigator of the Vision to Action Lab, for accepting me as a member of his lab and giving me the opportunity to develop such a challenging and ambitious project.

I wish to express my deepest respect and gratitude to João Marques, Associate Researcher at the Vision to Action Lab, who has guided me and taught me so much over the past months. I am especially thankful for his patience and support throughout my internship. This work could not have been completed without his help, especially during times when the experiments did not go as expected or when I was unsure of what to do. He explained everything as many times as necessary, and for that, I am truly grateful.

To all the other members of the Vision to Action Lab, thank you for everything. A special thanks to Catarina Matos, whose shadow I was during these months, and in particular for her support, insights, and good company throughout this time. Thank you as well to Lucas Martins, Joaquim Contradanças, and Pedro Tomás, from whom I learned so much. It was a real pleasure to share ideas and learn from your experience.

To the coordinating professors of the Biomedical Engineering Project course unit, Manuel Matos and Miguel Minhalma, thank you for your guidance throughout this semester and for providing the foundation on which this work was built.

A special thank you to my family for their unconditional support over the past three years of my undergraduate studies. In particular, to my parents and my brother, whose support has been my greatest source of motivation, always encouraging me to pursue my goals.

To my boyfriend, future biologist Manuel Pedro, thank you for your love, support, patience, and constant encouragement. Your insights into biology brought valuable perspectives that made this work truly unique.

Finally, to my friends, for every moment of help and companionship. You made these three years easier and more enjoyable, and it wouldn't have been the same without you. To all of you, my sincere and heartfelt thanks. This achievement would not have been possible without each and every one of your contributions and support.

Behavioral data analysis was conducted with the help of João Marques. Coding support was kindly provided by Adrien Jouary and João Marques.

## Resumo

A perceção espacial é uma capacidade fundamental para a navegação no mundo envolvente. Diversos estudos demonstraram que o cérebro consegue gerar representações internas da direção da cabeça, sustentando a orientação espacial através da manutenção e atualização de um mapa direcional interno. Recentemente, foi descoberto que larvas de peixe zebra com uma semana de idade possuem um sistema de direção da cabeça, mas até ao momento não foi descrito qualquer comportamento nestes animais que exija informação sobre a direção da cabeça.

Este estudo avalia se estes animais jovens utilizam a direção da cabeça para executar um comportamento em condições de incerteza sensorial. Quando expostas a um som intenso, as larvas de peixe zebra executam fugas (direita, esquerda), que se dividem em dois tipos: C-starts de latência curta e de latência longa. Uma vez que, nesta fase, não conseguem determinar a direção do som, qualquer influência na direção da fuga deverá provir de pistas idiotéticas, como a direção da cabeça. Para obter dados com uma ampla gama de ângulos de orientação, desenhámos ensaios comportamentais que combinaram alterações de luz com sinais sonoros. Utilizando rastreamento de alta frequência e classificação automática dos movimentos, quantificámos o tipo de nado e a direção da cabeça do movimento imediatamente anterior a cada fuga.

Os nossos resultados mostram que a direção do nado imediatamente precedente influência as trajetórias de fuga, com correlações consistentes observadas tanto em respostas de latência curta (SLC,  $\rho = 0.232$ , p < 0.001) como de latência longa (LLC,  $\rho = 0.278$ , p < 0.001). Este efeito depende do tipo de fuga e do contexto: no escuro, o padrão tornou-se mais acentuado, sugerindo um papel reforçado das pistas internas de orientação na ausência de informação visual. As LLCs apresentaram uma forte tendência para repetir a direção do nado anterior, independentemente da sua amplitude angular, enquanto as SLCs mostraram uma modulação gradual dependente do ângulo da cabeça do movimento anterior.

Estes resultados fornecem a primeira evidência comportamental de que as larvas de zebrafish integram sinais internos de direção da cabeça para guiar decisões motoras rápidas. Este trabalho abre caminho para a identificação direta dos circuitos neuronais subjacentes à seleção de ações baseadas na orientação, num modelo animal que permite registos neuronais de todo o cérebro e estudos de conectómica

Palavras-chave: direção da cabeça, larva de peixe zebra, comportamento de fuga, navegação, neurociência.

## **Abstract**

Spatial perception is a fundamental capacity for navigating the surrounding world. Multiple studies have shown that the brain can generate internal representations of head direction, supporting spatial orientation through the maintenance and updating of an internal directional map, a cognitive function shared across species. Recently, it was discovered that one week old larval zebrafish have a head direction system, but thus far no behavior has been described for these animals that requires heading information.

The goal of this study is to assess if these young animals use head direction to perform a behavior. To this end, we relied on the acoustic startle behavior under conditions of sensory uncertainty. When exposed to a loud sound, zebrafish larvae perform escapes to the right or left, which fall in two types: short latency C-starts (SLCs) and long latency C-starts (LLCs). Since larvae at this stage cannot determine the direction of sound, any influence on escape direction must arise from idiothetic cues, such as head direction. To generate a dataset spanning a wide range of heading angles, we designed behavioral assays that combined light changes and auditory beeps - stimuli known to elicit large-angle displacements. Using high-frequency tracking and automated movement classification, we quantified the swim type and head direction of the movement preceding each escape.

Our results show that the direction of the immediately preceding swim significantly shapes escape trajectories, with consistent correlations observed in both short-latency (SLC,  $\rho = 0.232$ , p < 0.001) and long-latency responses (LLC,  $\rho = 0.278$ , p < 0.001). This effect was escape-type and context dependent: under darkness, the pattern became more pronounced, suggesting an enhanced role for internal orientation cues in the absence of visual input. LLCs exhibited a strong tendency to repeat the direction of the previous bout, regardless of its angular magnitude, whereas SLCs showed a graded modulation dependent on the head angle of the prior movement.

These findings provide the first behavioral evidence that larval zebrafish integrate internal headdirection signals to guide rapid motor decisions. This work paves the way for the direct identification of the neural circuits underlying orientation-based action selection, in an animal model that permits brain wide neuronal recordings and connectomics.

Keywords: head direction, zebrafish larvae, escape behavior, navigation, neuroscience