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Collective behaviour of zibrafish and robot groups in a constrained environment.

Yohann Chemtob

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Laboratoire Interdisciplinaire des Energies de Demain

**Collective behaviour of zebrafish
and robot groups in a constrained
environment**

par

Yohann Chemtob

Thèse de doctorat d’Éthologie

Référents scientifiques : Pascal Hersen
et José Halloy

Présentée et soutenue publiquement le 10 Février 2020

Devant un jury composé de :

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Andrea Perna	Professeur	University of Roehampton	Rapporteur
Claire Detrain	Directrice de recherche	Université Libre de Bruxelles	Examinatrice
Muriel Mambrini	Directrice de recherche	INRA	Examinatrice
José Halloy	Professeur	Université de Paris	Référent scientifique

Abstract

Collective movement can be observed throughout the animal kingdom, particularly in fish. Yet, despite many studies on the subject, the decision-making mechanisms of these collective events remain poorly understood. In this thesis, we want to better understand collective movement by studying more precisely the decision-making process, the organisation and the cohesion of groups of social fish. Our study focuses on the zebrafish (*Danio rerio*), a model used in different areas of research. To highlight those behaviours, we have developed a specific constrained environment composed of two rooms connected by a corridor. Cohesion on groups of different sizes and the organisation of leadership have been examined. The collective behaviour of zebrafish in a constrained environment was then described throughout a multi-contextual stochastic model. We have also developed a robotic agent to determine the importance of aspect and behaviour in conspecific recognition. Finally, after its integration into the group, we influenced the movements of the fish group with this biomimetic and autonomous fish robot to test our hypotheses on the different rules underlying collective movements. We have achieved the following results. In a constrained environment, fish use the rooms as resting areas and frequently move from one area to another. We observed that the size of fish groups influences the structure and proportion of these transitions. Group size also changes the cohesion between individuals and their spatial distribution. We studied more precisely the decision-making process during transitions, and in particular the mechanics of leadership. We have shown that leadership is shared among all individuals in a group, with heterogeneous sharing modalities between the different groups studied. The stochastic model developed from these results correctly simulates fish group behaviour in a constrained environment, using different parameter values according to the position of the agent. We have succeeded in integrating an autonomous and biomimetic fish robot into a group of zebrafish. The use of the stochastic model to drive the robot has highlighted the importance of biomimetic behaviour in the process of recognising a conspecific. Finally, we modulated the behaviour of the zebrafish with the fish robot by inducing collective departures as well as significantly biasing the distribution of fish between the two rooms. These positive results allow us to validate the hypotheses about leadership and cohesion among social fish.

Keywords: collective behaviour, biohybrid systems, modeling, animal-robot interaction, biomimetic robotics, zebrafish

Résumé

Le mouvement collectif est un phénomène observable dans tout le règne animal et notamment chez les poissons. Néanmoins, malgré un grand nombre d'études sur le sujet, les mécanismes de prises de décisions durant ces événements collectifs sont encore mal compris. Dans cette thèse, nous avons cherché à mieux comprendre les déplacements collectifs en étudiant plus précisément les prises de décision, l'organisation et la cohésion de groupe de poissons sociaux. Nos travaux utilisent le poisson-zèbre (*Danio rerio*), qui est un modèle d'étude dans différents domaines de recherche. Pour analyser la cohésion au sein de groupes de différentes tailles ainsi que l'organisation du leadership, nous avons développé un environnement contraint spécifique composé de deux chambres reliées par un couloir. Le comportement collectif des poissons-zèbres en environnement contraint a ensuite été décrit dans un modèle stochastique multicontextuel. Nous avons également développé un agent robotique afin de déterminer l'importance de l'aspect et du comportement pour s'intégrer de manière autonome au sein d'un groupe de poissons. Enfin, après son intégration au groupe, nous avons utilisé ce robot poisson biomimétique et autonome pour tester nos hypothèses sur les différentes règles à l'œuvre dans les mouvements collectifs en influant sur les mouvements du groupe de poisson. Nous sommes parvenus aux résultats suivants. Dans un environnement contraint, les poissons utilisent les chambres comme zones de repos et transitent fréquemment d'une zone à l'autre. Nous avons observé que la taille des groupes de poissons a une influence sur la forme et la proportion de ces transitions. La taille des groupes modifie également la cohésion entre les individus et leur utilisation de l'espace. Nous avons étudié plus précisément les prises de décision lors des transitions, et tout particulièrement le fonctionnement du leadership. Nous avons fait apparaître que le leadership est partagé entre tous les individus d'un groupe, avec néanmoins des modalités de partage hétérogènes entre les différents groupes étudiés. Le modèle stochastique développé à partir de ces différents résultats simule correctement le comportement de groupe de poisson dans un environnement contraint, en utilisant des valeurs de paramètre différentes en fonction de la position de l'agent. Nous avons réussi à intégrer un robot poisson, autonome et biomimétique, au sein de groupe de poisson-zèbre. L'utilisation du modèle stochastique pour guider le robot a mis en évidence l'importance d'un comportement biomimétique dans le phénomène de reconnaissance d'un conspécifique. Enfin, nous avons modulé le comportement du poisson-zèbre avec le robot poisson en provoquant des départs collectifs ainsi qu'en biaisant de manière significative la répartition des poissons entre les deux salles. Ces succès nous permettent de valider les hypothèses émises sur le leadership et la cohésion chez les poissons sociaux.

Mots clefs : comportement collectif, systèmes biohybrides, modélisation, interaction robot-animaux, robots biomimétique, poisson-zèbre

Contexte de la thèse

L'introduction qui suit fait état des principales étapes de mon parcours de jeune chercheur, du contexte dans lequel j'ai travaillé et des compétences que j'ai eu l'occasion de développer. Le reste du manuscrit, en anglais, présente mes travaux scientifiques et leurs apports au domaine de l'éthologie.

De 2015 à 2018 j'ai exercé en tant qu'ingénieur biologiste au Laboratoire Interdisciplinaire des Énergies de Demain sous la direction du Professeur José Halloy. J'ai été recruté pour travailler au sein du projet européen "Animal and robot Societies Self-organise and Integrate by Social Interaction (bees and fish)" (ASISSIbf). Ce poste, dont je développerai le détail dans la suite de cette introduction, faisait suite à mon stage de recherche de Master 2 en Modélisation des Systèmes Écologiques effectué dans la même équipe. A l'issue de ce stage, nous avons envisagé avec le Pr Halloy de prolonger mon travail par un doctorat, malheureusement, le projet européen devait s'achever deux ans et demi plus tard, durée trop courte pour un contrat doctoral. Par la suite, le projet européen a été prolongé, portant la durée de mon contrat d'ingénieur à trois ans. La procédure de validation des acquis de l'expérience me permet aujourd'hui de valoriser les recherches que j'ai menées à ce poste, équivalentes à celles d'un doctorant, et d'obtenir le diplôme de docteur en Ethologie en validant *a posteriori* mes travaux qui contribuent aux avancées de ce domaine scientifique.

Le projet européen ASISSIbf avait pour objectif d'intégrer des agents robots autonomes au sein de groupes d'animaux sociaux notamment afin de développer de nouveaux outils en éthologie. Plus précisément, le projet a démontré la possibilité de moduler grâce à la robotique le comportement des animaux, mais également la communication entre deux espèces sociales très différentes : les abeilles et les poissons. Commencé en janvier 2013 et terminé en septembre 2018, ce projet a obtenu des évaluateurs la plus haute notation, "Excellence", après avoir atteint avec succès ses différents objectifs. La nature interdisciplinaire du projet a nécessité l'expertise et la collaboration de six laboratoires européens (figure 1). L'équipe de Paris a ainsi travaillé avec les ingénieurs de l'École Polytechnique Fédérale de Lausanne (EPFL) pour concevoir un robot poisson capable d'interagir de manière autonome et biomimétique avec le poisson-zèbre (*Danio rerio*), une espèce de poisson social. Le reste du consortium a effectué en parallèle les mêmes travaux sur les abeilles. Un projet européen diffère d'un projet de recherche plus classique

par le nombre d'acteurs impliqués, l'ambition de ses objectifs et en lien, la taille de son financement. Les différentes équipes s'engagent à respecter une série d'objectifs précisément programmée dans le temps, et permettant la réalisation du but final, ici la modulation du comportement de deux espèces sociales via la robotique. Cela nécessite de tenir un planning serré parfois au détriment du recul ou des questions scientifiques secondaires.



Figure 1: Le projet ASSISIfb était composé d'un consortium de six laboratoires.

J'ai été séduit par ce programme de recherche extrêmement novateur et pluridisciplinaire, couvrant à la fois les domaines de l'éthologie, du machine learning et de la robotique. La combinaison entre les aspects du comportement collectif qui me passionnent et dont j'ai fait ma spécialité, et l'utilisation d'outils robotiques et électroniques de pointe, convenait parfaitement au biologiste féru d'informatique que je suis. L'équipe de recherche que j'ai rejointe en septembre 2015 était composée de deux doctorants et d'un post-doctorant. C'est dans ce cadre et avec ces collaborateurs notamment que j'ai mené mes propres recherches, intégrées au projet européen. La forme de l'équipe et mes responsabilités ont évolué au cours de ces trois années de travail : les paragraphes qui suivent présentent cette évolution.

Durant la première année de mon contrat, mes travaux se sont concentrés sur le comportement collectif des poisson-zèbres (étudié sans robots). Ils sont présentés dans la partie I de ce manuscrit. Au cours des deux années qui ont suivi, j'ai travaillé sur la modélisation du comportement du poisson-zèbre (partie II) et sur l'intégration des robots au sein des groupes de poissons sociaux (parties III et IV).

J'ai utilisé les principes méthodologiques suivants pour aborder les différentes questions scientifiques de mes travaux. Pour commencer, le dispositif expérimental est un élément crucial dans la recherche, car son développement est la formulation concrète des questions scientifiques posées. Précisément, les travaux présentés dans ce manuscrit, utilisent un

type d'arène, un "labyrinthe" simple composé de deux chambres reliées par un couloir. Celui-ci matérialise un choix à deux options, qui permet d'étudier les dynamiques et les prises de décision au sein d'un groupe. Cette arène a été développée en équipe et légèrement remaniée pour les expériences impliquant les robots. J'ai notamment travaillé à la construction des différentes versions, pour laquelle j'ai mobilisé mes compétences en dessin 2D et je me suis investi dans un nouveau Fablab de l'université Paris Diderot. J'ai également collaboré avec des entreprises spécialisées pour les réalisations les plus complexes.

Au sein de l'équipe, nous travaillions généralement de manière indépendante et autonome pour la réalisation des expérimentations et leurs analyses. Ce mode de fonctionnement a été facilité par le développement de l'environnement du laboratoire, auquel j'ai contribué. Cet environnement nous permettait une forte automatisation des différentes tâches, ainsi que de gagner du temps et de réduire les biais. Par exemple, chaque expérience est enregistrée via une caméra avec une identification en temps réel de la position des individus puis la vidéo est traitée par un logiciel spécialisé pour obtenir en plus de la position, l'identité de chaque poisson. Cela nous permet de minimiser les interactions avec les animaux pendant l'expérience et les erreurs de localisation. Afin d'analyser les expériences, j'ai développé des scripts en Python adaptés à mes questionnements qui nettoient, analysent et produisent les graphiques de manière automatisée sur l'intégralité des données.

Le Pr José Halloy organisait régulièrement des réunions afin d'échanger sur nos avancements respectifs et définir les objectifs à atteindre. Ces réunions m'ont permis de développer mes capacités de synthèse, de valorisation de mes résultats, mais aussi d'estimation du temps à consacrer à l'expérimentation et à l'analyse des données. L'acquisition de cette dernière compétence me semble particulièrement importante, car les expérimentations en recherche fondamentale sont souvent au moins en partie inédites, et cette qualité de prototype rend l'estimation de leur durée délicate.

Après un an dans le laboratoire, mes travaux et mes tâches se sont complexifiés avec le passage de la partie biologie pure aux expériences d'interactions robots animaux. Ce travail interdisciplinaire m'a amené à collaborer avec deux doctorants, le premier travaillant au sein de l'équipe de Paris sur le machine learning, et le second, roboticien, travaillant dans l'équipe suisse du projet européen localisée à Lausanne. Venant d'une formation de biologie, mon expérience avec les robots était d'abord limitée ; cependant, mon intérêt personnel pour le domaine m'a incité à communiquer intensément avec ce collègue concepteur du robot utilisé dans nos expériences sur les poisson-zèbres. Cette collaboration a constitué un des apprentissages importants de ces trois années de recherche. D'une part, je n'avais pas la même vision du robot que son concepteur : pour moi celui-ci était un outil, pour lui c'était une finalité de ses propres recherches. J'ai donc pu lui apporter une perspective différente sur le comportement du robot, une perspective de biologiste, pour lequel le robot devait se comporter comme un poisson. J'ai appris à

percevoir les limites de cet outil et trouver de nouvelles solutions, fort des connaissances du comportement du poisson-zèbre acquises durant la première année de mes recherches. De plus, j'ai appris, empiriquement et grâce à nos échanges, à entretenir et réparer cet outil et acquis quelques compétences dans ce domaine (pour plus de détails sur le robot, voir le Chapitre 4). Cette expérience m'a également formé à collaborer efficacement avec des experts spécialistes d'un autre domaine de compétence.

Enfin, à partir de fin de 2017, les travaux menés se sont encore complexifiés : l'intégralité du consortium a été amenée à collaborer activement pour produire le démonstrateur final. Il s'agissait d'une expérience ayant pour but de démontrer la faisabilité d'une connexion inter-espèces via des robots animaux intégrés. Mes travaux sur la modulation du comportement collectif des poisson-zèbres par les leurs robots ont alors été indispensables pour la moitié du démonstrateur. Mes responsabilités ont donc augmenté en proportion, car mon expertise m'a mis en position de représentant de la partie "poisson" du projet européen. J'ai ainsi initié et participé à la conception du démonstrateur et j'ai piloté les expériences qui étaient réalisées simultanément dans deux laboratoires à mille kilomètres distance. Celles-ci consistaient à établir une communication entre robots interagissant simultanément avec les poissons et les abeilles. Relever ce défi scientifique et technologique a été un accomplissement professionnel et personnel, et ce travail n'aurait pas été possible sans le grand effort de coopération avec mes collègues étrangers pour la conception, mais également la réalisation des expériences.

J'ai également participé aux rapports internes du projet ASSISIBf. En effet, le consortium doit produire périodiquement un rapport condensant les résultats obtenus. La rédaction du rapport demande un effort de communication important, car chaque partie du projet est un entremêlement de tâches produites par différentes équipes. J'ai été en charge de certaines parties de ces rapports et il faut une certaine ténacité pour obtenir des différents partenaires les informations sur les résultats attendus, mais également une bonne compréhension des résultats pour résumer en quelques lignes plusieurs mois de recherche. En parallèle et pour faciliter la coordination, des assemblées générales rassemblant toutes les équipes ont été régulièrement organisées. Lors des assemblées générales, j'ai préparé et présenté des résumés de l'avancement des recherches de l'équipe française en anglais, complétant ainsi mes compétences de synthèse et d'exposition des résultats.

Durant ces trois ans, j'ai communiqué à plusieurs reprises sur mes travaux et sur le projet européen. Une des expériences de communication scientifique les plus marquantes a été le Festival Ars Electronica à Linz en Autriche. Ce festival d'art et de technologie de grande envergure est destiné au grand public. Les travaux du projet européen ont été présentés au cours de deux éditions auxquelles j'ai participé, en 2016 et 2018. Notre stand a été conçu par une designeuse professionnelle en communication au sein de l'équipe suisse avec qui j'ai pu collaborer durant le festival. Communiquer avec le grand public a été très instructif pour moi ; j'ai dû simplifier les questions scientifiques présentées jusqu'à leurs enjeux très fondamentaux, tout en étant très bref, face à un public versatile et varié.



Figure 2: Maintien du stock de poissons durant le Festival Ars Electronica 2018
(© Tom Mesic)

J'ai en effet dû répondre à un grand nombre de questions de visiteurs de tous milieux et de tous âges, mais également à des journalistes, le plus souvent en anglais. Ce festival a également été un défi logistique entre différentes équipes du projet européen, mais aussi avec les organisateurs du festival. Il m'a fallu improviser pour produire un environnement suffisamment proche d'un laboratoire pour mettre en place le dispositif expérimental que mon équipe présentait. J'ai également dû former mes collègues à la préparation des expériences biologiques pour qu'ils soient capables de tenir le stand. De nombreux imprévus mécaniques et informatiques ont rendu l'édition de fin 2018 encore plus ardue en termes d'organisation. J'ai relevé le défi seul, car la partie scientifique de ASSISbf s'étant terminée, mes collègues des équipes poissons avaient quitté le projet. Sans interlocuteur francophone, j'ai pu également constater avec plaisir les progrès que j'avais réalisés dans la pratique de l'anglais.

Le projet européen a également organisé deux ateliers à destination des étudiants ingénieurs et des doctorants. J'ai pris en charge un cours du second atelier, qui avait lieu à Graz (en Autriche) avec mon collègue roboticien de l'EPFL sur le thème de l'utilisation de robot en éthologie, de modélisation et d'intelligence artificielle. Je devais préparer les conférences et encadrer les participants, expériences d'enseignement que j'avais déjà eues de manière plus confidentielle avec les stagiaires du laboratoire. J'ai ainsi pu affiner mes méthodes pédagogiques auprès d'étudiants de niveau universitaire.

J'ai également eu l'occasion de postuler à plusieurs demandes de bourse de thèse avec

l'aide du Pr Halloy, afin de pouvoir prolonger mes travaux sur le comportement collectif du poisson-zèbre à la fin du financement européen (nous ne savions alors pas que ceux-ci allaient être prolongés). Ces propositions ont été très loin dans la sélection, sans toutefois être finalement retenues. J'ai cependant appris à définir et développer de façon formelle un projet de recherche : élaborer les questions scientifiques, les expériences nécessaires pour y répondre, anticiper les modèles de simulation, préparer un planning étalé sur trois ans et définir un budget prévisionnel. Dans l'optique de démontrer la faisabilité technique de mon projet, j'ai produit un prototype de dispositif expérimental. J'ai également défendu ces projets de recherche devant un jury d'experts.

Les travaux de recherche auxquels j'ai participé et qui sont présentés dans mon manuscrit ont tous donné lieu à des publications scientifiques dans diverses revues. J'ai ainsi pu me familiariser avec les différentes étapes de la publication et de la rédaction et contribuer par mes relectures à l'évolution des articles, lors des échanges et retours avec l'éditeur. Enfin, mes propres travaux ont donné lieu à deux articles en cours de publication. Sous la direction de José Halloy, j'ai rédigé et mis en forme mes résultats, tout en situant mes travaux dans la continuité des recherches existantes et en exposant les avancées fondamentales qu'ils apportent au domaine d'étude.

Ces trois années au sein du laboratoire LIED et du projet européen ASSISbf ont donc été riches d'enseignements : j'ai acquis de connaissances fondamentales en biologie et en éthologie, et des compétences en robotique, pédagogie, gestion de projet, présentation des résultats, et en rédaction de rapports et d'articles scientifiques. Réciproquement, j'ai pu contribuer à la compréhension du comportement collectif des poisson-zèbres, en développant mes propres expériences et analyses. Celles-ci ont été communiquées aux parties prenantes du projet, mais également à la communauté scientifique par les articles publiés ou en cours de publication. Mes travaux en éthologie sont présentés dans le manuscrit de thèse qui suit.

Publications

- **Chemtob Y**, Cazenille L, Bonnet F, Gribovskiy A, Mondada F, Halloy J. *Strategies to modulate zebrafish collective dynamics with a closed-loop biomimetic robotic system*. bioRxiv 2019. [44]
- Séguret A, Collignon B, Cazenille L, **Chemtob Y**, Halloy J. *Loose social organisation of AB strain zebrafish groups in a two-patch environment*. PloS one 2019. [211]
- Collignon B, Séguret A, **Chemtob Y**, Cazenille L, Halloy J. *Collective departures and leadership in zebrafish*. PloS one 2019. [48]
- Cazenille L, Collignon B, **Chemtob Y**, Bonnet F, Gribovskiy A, Mondada F, Bredeche N, Halloy J. *How mimetic should a robotic fish be to socially integrate into zebrafish groups?* Bioinspiration & biomimetics 2018. [43]
- Cazenille L, **Chemtob Y**, Bonnet F, Gribovskiy A, Mondada F, Bredeche N, Halloy J. *How to blend a robot within a group of zebrafish: Achieving social acceptance through real-time calibration of a multi-level behavioural model*. InConference on Biomimetic and Biohybrid Systems 2018. [42]
- Cazenille L, **Chemtob Y**, Bonnet F, Gribovskiy A, Mondada F, Bredeche N, Halloy J. *Automated calibration of a biomimetic space-dependent model for zebrafish and robot collective behaviour in a structured environment*. InConference on biomimetic and biohybrid systems 2017. [41]
- Noël E, **Chemtob Y**, Janicke T, Sarda V, Péliissié B, Jarne P, David P. *Reduced mate availability leads to evolution of self-fertilization and purging of inbreeding depression in a hermaphrodite*. Evolution 2016. [170]

Expériences professionnelles

Ingénieur d'études

Paris, France

Laboratoire Interdisciplinaire des Énergies de Demain

Oct. 2015 - Oct. 2018

Projet européen ASSISIBf “**Animal and robot Societies Self and Integrated by Social Interaction**”, sous la direction du Pr José Halloy. Ce projet de recherche a reçu la notation “Excellence” en Septembre 2018.

- Recherche sur les comportements sociaux des poissons (décision de groupe, stratégies d'exploration et leadership), modèle de poissons en environnement contraint et développement de stratégies biomimétiques pour moduler des groupes d'animaux sociaux à l'aide robots
- Conception et construction d'environnements contraints pour les études comportementales, amélioration des équipements robotiques en coopération avec les ingénieurs de l'EPFL et développement de scripts d'analyse automatisés
- Entretien des stocks de poissons et maintenance des robots poissons
- Rédaction des rapports du projet, coopération au sein d'un consortium européen de laboratoires de recherche

Stage de Master 2

Paris, France

Laboratoire Interdisciplinaire des Energies de Demain

Janv. 2015 - Juin 2015

“**Prise de décision et comportement collectif chez le poisson-zèbre (*Danio rerio*), effets des variabilités individuelles et environnementales**” sous la direction du Pr José Halloy.

- Expérimentations : groupes de poissons filmés et suivis dans un dispositif multi-chambres
- Analyses statistiques automatisées (script Python)
- Modélisation des mécaniques de choix et du transfert d'information (Python)

Stage de Master 1

Toulouse, France

Centre de Recherches sur la Cognition Animale

Mars 2013 - Juin 2013

“**Peut-on étendre la loi de Weber aux organismes unicellulaires ? Mise en évidence de la loi de Weber chez *Physarum polycephalum***” sous la direction du Dr Audrey Dussutour.

- Expériences de perception et de choix binaire sur des patches de myxomycètes
- Traitements et analyses statistiques (logiciel R)

Stage Volontaire Montpellier, France
 Evolutionary and Functional Ecology Centre Janv. 2012 - Juin 2012

“Étude de l'évolution de l'hermaphrodisme par une approche expérimentale sur *Physa acuta*” sous la direction du Dr Patrice David.

- Mesures des traits d'histoire de vie des lignées consanguines de *Physa acuta*
- Entretien des élevages

Formations

Master en Ecologie spécialisation Modélisation des Systèmes Écologiques Toulouse, France
2013-2015
 Université Paul Sabatier

Licence en Biologie des Organismes, des Populations et des Ecosystèmes Toulouse, France
2011-2012
 Université Paul Sabatier

DUT Génie Biologique option Génie de l'Environnement Toulon, France
 Université de Toulon 2008-2010

Conférences scientifiques

Festival Ars Electronica Linz, Autriche
 Représentant du projet ASSISIbf Sept. 2016 et Sept. 2018

- Présentation du projet européen ASSISIbf au grand public
- Démonstration d'un dispositif expérimental interconnecté entre des abeilles, des zebrafish et des groupes de robots

“Engineering and evolution of bio-hybrid societies” Graz, Autriche
ASSISIbf Summer School Août 2017
 Instructeur

- Présentation : Quelle leçon tirer des expériences poissons-robot ?
- Atelier : Adaptation automatique des contrôleurs du Fishbot dans des expériences de groupes mixtes de poissons et de robots

“From bio-inspired to bio-hybrid (robotic) systems” Lausanne, Suisse
ASSISIbf Winter School Janv. 2016

Assistant



ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

- Présentation : Interaction robots-poissons/abeilles
- Atelier : Éthogramme automatique sur le zebrafish



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Introduction

i.1 Collective group and decision making

Over the course of evolution, group life has appeared in different taxa and has reached varying degrees of complexity since collective phenomena can be observed from bacteria to vertebrates. Collective movements are very present among fish species: it is assumed that 25% of fish species swim in groups throughout their lives and 50% during their juvenile states [213]. The cohesion of these groups varies widely, from groups of fish staying together for social reasons, called shoals, to groups of fish moving in a coordinated and polarised way, called school.



Figure i.1: A school of barracudas (*Sphyraena barracuda*). Work of Robin Hughes published on Wikimedia Commons (under licence CC BY-SA 2.0)

Living in a group provides an adaptive advantage in the face of environmental pressures [133]. Group size can provide protection from predators by improving the detection process (“encounter dilution” effect) [74, 88, 148, 241], reducing the probability of mortality relative to group size [209], but also by disturbing the predator in the presence of too many prey in its visual field [111, 143] (“predator confusion” effect). It can also improve foraging [62, 189, 222] and avoidance of pollutants [96, 153]. Another mechanism emerging from social groups is the “many wrong” mechanism where individual noise and errors are averaged at the group level and produce an accurate decision [46, 219]. For example, it can improve navigation in a peaty environment [12, 94]. In this example, a group of fish want to go to a preferential area, here the darkest zone. Despite deliberately noisy light variations, the cohesion and polarisation of the group make it possible to avoid individual errors and to find precisely the preferred area. The precision increases with the number of individuals in the group. The improvement of the cognitive performance in groups, which allows better and faster decision-making than for an isolated individual, is called Swarm intelligence and results from distributed, self-organised decision making [16, 72, 85, 202]. As part of a whole, individuality is partially or totally erased and the decisions of each individual are influenced to a greater or lesser extent by the behaviour of group members.

Collective movements can therefore improve collective intelligence, but it is necessary to understand how it is organized. Collective movements can be defined as “as a group of animals that decide to depart/move quite synchronously, move together in the same direction and maintain cohesion until the group stops moving or starts a new activity” [183] and were often studied [75, 100, 103, 176, 183, 197, 231, 233]. Collective movements are active in different situations such as food collections [65, 112], nest site selections [4, 59, 203, 253], predator detection [13, 28, 45, 145, 149, 263], habitat or territory selection [82, 212, 252].

This implies that animals have to choose between different alternatives and therefore reach a consensus to select the right one [50], by making a collective decision. To understand how it works, we need to study how information is perceived and shared, but also who makes the decision.

The transfer of information is quite well understood in insects. The information can be shared with the use of chemical signals such as bumblebees that will take a pheromone into the nest to recruit new workers as soon as new flowers are discovered [69]. In ants, a chemical trail will be used by scouts to recruit workers to the food source [107, 178]. In this case, the information is completed by the recruited individuals who will reinforce the lead as long as the resource is available. If it disappears, the track will also disappear by evaporation due to its non-activity. In fish, the transfer of information from trained individuals to the naive individual has been shown several times [155] but the underlying mechanism remains unclear. While some of the signals used by fish are obvious, such as colour changes [221] or sounds [135], current knowledge suggests that in most collective

movements, information transfer is done by passive cues [110]. They are based on the relative alignment between individuals and water movements and allow fish to react quickly to the movements of neighbours. To allow this passive information transfer, fish have, in addition to vision, a lateral line to detect disturbances in water such as currents, prey, predators, congeners and obstacles [78]. They also have an inner ear to detect sound in water.

Leadership is defined by one or more individuals initiating the group movement towards a new direction [130]. Leadership can be designated, emerging spontaneously because some individuals have knowledge appropriate to the situation or possess traits that give them a tendency to make decisions [71].

Personal leadership is when an individual initiates the group activities alone [141]. This capacity is inherent in hierarchical groups. The status of dominant can be vested in the oldest individual such as the mountain gorillas (*Gorilla beringei beringei*) [6,255] or to a dominant couple like wolves (*Canis lupus*) [154] and the common dwarf mongoose (*Helogale parvula*) [5]. Leadership can also be established by several individuals at the top of the hierarchy, for example, rhesus macaques (*Macaca mulatta*) [234] and dolphins (*Tursiops sp.*) [147]. Being more autonomous, the dominant increases his chances of being the first to act, especially since he exerts a very strong influence on the group [131]. This implies that the interests of the group may differ from those of the leader.

Conversely, when leadership is not attributed to a particular individual, it is said to be distributed. All members of the group can initiate a collective activity regardless of their position in the hierarchy. These temporary initiators may be individuals who are momentarily more motivated than their congeners, have a particular spatial position [141] or even more informed. In zebra (*Equus burchellii*) [80], european bass (*Dicentrarchus labrax*) [160] or stickleback (*Gasterosteus aculeatus*) [166], temporarily more motivated individuals initiate collective movements. In colombian white-faced capuchin (*Cebus capucinus*) [141] and rhesus macaque (*Macaca mulatta*) [234], individuals in the centre of the group initiate collective movements while in golden shiner (*Notemigonus crysoleucas*) [199,205], roach (*Rutilus rutilus*) and stickleback [33,130] are the individuals on the periphery of the group. For sticklebacks, individual personality can influence leadership [98]. Some sticklebacks are bolder than others, explore their environment more willingly and initiate collective travel more often by easily recruiting so-called shy individuals. Finally, an informed individual will be able to take the leadership more easily, as for example in the case of predator detection. It will then initiate an escape behaviour that will be followed by the other members of the group [61,216].

i.2 Mathematical models

In order to understand collective movements, mathematical models are often used as tools to describe and validate by simulation hypotheses that underlay the cohesion of these movements. To do this, it is necessary to formalise in a mathematical formulation the empirically observed rules of behaviour. The solutions in these models can help to better understand or highlight interactions within the group, but also to predict or refine experiments that are difficult to perform.

Models can describe situations at different scales. At the macroscopic level, models describe collective movements at the group level, for example to describe the distribution of agents between different sites [4]. At the microscopic level, it is the individual behaviours that are described, each individual being described independently and reacting to the behaviour of other individuals described by the system. Due to the difficulty of obtaining detailed data on collective group movements until recently, mathematical models have made it possible to explain the theoretical rules of collective movements by simulation. In particular, physical models of particle displacement have revealed that complex collective movements can be summarised as simple rules of attraction and repulsion.

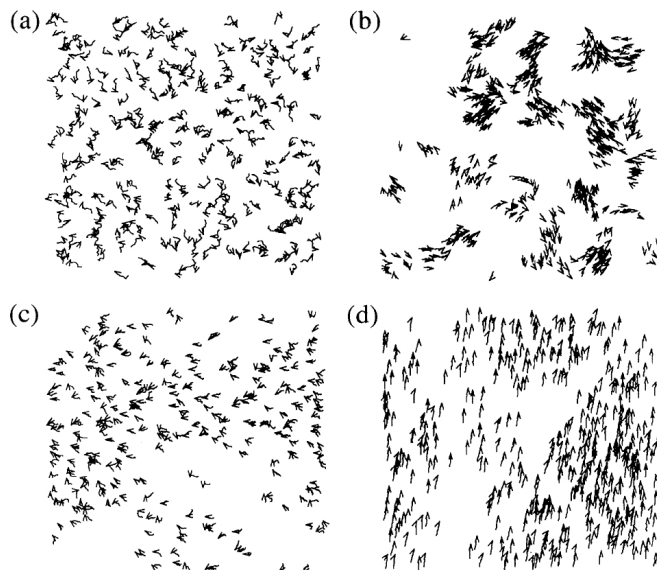


Figure i.2: Simulation of Vicsek model by Vicsek [250] in *Novel Type of Phase Transition in a System of Self-Driven Particles*. (a) represents the starting position of the simulation, where the velocity of the particles is represented by an arrow. (b), (c) and (d) represent simulations after some time, with each different values of density and noise. This demonstrate that it is possible to simulate collective displacements with simple individual rules.

For example, one of the simplest models for reproducing collective movements is the Vicsek model [250]. In this model, at each time step, the particles choose a direction that align them with their neighbours and moves at a constant speed. From this base,

by adding rules of repulsion [90], it is possible to maintain group cohesion. By using similar rules but applied in 3D, it is also possible to reproduce the collective movements produced by large fish groups as they had been observed in nature [53]. In this study, the agents have a blind spot in their visions in order to get close to the limits of the biological model. These models are defined as self-propelled particle models and a wide variety have been studied [146]. They are sufficient to generate movements similar to those observed in groups of animals. Several studies have shown that the features of these models, such as nearest-neighbour distance, polarisation, group speed, and turning rate, can be correlated with biological experiment data [101, 106, 109, 175, 251].

However, it has now become possible to obtain precise data on the movement of collective animals [9, 31, 163, 169, 215, 247]. Tools such as *idTracker* [181] precisely track the positions and identity of the animals studied. Thanks to this, it is therefore possible to produce new models closer to the observed behavioural rules. Some studies [86, 87] propose a bottom-up data-driven approach, which consists of developing a model by comparing each hypothesis with biological data. For example, the first iteration of the model will only take into account the swimming of a single fish [87], before a subsequent iteration adds cohesion parameters by studying two fish [86]. Since, several models have reproduced the social behaviours observed experimentally in fish [38, 49, 79, 142, 259, 261], like U-turns, hydrodynamic interactions, leadership, migrations, burst-and-coast (a swimming feature of some fish species like zebrafish) or wall following.

i.3 Mixed groups of animals and robots

Ethology researchers have been using for a long time decoys to interact with animals. This simple tool allows scientists to simulate a specific aspect of a behaviour, like feeding, in order to understand how animal interactions work [243–245, 248]. For example, in the figure i.3 based on an experiment from Turner 1964 [248], a decoy is used to simulate a bird food collection. The lure picks a food of a specific colour from among several and the experiment seeks to determine if a chick observing the action chooses the same food. In this experiment the lure is a simple flat shape that the experimenter moves by hand.

Technological advances have made more complex experiments possible, particularly with the appearance of the first robotic decoys. Lures can now move, as Martins *et al.* shows it with a lizard robot reproducing head movement patterns [151]. They can produce heat like in the study of Rundus *et al.* 2007 [207] with its squirrel robot emitting infrared through the tail to reproduce predator alert systems (figure i.4A). They can even produce sound like the frog robot in the study of Taylor *et al.* 2008 [242], which reproduces territory defence/reproduction vocalisations and moving vocal sac (figure i.4B).

However, these robots remain limited, often controlled by human operators and only able to operate on one animal at a time and during short periods of time. They are not able to

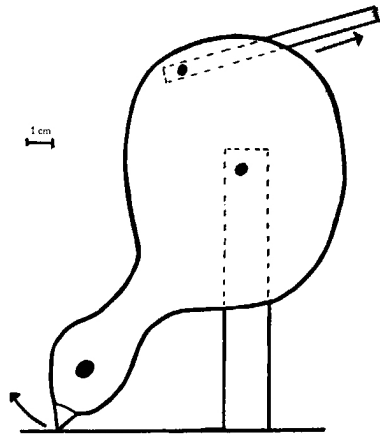


Fig. 15. Exp. 7. The Mechanical Hen.

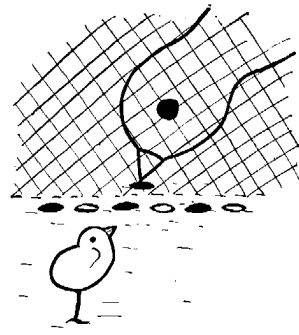


Fig. 16. Exp. 7. The mechanical hen pecked at a green coloured grain and the chick had the choice of green or orange grains. All grains were firmly glued down.

Figure i.3: Mechanical hen used by Turner [248] in *Social feeding in birds*.



(A) Rundus *et al.* 2007 [207]



(B) Taylor *et al.* 2008 [242]

Figure i.4: Advance robotised lures used in behavioural studies. (A) Robotic squirrel to study predator avoidance. (B) Robotic tungara frog to study mate selection.

respond autonomously to an animal interaction. It was only in 2000 that the first study using a robot capable of interacting with animals autonomously appeared. Vaughan *et al.* 2000 [249] shows that a robot can gather ducks by acting like a sheepdog. The robot uses a model to predict the behaviour of the ducks, and moves accordingly to force the group to reach a specific position. This study highlights that autonomous robots can allow more complex interactions by interacting with groups of animals and no longer a single individual. Since then, the number of studies in the field has increased significantly [204]. For example, researchers have developed a chicken robot capable of interacting with chicks [92] to study the imprinting mechanism. Similarly, they also developed a cockroach robot capable of modulating cockroaches collective decision making to get them to move from a safe shelter to an exposed shelter [97]. These studies all present robots cooperating with a group of animals in a closed-loop interaction: robots influence animals, which in turn will influence the behaviour of robots.

Robots do not necessarily need to be mobile to interact in closed-loop with animals.

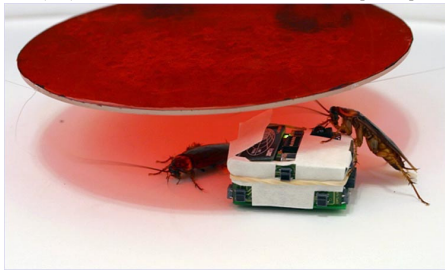
(A) Vaughan *et al.* 2000 [249](B) Halloy *et al.* 2007 [97](C) Gribovskiy *et al.* 2015 [92]

Figure i.5: Autonomous robots interacted socially with animals. (A) The robotic sheepdog with a ock of ducks. (B) The InsBot mobile robot with cockroaches. (C) The PoulBot mobile robot with chicks.

For example, in Stefanec *et al.* 2017 [228] the robot developed to interact with bees is actually a fixed grid of robots that simulate the presence of bees in a hive environment through vibration and heat. Finally, the robot can be mounted directly on the animal to monitor the animal activities or send stimuli. Studies using smart collars containing a GPS and various environmental sensors as well as speaker and electric shock systems. In Correll *et al.* 2008 [52], they used these collars on cows to influence them to stay in a virtual enclosure and to cause stress in animals leaving the area. This stress will be transmitted within the group by the cows themselves and will increase the aggregation of individuals.

Based on these studies, three types of autonomous robots can be described:

- (i) First, the artificial systems do not copy any feature of the animal but send cues that the animal responds to. Being biomimetic is not a necessity to interact with animals [52].
- (ii) Second, the artificial agent acts as a different animal species such as a sheepdog. The robot can be biomimetic but to another species like a dog for the sheep [249].
- (iii) Third, the artificial agent is mimicking the animal, luring it as if being the same animal species and using similar signals and behaviours [97]. We call this approach biomimetic and it will be the one developed in this manuscript.

A large number of studies focus on fish species, particularly for the easier conditions of fish maintenance in laboratory [204]. However, it is more difficult to understand

collective behaviours within fish groups as the transfer of information is less clear and more complex than, for example, in insects. In addition, the heterogeneity of individual behaviours increases the difficulty of studying the behaviour of the entire group [110]. In different fish species, work has been carried out to replicate locomotion [19,20,22,126,150] or the visual aspects [1, 10, 17, 138, 190, 192] to show their importance in attracting conspecifics. Other cues have been investigated, like on the electric fish *Mormyrus rume* where a robot was able to interact by displaying prerecord electric organ discharges (EODs) in answer of EODs made by the live fish [258].

i.4 Thesis overview

In this thesis, I investigated the mechanisms of collective movement and decision-making of social fish. This study focused on zebrafish (*Danio rerio*) in constrained environment. This thesis aims to answer the following questions:

- **How do social fish behave in constrained environments?** Group size has an effect on individual behaviours, group cohesion and transitions. During collective departures, the order of exit of the fish corresponds to the topology of the group a few seconds before the departure [211]. Leadership is shared among individuals: each fish has the same ability to initiate a collective movement. Nevertheless, there is a strong heterogeneity between groups on the proportion of each individual to be leader [48]. These topics will be covered in Chapters 1 and 2 of this manuscript.
- **How to model collective movement in constrained environments?** Our agent-based model takes into account the modulation of the collective behaviours depending of the location in the environment and the social context. It will be presented in Chapter 3.
- **How to integrate autonomous robots with groups of fish in a constrained and complex environment?** Using a biomimetic robot driven by the context-dependent model, we were able to integrate this biomimetic agent into a zebrafish group. The robot interacts with fish in a closed loop and is recognised as a conspecific [41–43]. The features used to achieve this result will be detailed mainly in the Chapter 4 but also in Chapters 5 and 6, which show that the improvement of the model also improves the integration of the robot with social fish.

- **How to modulate groups of fish using robots?** We use robots to initiate collective movements and lead the group of fish from one room to another. We also modulated the time spent in each room by the fish using the robot as an attractor [44]. This will be explained in the Chapter 7.

My work focused on the use of a constrained environment composed of two rooms connected by a corridor. This experimental set-up can be used to highlight decision-making and collective movements from one site to another. This study focused on a social fish species, the zebrafish (*Danio rerio*). Highly appreciated in laboratory for its robustness, it is a model organism [171, 172] living in a shoal-type group. I also produced a model simulating the movement of these fish in a constrained environment. The advantage of developing a context-dependent model is the inclusion of complex dynamics that are less apparent in a simple homogeneous environment. Finally, I used autonomous robotic agents, reproducing the patterns of a zebrafish and controlled by the model, to create a closed loop system where the robots are recognised as conspecific by the fish. I was then able to study leadership and collective movements using robots to modulate the collective behaviour of zebrafish.

i.5 Project ASSISIbf

This work was part of the European project (FP7) ASSISIbf (Animal and robot Societies Self-organise and Integrate by Social Interaction with bees and Fish).

The goal of ASSISIbf was to conceive autonomous and self-organised mixed-groups of animals (in this case, with fish and bees) and robots, with robots capable of learning how to interact with the animals, of adapting their behaviour to the animals response, and of modulating their collective behaviour. Six partners were part of the ASSISIbf project. Our team at the Université Paris Diderot (LIED lab) developed fish behavioural models and performed experiments involving fish and robots. The roboticists at the EPFL (LSRO lab) designed and built the robots used during our experiments. Meanwhile, the four other partners were involved with mixed-groups of bees and robots. The roboticists from the university of Zagreb (LARICS lab) designed and built robots and software tools used to conduct experiments involving mixed-groups of bees and robots, with some parts developed by another partner, the company Cybertronica. The ethologists from the University of Graz (Artificial Life lab) performed these experiments. The group from the University of Lisbon (FCiências.ID lab) developed multi-agents simulation tools and optimisation frameworks.

Part I

Zebrafish collective behaviour

Zebrafish (*Danio rerio*) are a gregarious model fish. This species is a prime candidate for research laboratories due to how easy it is to breed, its robustness, its gigantic spawning and its rapid maturity. It is now a model organism [171,172] and its DNA was fully sequenced in 2013 [108]. There are a multitude of zebrafish strains, with various genetic and phenotypic properties, used according to research needs, in oncology [208], neurology [95], toxicology [73], etc.

This freshwater fish of the Cyprinidae family is small in size (3.5 to 4.5 cm). Originally from India, zebrafish are diurnal. Their habitat is very varied and ranges from simple shallow and weak flowing rivers to non-permanent ponds or even irrigation canals. These fish are very robust and can adapt to very variable water temperatures ranging from 6°C in winter to 38°C in summer [226]. *Danio rerio* is omnivorous. Its diet mainly includes zooplankton and insects, but it sometimes feeds on phytoplankton, algae, detritus, sand, fish scales and other invertebrate eggs [70,152,224]. Zebrafish are oviparous; every two to three days, depending on their age, females can lay several hundred eggs at a time. On average, zebrafish mature 75 days after fertilisation [226].

In a previous study [212], the difference in behaviour was studied between two wild-type zebrafish lines, AB with short fins, and TL with longer fins. AB zebrafish has been used in all the works presented in this manuscript. The study takes place in a square arena and includes two identical spots (Fig. 6) over a period of one hour. This study highlights greater cohesion within TL groups and greater interest in spots among AB. It also shows that zebrafish, AB or TL, are constantly in motion, and would rather swim near walls or spots than in the empty space of the set-up. The fish swam mainly together and oscillated from one landmark to another with short rest periods. No selection of a specific spot was observed.

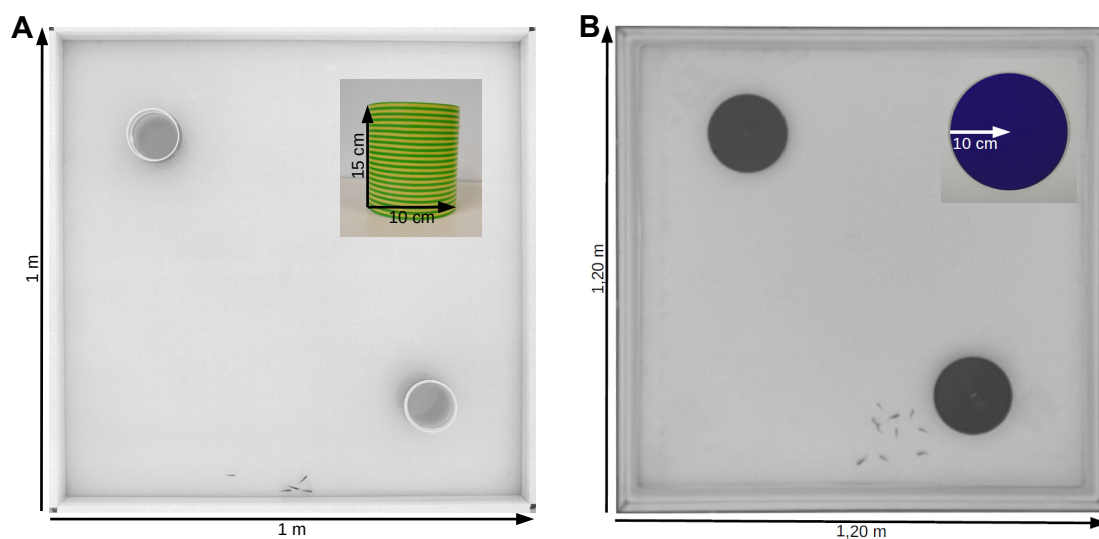


Figure 6: Experimental set-up with spots. (A) Cylinders, (B) Discs.

With this knowledge and in order to be able to study more specifically the cohesion, decision-making, organisation and use of space by the group during exploration events,

the work presented in this first part focuses on the study of zebrafish in a constrained environment developed for this purpose. The set-up consists of two rooms (patches) separated by a corridor where fish can swim in groups and move freely between areas. This corresponds to their natural fragmented habitat and allows us to observe repetitive social interactions leading to collective departures.

In Chapter 1, we study the use of space and the cohesion of groups according to their size in this maze. We present the experimental results of pair interactions and collective departure organisations of seven sizes of zebrafish groups. Using a tracking system that gives the position and identity of each fish without marking them, we computed individual measurements such as speeds, distances travelled and respective pair interactions. We analysed collective departures by sorting the fish by their exit rank and distance rank to the initiator (the first fish leaving a room) and we quantified site transitions. Our results show that population size has an impact on individual speeds and distances travelled. We show that there are preferential interactions between respective pairs and that the larger the population, the weaker these preferential interactions are. The study of area transitions reveals several social structures: “collective transitions”, “one-by-one transitions” and “u-turns”. We show that population size influences the proportion of these transitions. Finally, the results of this chapter show that the zebrafish transition is determined by the topological structure before departure, where the second ranked fish and the last ranked fish leaving a room are, most of the time, the closest and the farthest fish from the initiator, respectively.

This work is based on the publication [211]:

Séguret A, Collignon B, Cazenille L, **Chemtob Y**, Halloy J. Loose social organisation of AB strain zebrafish groups in a two-patch environment. *PloS one* 2019

The experimental set-up was designed and built by Bertrand Collignon, Axel Seguret, Leo Cazenille, José Halloy and myself. The experimental arena was built by Bertrand Collignon, Axel Seguret and myself. The experiments were carried out by Axel Seguret, Bertrand Collignon and myself. I produced part of the preliminary analysis. This document was written mainly by Axel Seguret, with the help of José Halloy and Bertrand Collignon.

Chapter 2 focuses on decision-making and leadership. We present an analysis of the collective departures of twelve groups of two, three, five, seven and 10 zebrafish swimming in the two rooms maze. We focus our analysis on the identity of the first fish that exit the rooms, the initiators, and on the possible features that favour them to take the lead of the shoal. Our results show that groups of similar sizes display a wide range of distribution of the leadership ranging from a homogeneous to a strongly asymmetrical sharing of the initiative. However, in most of the group the number of departures is not homogeneously

distributed among the group members, some fish leading more often than others. We highlight that those initiators do not have a stronger influence on their congeners but perform more attempts than other fish. Indeed, the number of successful departures is linearly correlated with the number of attempts, giving the same success rate for all fish. This conclusion is observed for all group sizes but the success rate decreases for larger populations. Finally, we provide evidence that the number of attempts performed by the fish is related to their intra-group ranking for average speed, the most mobile individual leading more attempts than slower fish.

This work is based on the publication [48]:

Collignon B, Séguret A, **Chemtob Y**, Cazenille L, Halloy J. Collective departures and leadership in zebrafish. PloS one. 2019

The experimental set-up was designed and built by Bertrand Collignon, Axel Seguret, Leo Cazenille, José Halloy and myself. The experimental arena was built by Bertrand Collignon, Axel Seguret and myself. The experiment was done by Bertrand Collignon, Axel Seguret and myself. I produced some of the preliminary analysis. This document was written mainly by Bertrand Collignon.

Chapter 1

Loose social organisation of AB strain zebrafish groups in a two-patch environment

Abstract

We study the collective behaviour of zebrafish shoals of different numbers of individuals (1, 2, 3, 5, 7, 10 and 20 AB zebrafish *Danio rerio*) in a constraint environment composed of two identical square rooms connected by a corridor. This simple set-up is similar to a natural patchy environment. We track the positions and the identities of the fish and compute the metrics at the group and at the individual levels. First, we show that the number of fish affects the behaviour of each individual in a group, the cohesion of the groups, the preferential interactions and the transition dynamics between the two rooms. Second, during collective departures, we show that the rankings of exit correspond to the topological organisations of the fish prior to their collective departure. This spatial organisation appears in the group a few seconds before a collective departure. These results provide new evidences on the spatial organisation of the groups and the effect of the number of fish on individual and collective behaviours in a patchy environment.

1.1 Introduction

Across the collective behaviours observed in social animals, collective movements [75, 100, 103, 176, 183, 197, 231, 233], nest site selections [4, 59, 82, 203] and site transitions [98] have been evidenced in many species. In this latter case, the groups face several alternatives and transit between them. The study of these transitions relies on decision-making processes and individual or collective preferences for environmental [50] or group members characteristics [75, 76, 106, 195] like leadership [56], motion [27] or behavioural traits, for example bold and shy individuals [59, 201].

Numerous animal species have been observed in different sorts of constraint setups or mazes to study collective movements from one site to another: corridor type [75,76,185], Y-maze [253], T-maze [124] or Plus-maze [155,220]. Such constraint set-ups engage the animals to transit alone or in group from site to site and allow the observation of leadership [26,48,252], initiation of group movements [27,205,252], followers organisations [252], pre-departure behaviours [26,27] and sites transitions [98,164,167]. In these latter cases the authors studied the transitions from one site to the other of one and two fish separated by a transparent partition (*Gasterosteus aculeatus* and *Sciaenops ocellatus*). Although such experimental procedure provided evidence of different leader/follower behaviours in fish, they prevent the fish from direct interactions between each other during the departures.

On the one hand, studies performed with groups of fish swimming together have evidenced that the group size can impact swimming behaviours with a variety of results. Several papers showed that the speed, the turning speed, the nearest neighbour distances, the milling or the alignment are affected by the number of group members [11,102,247]. The authors present opposite results depending on the species: increasing the group size of *Oreochromis niloticus* (330 and 905 fish), makes a stronger alignment [11], while for *Notemigonus crysoleucas* (30, 70, 150 and 300 fish) alignment decreases [247]. On the other hand, *Shelton et al.* [214] have shown that the density influenced nearest neighbour distances in *Danio rerio* when *Frommen et al.* [83] noticed that shoaling preferences might not always be influenced by a higher number of group members but also by the density and cohesiveness of the respective groups.

We focus on the collective movements between two environmental patches of different numbers of zebrafish. We have shown in a previous study that zebrafish transit without interruption from one landmark to another one in an open environment during trials of one hour [212]. Moreover, we have shown that groups of fish were swimming along the border of the tank and thus had a strong thigmotactic tendency [49]. Inspired by the experiments developed for highly dynamical groups of animals like the ants [113] or the fish [75,76,185] in constraint set-ups, we created a binary choice set-up able to channel the groups of zebrafish and to increase their stabilisation in the patches. Our experimental set-up is composed of two environmental patches (rooms) linked by a corridor. The geometry of the setup is designed to study collective transitions between patches allowing to quantify the group cohesion and collective decision-making. In this study, we aim at characterising the dynamics of departure during sites transitions for several group sizes (1, 2, 3, 5, 7, 10 and 20 individuals) of AB zebrafish swimming in a constraint environment. Here we consider group size as the number of fish in a group.

Zebrafish are gregarious vertebrate model organisms often used in behavioural studies [171,172]. In the laboratory as much as in the nature, the zebrafish behave in groups [75,152,226]. They are native to the Indian sub-continent and live in small groups or in

big shoals of several hundreds of fish depending on the region and the water or the environmental features (temperature, pH, human activity, predators, ...) [174,195,225,238]. Zebrafish live in a wide variability of habitats with varying structural complexities [7,238] (from river channels, irrigation canals to beels) and we based our experimental method on the observations of fish swimming in a constraint set-up composed of two identical squared rooms (evoking patchy environments [257]) connected by a long corridor. The goal of the paper is to measure the impact of the groups of fish on the collective decision making between two identical patches. This methodology has been developed in [4].

Here, we study the collective dynamics of group transitions in zebrafish with a new type of set-up. By observing groups composed of different numbers of fish, we evaluate the influence of the number of individuals in the shoals on the structure of the group (cohesiveness, inter-individual distances) and on the sequence of exit for each collective departure. By performing trials of one hour, we could observe a large number of successive transitions.

1.2 Results

1.2.1 Group structure and number of individuals

First, we studied the change of the group structure according to the location of the group and the number of fish by measuring the nearest neighbour distances for each individual. Fig 1.1 shows the boxplots of the medians of the nearest neighbour distance distributions for each fish in 5 shoal sizes (2, 3, 5, 7 and 10 fish). We chose to use the Nearest Neighbour Distance (NND) because we wanted to describe the shoal dynamics. If we took an average of all Inter-Individual Distance (IID), this would have been higher in larger shoals than smaller shoals because larger shoals take up more volume. Also the NND lowers the effect of the geometry of the set-up compared to IID. Thus, the boxplots for each area (rooms or corridor) and each number of individuals consist in 12 values of medians. For groups of 2 to 3 individuals, the increase of the number of individuals made the medians of the nearest neighbour distances decrease until a plateau value of approximately 4 cm. For groups of 5, 7 and 10 individuals, the medians of the nearest neighbour distances remained very close from each other.

We compared with a Two-way ANOVA the distributions of the medians of the nearest neighbour distances for each fish focusing on each area (room 1, room 2 and corridor) or each number of individuals. The test shows that there is an effect of the number of individuals on the medians of the nearest neighbour distances ($p - value < 0.005$, $F = 3.87$, $MS = 0.00092$ and $df = 4$). However, it does not show any significant effect of the type of the area – Room 1, Room 2 or Corridor – ($p - value > 0.1$, $F = 1.96$,

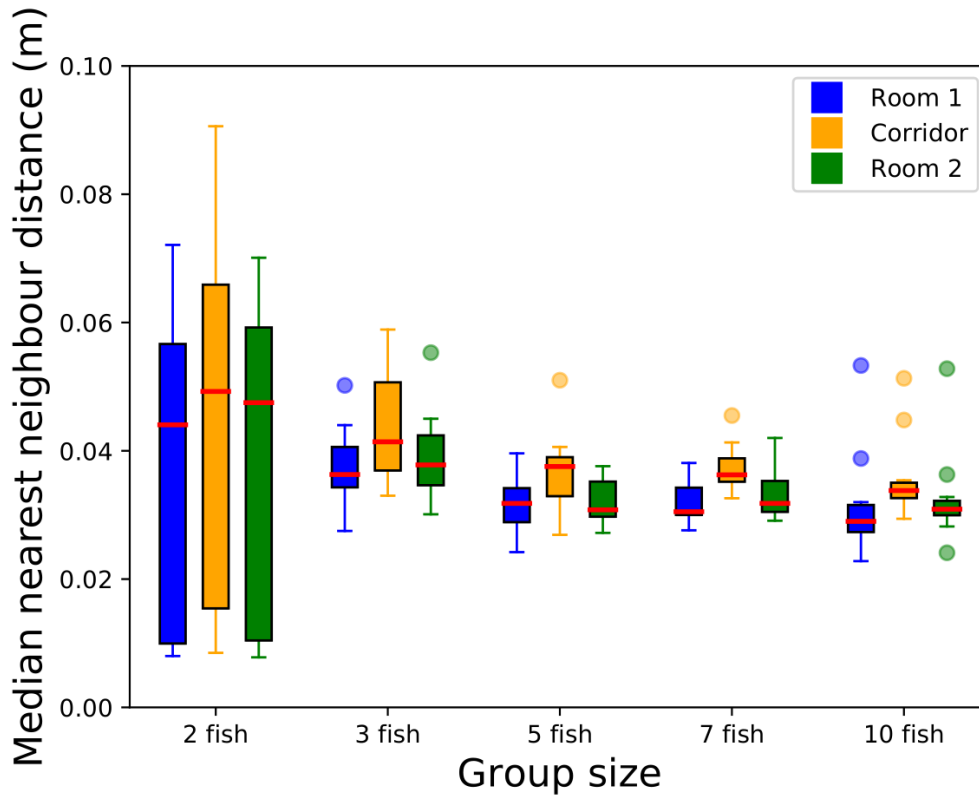


Figure 1.1: Boxplots of the medians of the nearest neighbour distance distributions for each zebrafish (blue) in the room 1, (green) in the room 2 and (yellow) in the corridor. The red line is the median. The higher the number of individuals, the lower the nearest neighbour distances between fish.

$MS = 0.00047$ and $df = 2$) nor of an interaction between the number of individuals and the type of the area ($p - value > 0.5$, $F = 0.15$, $MS = 0.00004$ and $df = 8$).

1.2.2 Oscillations and collective departures

Then, we characterised the collective behaviours of the fish. In particular, we focused our investigation on the oscillations between both rooms and the dynamics of the collective departures of the groups. First, we studied the repartition of the fish among the two rooms. Approximately 70% of the positions of the fish were detected in the rooms, independently of the number of individuals. In the Fig 1.2, we show that 80% of the time, less than 20% or more than 80% of the whole group is detected in the room 1. This result highlights that, as expected for a social species, the fish are not spread homogeneously in the two rooms but aggregate collectively in the patches, with only few observations of homogeneous repartition in both rooms. However, this analysis also shows that the proportion of observations with equal repartition between both rooms (40-60%) increases with the number of individuals. Thus, even if they are mainly observed together, fish in large group have a slightly higher tendency to split into subgroups.

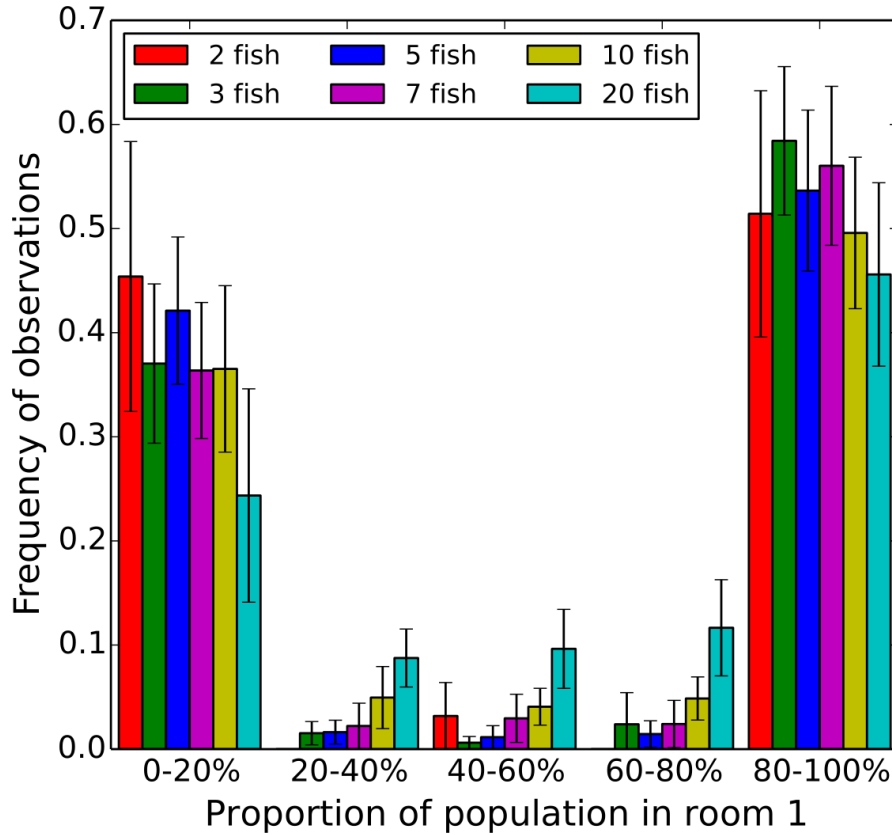


Figure 1.2: Frequency of the proportion of the whole group in room 1. Almost 35% of the time, 0 to 20% of the whole group is present in the room 1 when almost 50% of the times, 80 to 100% of the whole group is in the room 1. Focusing on more equal repartition of the fish between the rooms (40 to 60% of the whole group), larger groups lead to higher frequency of group splitting.

We show that the frequencies of observations for the proportions of 80 to 100% of the whole group in the room 1 are higher than 50% for all group sizes, except for 10 and 20 fish. For each trial, we defined the room 1 as the starting room where we let the fish acclimatize during 5 minutes in a transparent perspex cylinder. This may explain the observed bias of presence in favour of room 1 that may be a consequence of a longer residence time at the beginning of the trials.

Then, since the fish are observed most of the time forming one group in one of the two rooms, we studied the transitions of the majority of fish between the two patches during the whole experimental time. In Fig 1.3, we plot the number of transitions between both rooms for the plot of the means of the numbers of transitions and their standard deviations in a table). First, we present the total number of transitions (*All transitions*) for all group sizes (referred as *All transitions*). Then for groups with at least two individuals, we detailed these transitions into two subcategories: *Collective transitions* (they occur when the whole group transit between both rooms through the corridor, i.e. the majority of the group is detected successively in one room, the corridor and the other room) and *One-by-one transitions* (they occur when the fish transit one by one from one room to the other through the corridor, i.e. the majority of the group

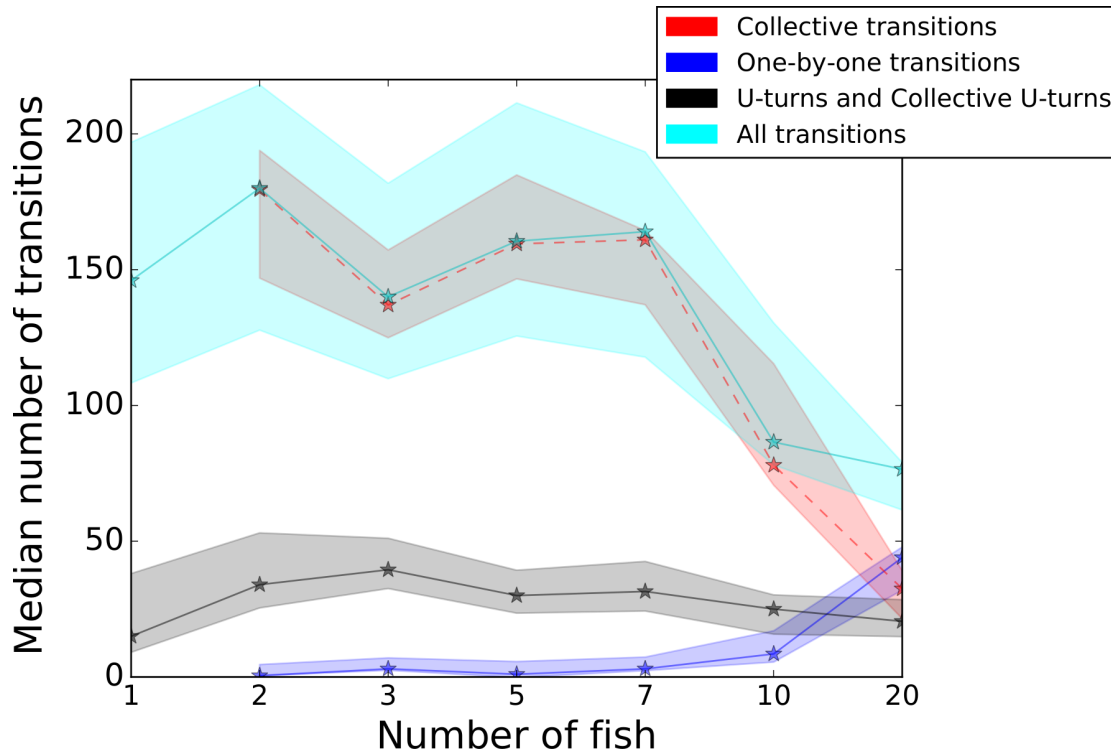


Figure 1.3: Mean and median number of transitions for groups of different numbers of individuals. The red curve shows *Collective transitions*, the blue curve shows *One-by-one transitions*, the black curve represents the *Collective U-turns* and the magenta (*All transitions*) is the sum of *Collective transitions* and *One-by-one transitions*. The stars show the medians. *One-by-one transitions* occur when the fish transit one by one from one room to the other through the corridor. *Collective transitions* appear when the whole group transit between both rooms through the corridor. *Collective U-turns* occur when the whole group go back to the previous room. The dashed lines facilitate the lecture. The figure shows that increasing the number of individuals makes the number of *Collective U-turns* and *Collective transitions* decrease and the number of *One-by-one transitions* increase. Each point shows the median of 12 values.

is detected successively in one room and then in the other room, indicating that the fish did not cross the corridor together). In addition, we also quantified the *Collective U-turns* that occur when the majority of the group was detected successively in one room, in the corridor and back to the previous room.

For larger groups, the numbers of *All transitions*, *Collective transitions* and *Collective U-turns* decrease while the number of *One-by-one transitions* increases. For the transitions (*Collective*, *One-by-one* and *All*), this tendency intensifies for bigger groups of 10 and 20 zebrafish. Also, for groups of 3 zebrafish, there are less *Collective transitions* (as well as *All transitions*) than for groups of 2, 5 and 7 zebrafish. *U-turns* remained rare and are very stable for all shoal sizes and their highest means are reached for groups of 2 and 3 zebrafish. *One-by-one transitions* are as well very rare for small groups and increase when the shoal size reaches 10 zebrafish.

For each number of fish (Fig 1.3), we compared with a Kruskal-Wallis test the distributions of the number of transitions (*Collective*, *One-by-one* and *U-turns*) and found: for

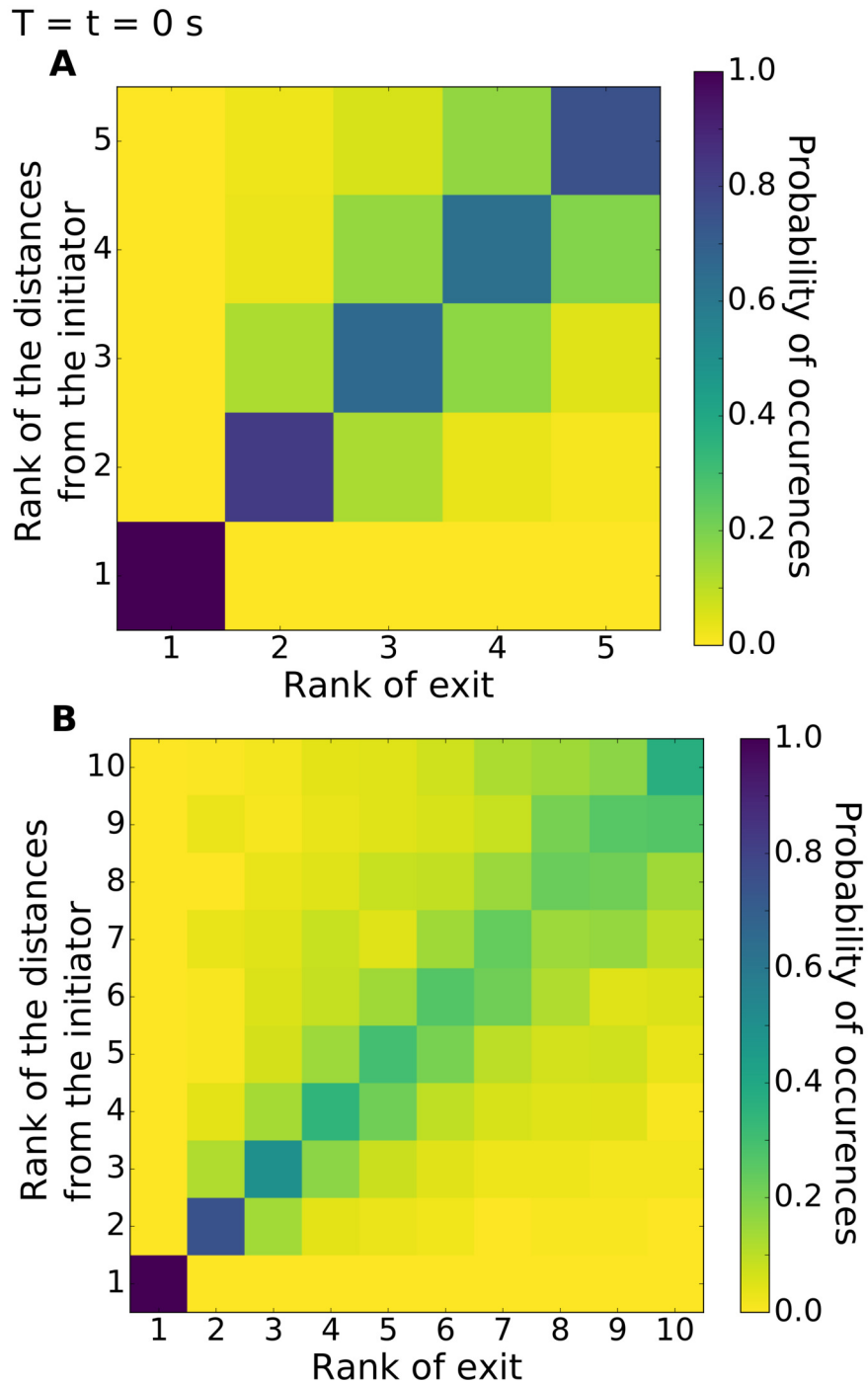


Figure 1.4: Probability of occurrence of the rank of exit with the rank of distance to the initiator for groups of 5 zebrafish (left column) and 10 zebrafish (right column). We counted $N = 1456$ exits for 12 replicates with 5 zebrafish and $N = 277$ for 12 replicates with 10 zebrafish. (A) and (B) show the map at the time when the initiator leave the room. As an example, in (A) the probability of occurrence where the second fish leaves the room and has the shortest distance from the initiator is 0.82. This probability decreases to 0.12 for fish with rank of 2 for exit and rank of 3 for distances (the second closest distance from the initiator).

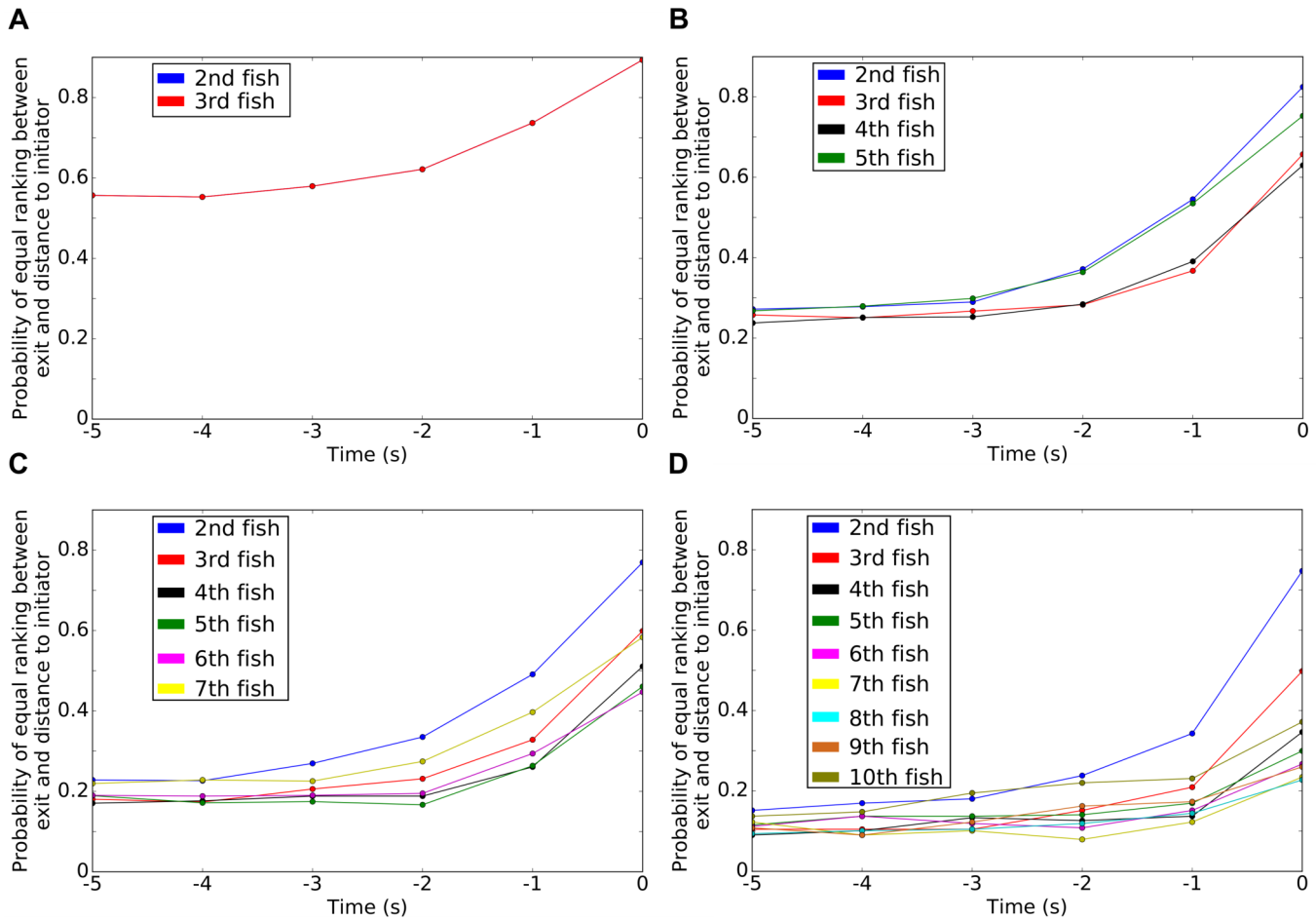


Figure 1.5: Time series of the probability of equal ranking between the rank of exit and the rank of distances from the initiator for groups of (A) 3 zebrafish, (B) 5 zebrafish, (C) 7 zebrafish and (D) 10 zebrafish. This figure is related to the results shown in Fig 1.4 (the diagonal). We plot a time series of the 5 seconds before the initiation. We show that the probability increases strongly 2 seconds before the initiation. We see also that this probability is the highest for the first ranked fish and higher for the 3 first ranked fish and for the last ranked fish. This behaviour is also valid even few seconds before the initiation. In (A) the line-plots for 2nd and 3rd fish are overlapping.

1 fish, $df = 2$, $\text{Chi-sq} = 31.62$ $p < 0.001$; for 2 fish, $df = 2$, $\text{Chi-sq} = 30.76$, $p < 0.001$; for 3 fish, $df = 2$, $\text{Chi-sq} = 30.94$, $p < 0.001$; for 5 fish, $df = 2$, $\text{Chi-sq} = 30.41$, $p < 0.001$; for 7 fish, $df = 2$, $\text{Chi-sq} = 30.54$, $p < 0.001$; for 10 fish, $df = 2$, $\text{Chi-sq} = 19.22$, $p < 0.001$ and for 20 fish, $df = 2$, $\text{Chi-sq} = 18.36$, $p < 0.001$. For each group size, we show that at least one of the distributions is significantly different from the others. The Tukey's honest significant difference criterion shows that: all the distributions are significantly different ($p < 0.05$) except in groups of 10 individuals between *Collective U-turns* and *One-by-one transitions* and in groups of 20 individuals between *Collective U-turns* and *Collective transitions*.

As most of the transitions occur in groups, we analysed the dynamics of collective departures from the rooms with a particular emphasis to the pre-departure period.

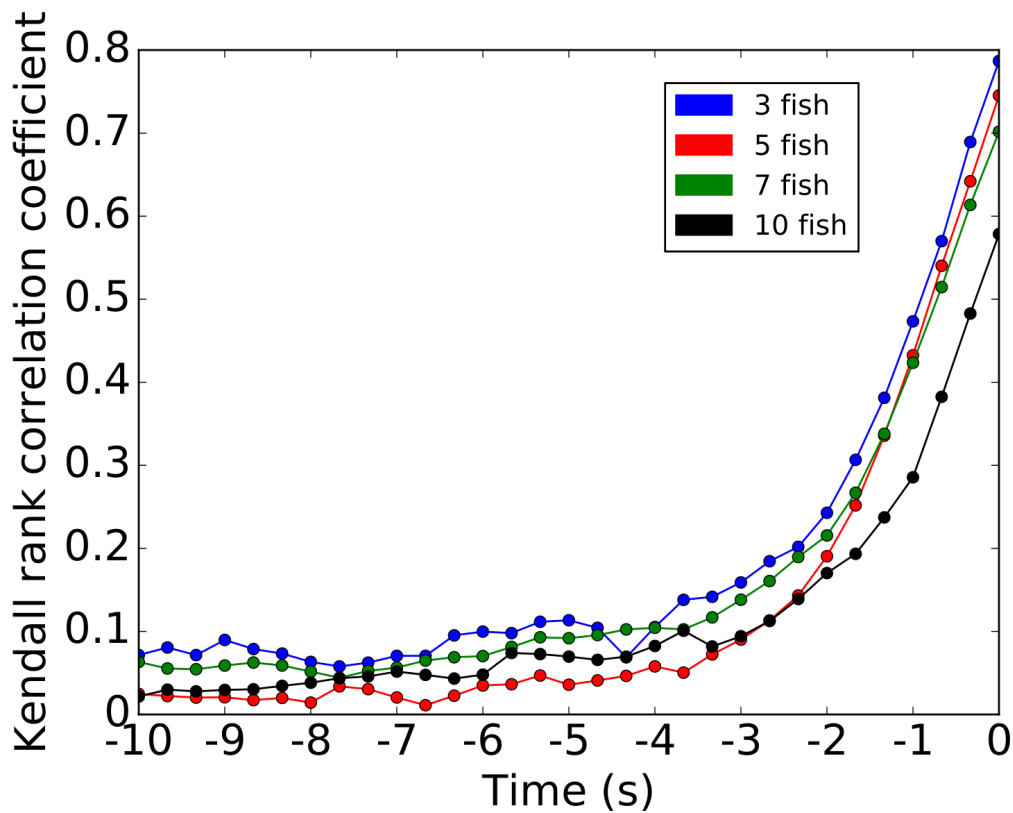


Figure 1.6: Time series of the Kendall rank correlation coefficient. It has been calculated every $1/3$ second starting 10 seconds before the initiation. The Kendall rank correlation coefficient is a measure of ordinal association between two measured quantities. It goes to 0 when the two quantities are independent and goes to 1 if they are correlated. For example with 5 zebrafish, the time series shows that from 4 seconds before the initiation the Kendall rank increases from 0.06 to 0.75 (at $T = t = 0$ s). Whatever the number of individuals, we conclude that the closer to the initiation the higher the correlation between the rank of exit and the rank of the distances with the initiator.

Thus, for each collective departure of the fish, defined as the whole group leaving one of the resting sites for the corridor towards the other one, we identified the ranks of exit of each fish and also their distances from the first fish leaving the room (i.e. defined as the initiator) measured at the departure timing of this initiator. Fig 1.4 represents the normalised contingency table of the rank of exit for all zebrafish from both rooms (without distinction) with the rank of the distances of all zebrafish to the initiator. These results correspond to 12 replicates of groups of 5 and 10 zebrafish. The initiator has a rank of exit and a rank of distances of 1. For example, in (A) the probability that the first fish to follow the initiator (rank 2) was also the closest fish of the initiator when it exited the room is 0.82. As evidenced by the darker diagonal of the contingency matrix, the rank of exit was closely related to the distance from the initiator at the beginning of the departure. In Fig 1.5, we plot for 3, 5, 7 and 10 zebrafish the values of the probability of equal ranking between the exit and the distances with the initiator (i.e. the diagonal of the previous plots – Fig 1.4) for different time-lag before the exit of

the initiator. In particular, we computed the ranks of the distances from the initiator at 1 to 5 seconds before the exit of the initiator. First, these measures show that the further from the time of the initiation the lower the probability of equal ranking. This assessment is valid for every shoal sizes. Second, we see that the probability of equal ranking is often higher for the first and for the last ranked fish even few seconds before the initiation (around 2 seconds before the initiation). In Fig 1.6, we use the Kendall rank correlation coefficient to see if the rank of exit and the rank of distances with the initiator are dependent (close to 1) or not (close to 0) through the time. For every group sizes, we show an increase of the Kendall rank correlation coefficient when closer to the initiation. For 3 zebrafish, the time series show that from 4 seconds before the initiation the Kendall rank correlation coefficient fully increases from 0.11 to 0.79 (at $T = t = 0$ s). For 5 zebrafish, it increases from 0.06 (at $T = t - 4$ s) to 0.75 (at $T = t = 0$ s), for 7 zebrafish, it increases from 0.10 to 0.70 and for 10 zebrafish, it increases from 0.08 to 0.58. These results show that for all group sizes, the closer to the initiation the higher the correlation between the rank of exit and the rank of the distances with the initiator.

1.3 Discussion

We studied the impact of the number of individuals in the shoals (1, 2, 3, 5, 7, 10 or 20 individuals) on the collective motion and the collective departure between two environmental patches in adult AB zebrafish.

Here we consider group size as the number of fish in a group. By changing that number of fish it may lead to size or density effects. We do not address the question of untangling size or density effects in this study. The density is related to a need of individual space and the group size is related to a limitation in the considerations, for each individual, of the other members of the group. Our set-up reached a compromise between reducing the size of the set-up for small numbers of individuals, that would have an effect on the small group collective transitions, and increasing the size of the set-up, that would have led to tracking issues (less pixels by fish) [181]. Actually, if we resize the set-up proportionally to the density, we will modify the lengths and the widths of the rooms and the corridor. This would have an effect on the time of residency in the rooms or on the decision to cross the corridor and would introduce new variables in the experiment.

Furthermore, the Fig 1.1, showing that the more individuals the lower the nearest neighbour distances, elucidates the previous statement. For small groups, the medians of the nearest neighbour distances distributions decrease when increasing the numbers of individuals. This result could be due to an effect of the set-up where the environment is not resized in proportion of the number of the individuals. For groups of at least 5 fish, it seems that the medians of the nearest neighbour distances distributions are very similar. Here, it is possible that the threshold value of the nearest neighbour distance

for the AB zebrafish has been evidenced. In this latter case, it means that the zebrafish spread more in the set-up which show that the groups are not denser when we increase their sizes. Moreover, the results of the Two-Way ANOVA evidenced that the influence of the number of individuals over the nearest neighbour distances was significant when the influence of the type of the area (rooms or corridor) is not. In [67], the authors have shown that the mean of the nearest neighbor distances is about 30 mm for groups of 8 wild type zebrafish (presumably AB). Their results are very close from ours and the experiments were performed in a circular shape bowl of 20 cm in diameter. Both of our results may show that the environment has no influence on the compact structure of big groups. In parallel, if we focus on the distances between all pairs within a group we show that the higher the number of individuals the higher the medians of the distances between all respective pairs of zebrafish (S4, S5, S6, S7, S8 and S9 Figs). Finally, we show that the higher the number of individuals the lower the time the fish stay with their nearest neighbours. The combination of these results shows that there is a clear effect of the number of individuals on the cohesion; an effect that we already have shown in [212] where the bigger the group the higher the cohesion of the whole group.

It seems that there are preferential interactions between zebrafish (S4, S5 and S6 Figs) and increasing the number of individuals will affect these interactions: respective pairs are less cohesive in larger groups. We consider that revealing the time spent by the fish with their nearest neighbours show stronger informations about preferences between pairs than the median distances of each couple, which are already approximations. Hence, we show that for groups of 3 fish the distribution of the number of times the fish stay with their nearest neighbours is not significantly different from a random uniform distribution. It means that for such number of individuals there is no preferential interaction. For bigger groups, these distributions are significantly different from a random uniform distribution which means that there are preferential interactions. Preferential interactions have been evidenced in other species: Briard et al. [32] show affinities, hierarchy and pairs interactions in a group of domestic horses, [114, 123, 234, 236] show that the affinity between individuals (monkeys, *Macaca mulatta*, *Macaca tonkeana*, *Papio ursinus*; or fish, *Gasterosteus aculeatus*) play a role in the collective movements. We propose two hypotheses that could explain the change of the interactions between pairs of zebrafish when changing the number of individuals. On the one hand, in groups larger than two fish, each zebrafish has to choose the preferred partners, between all other fish. In larger groups there are more individual choices and more preference tests. On the other hand, the patchy environment may break pair interactions and may force the emergence of new pairs. These two hypotheses could explain the dynamics of the pair interactions observed during the experiments.

The fish are detected 70% of the time in the rooms. On average, they spend about

10 seconds in a room, transit to the other room through the corridor (4 seconds on average) and then come back. They oscillate between the rooms. In a previous study we showed that zebrafish also transit and oscillate between landmarks in an open environment [212]. The Fig 1.3 shows that most of the transitions are collective when the Fig 1.2 shows that the whole group swim generally together in both rooms. This observation is strengthened by the very rare number of *One-by-one* transitions between the rooms. However, groups of 10 and 20 zebrafish show sharp decreases in the number of collective transitions. This drop could be due to the topology of the set-up and congestion effects. Larger groups can split into smaller subgroups. The threshold we imposed in the analysis of the collective transitions (below 70% of the whole group, the transitions were not taken into account) may reinforce this effect. This seems to be confirmed by the mean and median number of transitions for different numbers of individuals when all the fish start to move from a room: the larger the group, the lower the number of transitions with the whole group. Also the Fig 1.2 shows that the bigger the groups, the higher the frequency of observations of sub-groups located in the rooms and in the corridor (20-40%, 40-60% and 60-80%). Many studies have analysed the fusion-fission mechanisms occurring in groups of fish or mammals. In [57, 105, 133], the authors show that these mechanisms are frequent in the wild and generate body length assortment within groups of fish (*Fundulus diaphanus*, *Notemigonus crysoleucas*, *Catostomus commersonii*, *Poecilia reticulata*). Sueur et al. [233] show that fission-fusion mechanisms participate in the information transfer between subgroups and the group of *Myotis bechsteinii*.

In the corridor, we observe few U-turns. The zebrafish swimming preferentially along the walls and a canalisation effect of the corridor may explain this observation. As expected, connecting the two patches, the corridor is used as a mere transit area.

We show that the organisation of the group during collective departures takes place during a short pre-departure period and is related to the distances between the initiator (of the exit from the room) and the other fish. Ward *et al.* have shown that the first fish (of a group of 5 *Dascyllus aruanus*) to follow the initiator is generally (rank = 2: 53% of the trials over 2 trials for each 15 groups of fish) the nearest neighbour of the initiator and that the frequency of equality between the rank of exit and the rank of the distances from the initiator decreases, with these results rank = 3: 27% and 33%, rank = 4: 20% and 7% then rank = 5: 0% and 7%) [252]. We tested four different numbers of individuals (3, 5, 7 and 10 zebrafish) and show similar results especially on the decreasing trend of the probability when focusing on the next ranked fish and Fig 1.5). However, we observed an extremely high probability of equal ranking for the first fish that follows the initiator (rank = 2: 75% to 90%), high probabilities of equal ranking for the second and the last fish that follow the initiator (rank = 3: 50% to 65% and rank = last fish: 38% to 75%) and showed that the probabilities of equal ranking for the other fish are quite similar to each others. The rank of exit and the rank of

the distances from the initiator are strongly correlated at the moment of the exit ($T = 0s$, Fig 1.6), from 60% to 80%. Hence, it seems that the organisation of the zebrafish groups (2nd, 3rd and last ranked fish) during the collective departures is topological. Other studies about the organisations of collective departures show a joining process for *Equus ferus caballus* that is related to affinities and hierarchical ranks [32]. Rosenthal *et al.* show that, in groups of *Notemigonus crysoleucas*, the initiator is the closest fish from the group boundary in 27% of the cases and the first responder is the closest fish from the group boundary in 19% of the cases [205]. Moreover during the initiation, when fish leave the rooms, our results suggest the idea of cascades of behavioural changes already developed by Rosenthal *et al.* [205]: the initiator drags another fish along that drags another one, etc.

This organisation appears a few seconds before the fish leave a room to transit to the other one. Two seconds before the initiation, the groups show a structure that prepares for the exit (Fig 1.5). The Kendall rank correlation coefficient confirmed the idea of the organisation as it reaches 18% to 25% two seconds before the departure and 30% to 50% one second before the departure (Fig 1.6). In the literature we found other cases of initiations: [188, 201] have shown that a three-spined stickleback *Gasterosteus aculeatus* or a sheep *Ovis aries* alone moving away from the herd can initiate a collective departure, [37] have noticed a large variety of initiations for groups of mountain baboons *Papio ursinus* where the initiator can be joined by the group immediately or [141, 184] have observed for white-headed capuchins *Cebus capucinus* a synchronization of their behaviours and also that a minimum proportion of the whole group is needed to launch a collective departure.

In conclusion, this study showed that the number of fish affects the motion of each individual in the groups and the group cohesion. The analysis of the dynamics showed that the zebrafish oscillate mainly in groups between the two patches in the environment and that the majority of the departures is collective. During the collective departures, we observed that an intra-group organisation appears prior to the transition. Increasing the number of individuals makes this organisation less predictable. Finally, we noticed that a few seconds before the collective departures the groups have a particular spatial organisation.

1.4 Methods

1.4.1 Fish and housing

We bred 600 AB strain laboratory wild-type zebrafish (*Danio rerio*) up to the adult stage and raised them under the same conditions in tanks of 3.5L by groups of 20 fish in a zebrafish aquatic housing system (ZebTEC rack from Tecniplast) that controls the water quality. It changes 10% of the water in the breeding tanks every hour. Zebrafish descended from AB zebrafish from different research institutes in Paris (Institut Curie and Institut du Cerveau et de la Moelle Épineière). AB zebrafish show zebra skin patterns and have short tail and fins. They measured in mean = 3.0 cm \pm 0.36 cm, median = 2.9 cm long. All zebrafish used during the experiments were adults from 7 to 8 months of age. We kept fish under laboratory conditions: 27°C, 500 μ S salinity with a 10:14 day:night light cycle, pH is maintained at 7.5 and nitrites (NO²⁻) are below 0.3 mg/L. Zebrafish are fed two times a day (Special Diets Services SDS-400 Scientific Fish Food).

1.4.2 Experimental setup

The experimental tank consisted in a 1.2 m x 1.2 m tank confined in a 2 m x 2 m x 2.35 m experimental area surrounded by white sheets, in order to isolate the experiments and homogenise luminosity. A white opaque perspex frame (1 m x 1 m x 0.15 m - interior measures) is placed in the center of the tank. This frame helped us to position the two rooms and the corridor. The squared rooms (0.3 m x 0.3 m) and the corridor (0.57 m x 0.1 m) have been designed on Computer-Aided Design (CAD) software and cut out from Poly(methyl methacrylate) (PMMA) plates of 0.003 m thickness. Each wall are tilted, (20° from the vertical) to the outside with a vertical height of 0.14 m, to avoid the presence of blind zones for the camera placed at the vertical of the tank (Fig 1.7). The water column had a height of 6 cm, the water pH is maintained at 7.5 and Nitrites (NO²⁻) are below 0.3 mg/L. The experiments are recorded by a high resolution camera (2048 px x 2048 px, Basler Scout acA2040-25gm) placed above the experimental tank and recording at 15 fps (frame per second). The luminosity is ensured by 4 LED lamps of 33W (LED LP-500U, colour temperature: 5500 K - 6000 K) placed on each corner of the tank, above the aquarium and directed towards the walls to provide indirect lightning.

1.4.3 Experimental procedure

We recorded the behaviour of zebrafish swimming in the setup during one hour and did 12 replicates with groups of 1, 2, 3, 5, 7, 10 and 20 zebrafish. Each fish is tested only once. Every six replicates out of twelve we rotated the setup by 90° to avoid a potential bias associated with the initial position of the tank or environmental bias

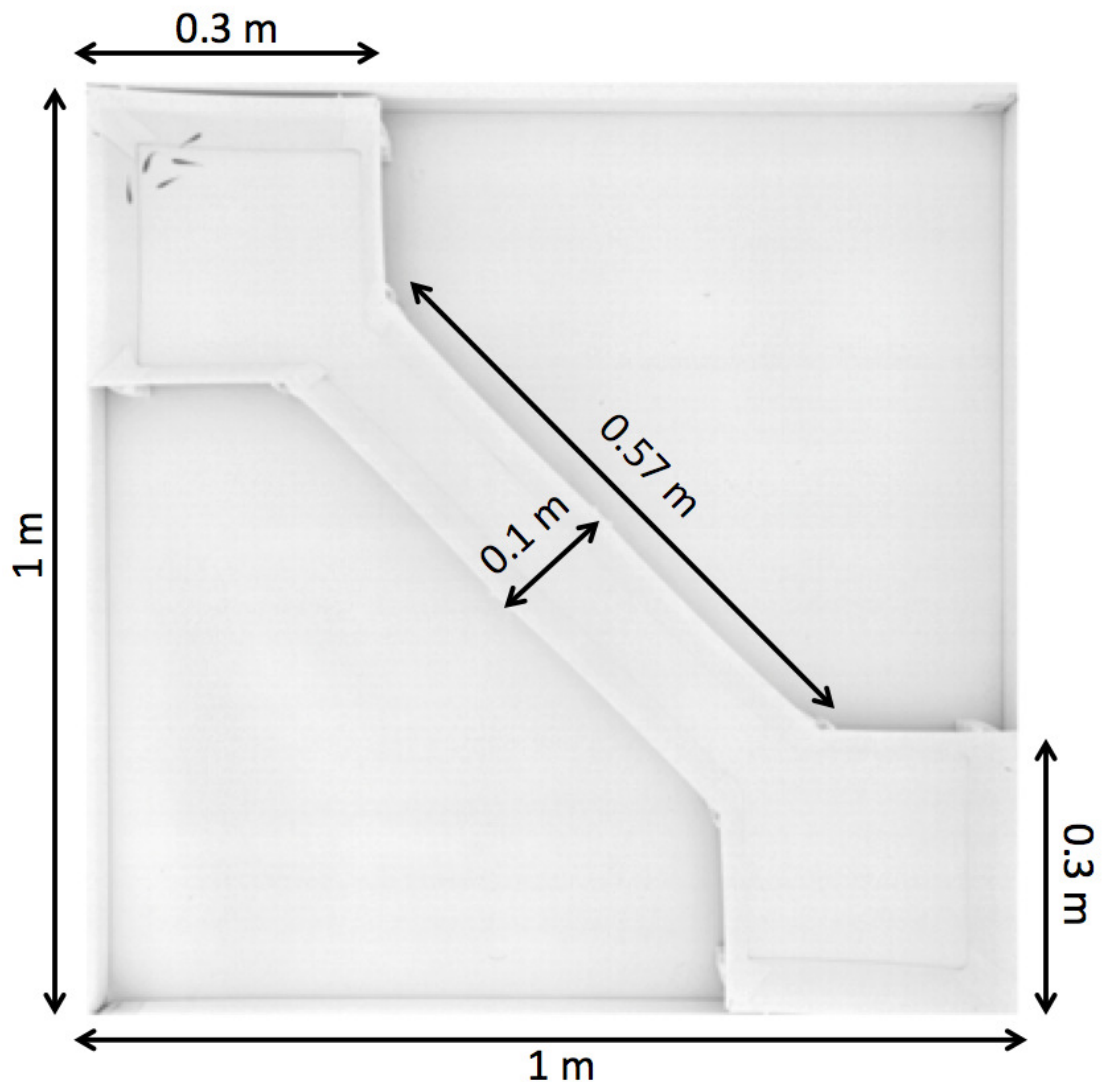


Figure 1.7: Experimental setup. A tank of 1 m x 1 m is divided into three areas: two rooms (0.3 m x 0.3 m) connected by a corridor (0.57 m x 0.1 m). The water column has a height of 6 cm. The luminosity is ensured by 4 LED lamps of 33W (LP-500U) placed on corners of the tank and directed towards the walls to provide indirect lighting. The whole setup is confined in a 2 m x 2 m x 2.35 m experimental chamber surrounded by white sheets to isolate the experiments and to homogenise luminosity.

(noise, light, vibrations, ...). For each replicate we choose randomly the starting chamber by flipping a coin. We called the starting chamber *Room 1*. Heads or tails defined the starting chamber with a maximum of three starts in each chamber. This method gave us 3 experiments with each combination of orientation of the setup x starting chamber. Then, the fish are placed with a hand net in a cylindrical arena (20 cm diameter) made of Plexiglas in the centre the selected rooms. Following a five minutes acclimatisation period, this cylinder is removed and the fish are free to swim in the setup. The fish are randomly selected regardless of their sex and each fish is never tested twice to prevent any form of learning. The water of the tank is changed every week and the tank and the set-up are cleaned during the process.

1.4.4 Tracking & data analysis

Today, many studies on animal collective behaviours use methodologies based on massive data gathering, for example with flies (*Drosophila melanogaster*) [31,60], birds (*Sturnus vulgaris*) [9,156,159], fish (*Notemigonus crysoleucas*) [229]. Our experiments are tracked in real-time ("on-line") by a custom made tracking system based on blob detection. Each replicate except experiments with 20 zebrafish is also tracked by post-processing ("off-line") with the idTracker software to identify each fish and their positions [181]. Each replicate consisted of 54000 positions (for one zebrafish) to 1080000 positions (for 20 zebrafish). The idTracker software is not used for groups of 20 fish due to higher number of errors and too long computing time. For example, for a one hour video with 2 fish idTracker gives the results after 6 hours of processing and for a one hour video with 10 fish it lasts a week to do the tracking (with a Dell Precision T5600, Processor : Two Intel Xeon Processor E5-2630 (Six Core, 2.30GHz Turbo, 15MB, 7.2 GT/s), Memory : 32GB (4x8GB) 1600MHz DDR3 ECC RDIMM).

Since idTracker solved collisions with accuracy [181] we calculated individual measures and characterised the aggregation level of the groups (except for groups of 20 individuals). We also calculated the distances between each pair of zebrafish respectively, the travelled distances of each individual and their speeds. The computing of the speed has been done with a step of a third of a second in sort of preventing the bias due to the tracking efficiency of idTracker that does not reach 100% (see S2 Table). The data gathered for groups of 20 individuals are only used in the analyses which focused on group behaviour and do not need the identities of the fish. The data analysts were not blind to group size. However, the data analysis was done automatically by custom scripts that did not differentiate between the different experimental conditions. Each .txt with the position (x,y,t) of the fish entered the pipeline analysis and received the same treatment. Thanks to this automated treatment, we ensured that there was no bias in the data analysis due to the observer.

The Fig 1.2 has been obtained following this process : At each time step with at least one fish detected in a room, we analysed the repartition of the group among the rooms by computing the proportion of fish present in room 1 = $R_1 / (R_1 + R_2)$ with R_1 and R_2 the number of fish in the respective room number.

When all fish were present in the same room, we identified which fish initiates the exit from the room, established a rank of exit for all the fish and calculated the distances between all zebrafish to the initiator to establish a rank of distances. Finally, we confronted these ranks and count the number of occurrences for each ranking case. We checked the results for different time steps before the initiation. The idea was to highlight a correlation between the spatial sorting and the ranks of exit and also a possible prediction of ranks of exit.

We looked at majority events defined as the presence of more than 70% of the zebrafish in one of the three areas of the setup, either in the room 1 or in the room 2 or in the corridor. To compute their numbers, we averaged the number of fish over the 15 frames of every second. This operation ensures that a majority event is ended by the departure of a fish and not by an error of detection during one frame by the tracking system. We then computed the durations of each of those events and counted the transitions from a room to the other one and sort them. All scripts were coded in Python using scientific and statistic libraries (numpy, pylab, scilab and matplotlib).

Finally, we computed the neighbour distances as the distances between each nearest fish.

1.4.5 Statistics

All scripts were coded in Matlab and Python using statistics libraries (numpy, pylab, scilab and matplotlib). For the Figs 1.1 and 1.3 we tested the distributions using Kruskal-Wallis tests completed by a post-hoc test: Tukey's honest significant difference criterion.

The Kendall rank correlation coefficient [119], τ , is a measure of ordinal association between two measured quantities. It goes to 0 when the two quantities are independent and to 1 if they are correlated. It is computed by:

$$\tau = \frac{\text{number of concordant pairs} - \text{number of discordant pairs}}{\text{number of concordant pairs} + \text{number of discordant pairs}}$$

We use the Kendall rank correlation coefficient to see if the rank of exit and the rank of distances with the initiator are dependant or not through the time.

Chapter 2

Collective departures and leadership in zebrafish

Abstract

In social animals, morphological and behavioural traits may give to some individuals a stronger influence on the collective decisions, even in groups assumed to be leaderless such as fish shoals. Here, we studied and characterized the leadership in collective movements of shoals of zebrafish *Danio rerio* by observing groups of 2, 3, 5, 7 and 10 zebrafish swimming in a two resting sites arena during one hour. We quantified the number of collective departures initiated by each fish and the number of attempts that they made. To do so, we developed an automated pipeline that analysed the individual trajectories generated by the tracking software. For all shoal sizes, the leadership was distributed among several individuals. However, it was equally shared among all the fish in some shoals while other groups showed a more asymmetrical sharing of the initiation of collective departures. To quantify this distribution, we computed the entropy associated with the time series of the identity of all initiators for each experiment and confirmed the presence of a continuum between a homogeneous and a heterogeneous distribution of the leadership. While some fish led more departures than others, an individual analysis showed that all fish had actually the same success rate to lead the shoal out of a resting site after an attempt. Thus, some individuals monopolized the leadership by attempting more often than others to exit a resting site. Finally, we highlight that the intra-group ranking of a fish for the initiative is correlated to its intra-group ranking for the average speed with mobile individuals more prone to lead the shoal. These results demonstrate that the collective behaviour of a shoal can be mainly driven by a subset of individuals even in the absence of higher influence of a fish on its congeners.

2.1 Introduction

Collective movements in animals often require that the group members make decision on the move. In this process, an individual generally initiates the movement of the group

towards a new direction or out of a resting site. The identity and motivation of this initiator vary widely according to the social organisation of the considered species [122,183]. The initiation of movement may be undertaken by a unique or a subset of individuals resulting in a consistent leadership over time. These individuals can be older [235], of a specific sex [5] or dominant in the group [182,235]. In societies that do not identify a specific individual as the group leader, initiations may be performed by any member of the group without consistency over time. In this context, fish are a traditional example of animal that form leaderless shoals or schools and rely on self-organized processes for information transfer and collective decision-making [55,100,130,142]. Indeed, a distributed mechanism in which any individual can potentially initiate a collective movement seems particularly suited for large schools of fish that lack global communication systems and share similar interests and costs [50,51]. For example, the first fish to spot a predator coming potentially from any direction can start an escaping manoeuvre that will be propagated from neighbour to neighbour in the whole school.

In this case, the emergence of a leader is favored by external factors (e.g. the direction of the predator's attack and its perception). However, internal factors can also cause an individual to act as a leader. Indeed, these individuals may be temporarily more motivated due to their physiological state [80,84,198,232], level of information [47,55,188] or position in the group [33,141]. In fish shoals, collective movements are mainly driven by the individuals located at the front of the shoal [33]. Several motivations might prompt a fish to occupy these leading positions. Starved fish that have temporary higher nutritional needs are observed at the front positions of the shoal [128] associated with a higher rate of prey capture and food intake [127,129]. In this case the preference for leading positions dissipates once the fish are fed [128]. Similarly, individuals that know the location of a potential food source can lead a group of naive fish towards foraging patches either by initiating departures [199] or favoring a particular swimming direction [54,55]. The success of this steering has been shown to be related to the size of the guiding individuals in golden shiners, larger individuals being more often followed than smaller ones [200]. In this case, the propensity of some fish to take the lead is related to an information that can be gained by other fish or that can become outdated, resulting in an ephemeral leadership by some group members. Finally, the initiation of collective departures has been related to the personality of the fish (mainly *bold* versus *shy*) by several studies. Indeed, while front positions are linked with higher food intake, they are also more exposed to attacks by ambushed predators [132]. Faced with this trade-off, bolder individuals are more prone to exit a shelter and search for food than shyer fish that will mostly follow them rather than initiate a departure [254]. This asymmetry can be reinforced by the social composition of the group with shy individuals enhancing leadership by bold ones [98]. In addition, bolder individuals show a lower behavioural plasticity than shyer ones, even when rewarded after following a partner rather than taking the lead [115,168]. Thus, although each individual can initiate a

collective movement, some characteristics may enhance the probability of some fish to take the leadership more often than others.

While the literature provides evidences for morphological and behavioural traits that lead some fish to become initiator more than others, the impact of this heterogeneous distribution of initiative on the collective dynamics of the group remains unclear. Indeed, most of the works rely on a preliminary binary classification of the individuals (e.g. bold or shy) that are then observed only in pairs with both fish being physically separated in two adjoined tanks or on the observation of groups during a short period of time, often due to tracking limitations preventing a reliable identification of the fish. Therefore, the relation between the individual characteristics of the fish and the distribution of the leadership is still clouded by the lack of repeated observations of collective departures in freely swimming shoals. However, the recent development of tracking techniques based on the individual recognition of specific patterns associated with each fish [181] allows us to overcome these limitations and to individually follow fish in larger groups and for longer time periods.

In this context, we studied the distribution of leadership during collective movement in small shoals of zebrafish *Danio rerio* swimming in an environment composed by two connected spots. At the collective level, the shoaling behaviour of zebrafish is already observed in larvae and shoaling preferences appear at the juvenile stage [75]. Once adult, zebrafish periodically oscillate from loosely connected groups to dense aggregates [157] and regularly transit from unstructured shoals to polarised schools (and inversely). During the school phases, they show a larger inter-individual distances and swim at a higher speed [158]. Here, we observed shoals of 2, 3, 5, 7 and 10 zebrafish swimming for one hour in an experimental arena consisting in two rooms connected by a corridor. We expected the fish to show a succession of mobile and static phases with frequent transitions from one spot to the other one, as observed for other heterogeneous environments [212]. For each experiment, we measured the number of collective transitions from one site to the other as well as the identity of the leading fish of each departure. In addition, we also quantified the number of attempts to initiate a collective departure made by each fish. As fish are generally characterized as leaderless, we expect that the number of initiated departures is proportional to the number of attempts made (i.e. the fish have a similar success rate). However, as mentioned above, individualities may incline some fish to initiate a collective departure more often than other ones. In particular, we expect that the individuals with the highest mobility would be more likely to steer the shoal by attempting to leave a resting site more frequently than the other members of the shoal. Therefore, we also characterized the mobility of the fish by computing their average speed as well as the related intra-group ranking from the fastest fish to the slowest one and related these traits with their tendency to lead their shoal out of the resting sites.

2.2 Materials and methods

2.2.1 Ethic statement

The experiments reported in this study were approved by the Buffon Ethical Committee (registered to the French National Ethical Committee for Animal Experiments #40) after submission to the state ethical board for animal experiments.

2.2.2 Animals and housing

Around 150 adult laboratory wild-type zebrafish (*Danio rerio* AB strain) were reared in housing facilities ZebTEC and fed two times a day (Special Diets Services SDS-400 Scientific Fish Food). The sex ratio was close to 1:1 with females and males randomly mixed in groups of 15 to 20 fish housed in 8l containers with continuous renewal of the water by the ZebTEC system. All zebrafish observed in this study were 6-12 months old at the time of the experiments. We kept the fish under laboratory conditions, 27°C, 500µS salinity with a 10:14 day:night light cycle. The water pH was maintained at 7 and the nitrite concentration (NO^{2-}) was below 0.3 mg/l.

2.2.3 Experimental setup

We observed shoals of zebrafish swimming in an arena consisting of two square rooms connected by a corridor starting at one corner of each room placed in a 100 cm x 100 cm x 30 cm experimental tank (Fig 2.1). This experimental set-up allowed us to observe a large number of collective departures for long duration experiments (1 hour) without human intervention. The walls of the arena were made of white opaque PMMA. The water depth was kept at 6 cm during the experiments in order to keep the fish in nearly 2D to facilitate their tracking. One lamp (400W) was placed on the floor at each edge of the tank which is 60 cm above the floor to provide indirect lightning. The whole setup was confined behind white sheets to isolate experiments and homogenise luminosity. A high resolution camera was mounted 1.60m above the water surface to record the experiments at a resolution of 2048 x 2048 pixels and at 15 frames per second.

2.2.4 Experimental procedure

We observed 12 shoals of two, three, five, seven and ten zebrafish during one hour for a total of 60 experiments. The shoals were formed so that no fish was tested twice the same day and no fish was tested twice for a particular shoal size. Before the trials, the fish were placed with a hand net in a cylindrical arena (20 cm diameter) in one of the two rooms. Following a 5 minutes acclimatisation period, the camera started recording

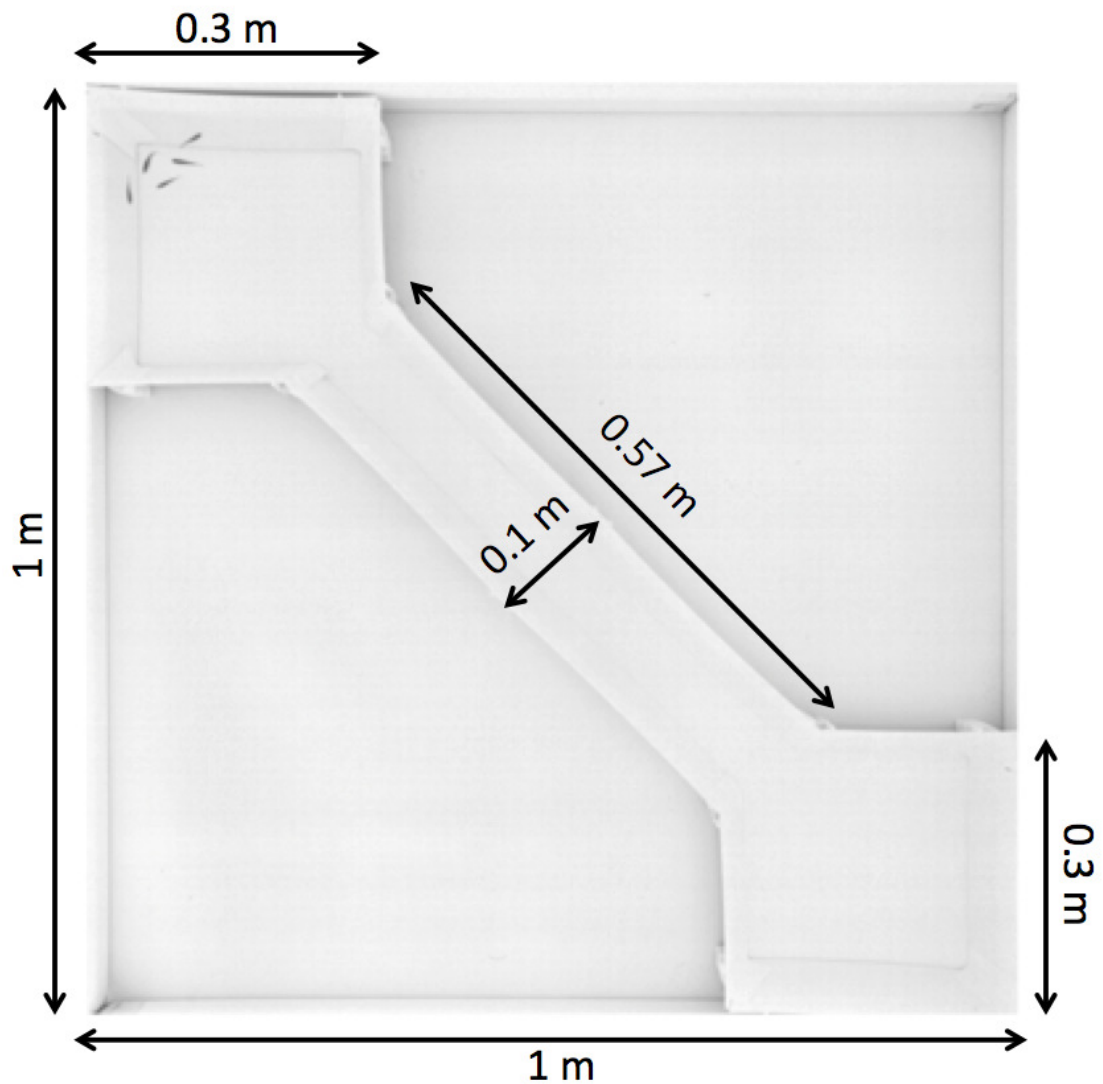


Figure 2.1: Experimental setup. The arena consists of two square rooms (30 cm x 30 cm) connected by a corridor (57 cm x 10 cm) placed in a 100 cm x 100 cm tank. Twelve groups of 2, 3, 5, 7 and 10 zebrafish were observed swimming freely during trials of 1 hour to study the collective departures of the fish from one room to the other.

and the fish were released and able to swim in the experimental arena. After one hour, the fish were caught by a hand net to be placed temporarily in a separated tank and replaced in the rearing facilities at the end of the day.

2.2.5 Tracking

The videos were analyzed offline by the idTracker software [181]. This multi-tracking software extracts specific characteristics of each individual and uses them to identify each fish without tagging throughout the video. This method avoids error propagation and is able to successfully solve crossing, superposition and occlusion problems. However, the tracking system failed to track correctly two experiments with two fish, two experiments

with five fish and four experiments with ten fish (some sections of 5 to 10 seconds were missing on the trajectories of one or two fish). Therefore, these four experiments were excluded from our analysis.

In addition, the software provides a confidence probability for the identification of the fish at each time step. We used this identification of the leader (see below)

2.2.6 Data analysis

For all other experiments, we obtained the coordinates $P(x,y,t)$ of all fish as well as a confidence probability for their correct identification by the software at each time step $\Delta t = 1/15s$. With these coordinates, we built the trajectories of each fish and computed their position in the arena.

To automatically detect collective patterns in the dataset, we developed an automated data analysis pipeline that analyses the trajectories of the fish (Fig 2.2). First, the algorithm converts the positions $P(x,y,t)$ into symbolic coordinates corresponding to the three regions of the experimental arena (Room 1, Corridor and Room 2, Fig 2.2A). From this derived dataset, the algorithm computes the total number of fish in each zone (Fig 2.2B). Then, it retains only the time steps at which the movement of a fish occurred (Fig 2.2C). In this time series, the algorithm searches for particular sequences that correspond to target collective behaviors. As we focused our analysis on the collective departures and the leadership, we targeted three types of event: (i) Collective Residence Events (*CRE*) defined by the entire shoal resting in one of the two rooms, (ii) Attempts of collective departures defined as one fish living one of the two room after a CRE and (iii) Collective Departure Events (*CDE*) defined by the whole shoal leaving one of the two rooms for the corridor after a CRE. For a shoal of *n-fish*, Collective Residence Events are identified by *n-fish* in Room 1 or Room 2. Attempts of collective departure correspond to a successive sequence *n-fish*, *n-fish-1* in Room 1 or Room 2. Finally, collective departures events are identified by a succession of *n-fish*, *n-fish-1*, ..., *1*, *0* fish in Room 1 or Room 2. Then, the algorithm identifies the first fish that left the room 1 or 2 for all attempts and collective departures (Fig 2.2D) and stores their ID and the departure time in two arrays. If more than one fish left the room at the same time step, the algorithm randomly select one of those fish as the leader (this case happened with a frequency of 2% for 2 fish, 2.5% for 3 fish, 14.5% for 5 fish, 6 % for 7 fish and 11 % for 10 fish). To make sure that we considered only events with a correct identification of the individuals, the algorithm check the confidence probability for the ID the potential leader. If the confidence is higher than 0.75, the candidate fish is definitely considered as the leader of the collective departure or the attempt, otherwise the event is discarded and the algorithm consider the next event of departure (this case happened with a frequency of 3% for 2 fish, 6% for 3 fish, 28% for 5 fish, %15 for 7 fish and 41% for 10 fish). Finally, it computes the total number of attempts and collective departures led by each fish.

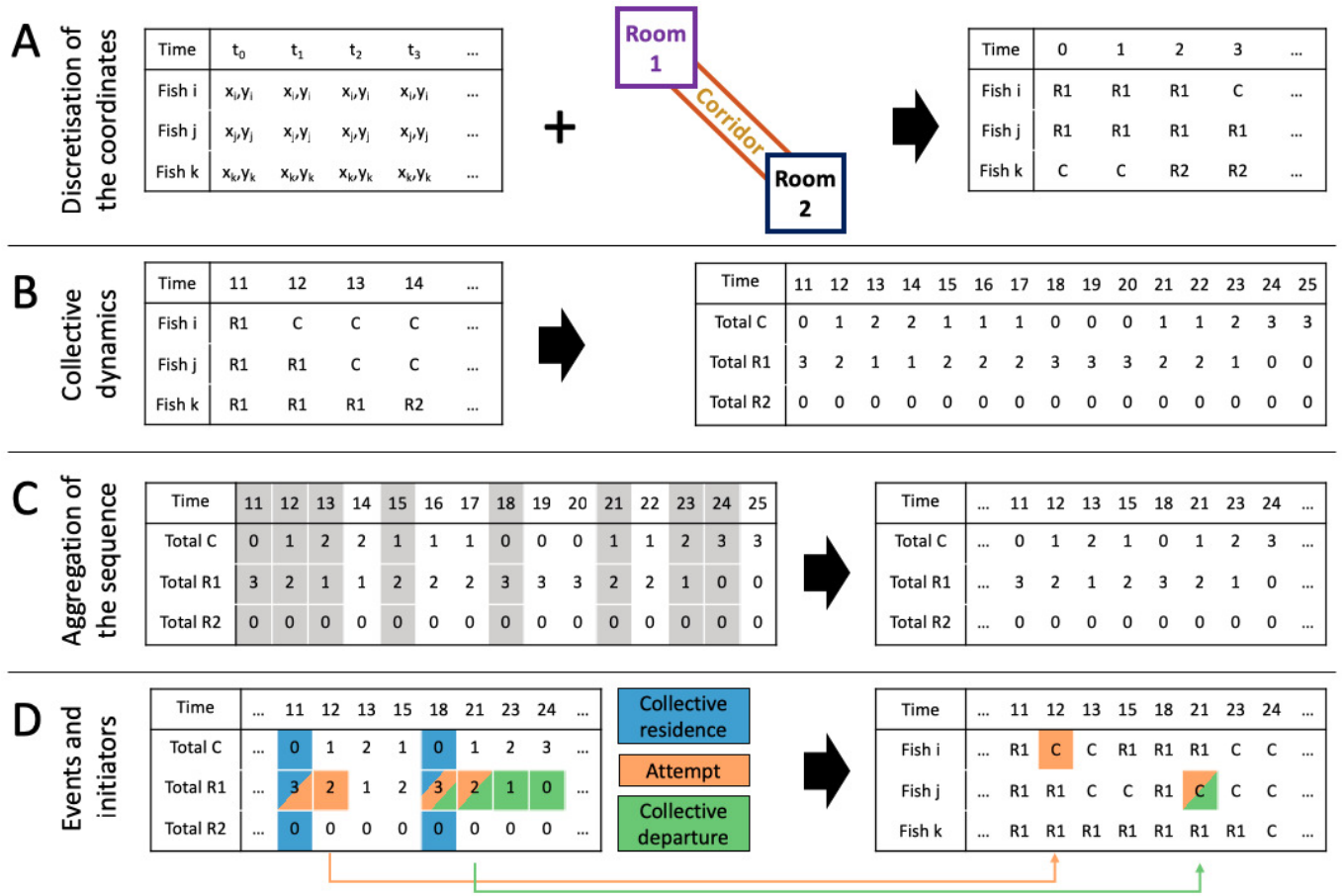


Figure 2.2: Automated data analysis pipeline of collective departure and leadership. Example for a shoal of three fish. (A) Transformation of the $P(x, y, t)$ positions of the fish into symbolic coordinates (C:corridor, R1:room 1, R2:room 2). (B) Computation of the total number of fish in each region. (C) Removal of successive duplicate time steps with no event. (D) Search for particular sequences: three fish in room 1 or 2 (collective residence event), sequences of 3-2 fish in room 1 or 2 (attempt of collective departure) and sequences of 3-2-1-0 fish in room 1 or 2 (collective departure), and identification of the first fish that left the room 1 or 2 for all attempts and successful departures.

Thanks to this analysis, we obtained the total number of collective residence events (*CRE*) and collective departure events (*CDE*) observed for each experiment. To study the effect of the shoal size on these events, we compared the number of events observed for each shoal size but also compared them with measures obtained from simulated non-social shoals in which fish do not pay attention to other shoal members. This null model allowed us to evaluate if any change in the number of collective events detected in our experiments was only due to a scaling effect independently of the social interactions between the animals. To design this non-social shoals, we combined the trajectories of fish originating from different experimental shoals of the same size. Then, we performed the same analysis on this artificial dataset and computed the number of collective residence events (*CRE*) and collective departure events (*CDE*).

Then, the distribution of the leadership was quantified by computing the entropy $H(l)$ (Eq. 2.1) of the time series of leadership events computed in Fig. 2.2G:

$$H(l) = - \sum_{i=1}^k \frac{L_i}{L} \log_k \left(\frac{L_i}{L} \right) \quad (2.1)$$

with k the number of fish in the shoal, L_i the number of transitions led by the fish i , L the total number of transitions observed in the experiment and \log_k the logarithm to base k . In this equation, the ratio L_i/L estimates the probability to observe the fish i as the leader of a departure. A perfectly uniform distribution of the leadership is associated with the maximal entropy $H(l) = 1$ while a totally despotic organisation is associated with the minimal entropy $H(l) = 0$. Similarly, we studied the temporal organisation of the leaders by computing the conditional entropy $H(l_t|l_{t-1})$:

$$H(l_t|l_{t-1}) = - \sum_{i=1}^k \sum_{j=1}^k p(l_{t-1} = i, l_t = j) \log_k \left(\frac{p(l_{t-1} = i, l_t = j)}{p(l_{t-1} = i)} \right) \quad (2.2)$$

with l_t the identity of the leader of departure t and l_{t-1} the identity of the leader of departure $t-1$. In this case, the quantity $H(l_t|l_{t-1})$ quantify the ability to predict the identity of the leader l_t of a departure given the identity of the fish l_{t-1} that led the previous departure. A totally random organisation is associated with the maximal entropy $H(l_t|l_{t-1}) = 1$ while a perfectly predictable organisation results in a minimal entropy $H(l_t|l_{t-1}) = 0$.

Finally, we studied the relationship between the tendency of the fish to initiate a collective departure and their motility. To do so, the instantaneous speed of the fish v_t was computed as the distance between $P(x,y,t-1)$ and $P(x,y,t+1)$ divided by two time steps.

2.3 Results

2.3.1 Distribution and temporal organization of the leadership

In all experiments, the fish mainly swam together and regularly transited from one room to the other. In the rooms, the shoal circled a few times before one fish decided to leave the room for the corridor. This fish was either followed by the whole shoal, only a part of the shoal resulting in a temporary split, or not followed at all. In this section, we focused our analysis on the distribution of the leadership and the sequential organisation of the leaders during collective departures from one room to the other. For each CDE,

the leader was identified as the first fish of the shoal that left the room. Therefore, we excluded from our analysis the departure of only a subgroup of fish while the rest of the fish remained in the room as well as the departure of a remaining subgroup to join the rest of the shoal.

First, we quantified the total number of collective departures events and collective residence events for the different shoal sizes. The number of CRE significantly decreases when the number of fish increases with an average number of 249 ± 38 CRE for two fish to 158 ± 36 CRE for groups of ten fish (Fig 2.3A, Kruskal-Wallis test, $\chi^2=15.39$, $df = 4$, $p<0.01$). Similarly, the number of CDE also decreases but with a stronger difference between the shoals from an average number of 228 ± 35 for two zebrafish to 49 ± 31 CDE for ten zebrafish (Fig 2.3B, Kruskal-Wallis test, $\chi^2=33.15$, $df = 4$, $p<0.001$). Therefore, an average of 92% of the CRE were followed by a collective departure in dyads while only 29% were in groups of ten fish (Fig 2.3C). This success rate was mainly influenced by the shoal size ($p<0.0001$) rather than by the number of residence events ($p>0.05$) that has only a marginally significant effect when coupled with the shoal size ($p=0.03$) as shown by the model $Success \sim shoal\ size + CRE + shoal\ size * CRE$ (Null deviance = 2152.38 with 51 df, Residual deviance = 261.22 with 48 df). Thus, larger shoals were more likely to split during departures while small ones remained cohesive most of the time. However, the shoals remained strongly cohesive compared to simulated non-social shoals. Indeed, the occurrence of these CRE and CDE decreases much more promptly in the null model, with only a few CRE and almost no CDE observed for shoals of five, seven, and ten non-interacting fish. Thus, the smaller decrease in CRE and CDE observed in real shoals compared to non-social agents confirms that the shoals remained strongly social, even with 10 individuals.

Thanks to the individual tracking of the fish, we determined the identity of the leaders for all collective departures and computed the sequence of successive leaders' identities along the experiments. While multiple leaders were observed in each shoal, the leadership was monopolized by only some members of the shoal (e.g. for a shoal of 5 fish Fig 2.4A) or equally distributed among all individuals (e.g. for a shoal of 5 fish Fig 2.4B). To better characterise the distribution of the leadership among the fish, we computed the entropy $H(L)$ associated with those sequence of leaders observed in each experiment. The distribution of the leadership was significantly influenced by the shoal size (Fig 2.4C, Kruskal-Wallis test, $\chi^2=13.77$, $df = 4$, $p<0.01$). Leadership in small shoals was more likely to be shared among all fish ($H(L)\approx 1$) while a subgroup of individuals was more involved than others in the leadership for larger shoals (intermediate values of $H(L)$). However, this result also shows that (i) a continuum from homogeneous to heterogeneous sharing of leadership was observed for all shoal sizes, but with different distributions and (ii) no shoal was organized despotically ($H(L)\approx 0$) with one fish leading all departures, independently of the shoal size. As several initiators were observed in each shoal, we studied the temporal distribution of the leading events to highlight a potential temporal

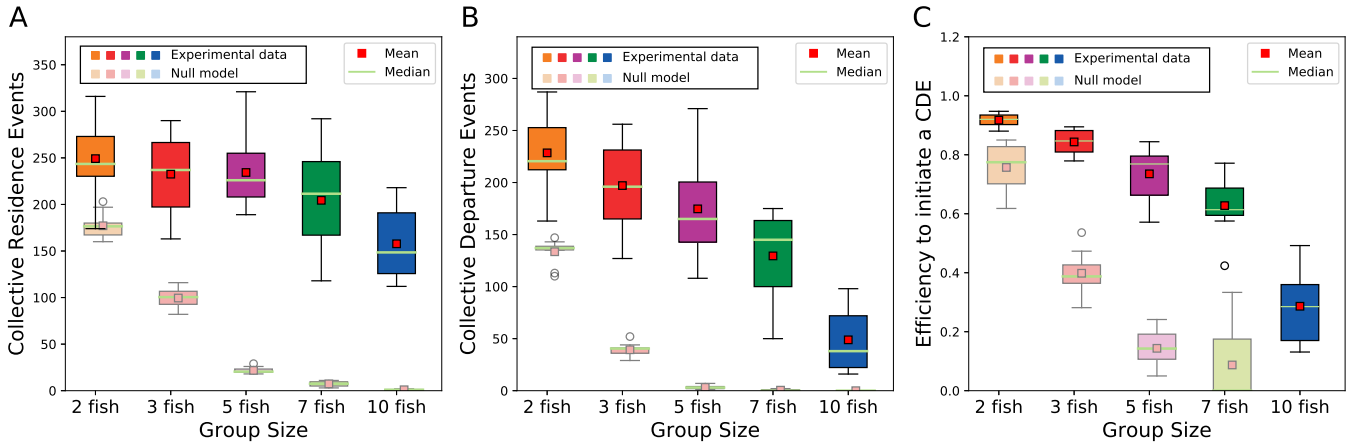


Figure 2.3: Collective behaviours observed in real shoals (experimental data) and simulated non-social shoals (null-model). (A) Number of collective residence events (CRE) and (B) collective departure events (CDE) for the 11 groups of two, 12 groups of three, 11 groups of five, 12 groups of seven and 10 groups of 10 zebrafish observed during one hour. Collective residence events are defined as the whole group resting in one of the two rooms and collective departures events are defined as the whole group leaving one of the resting sites. (C) Efficiency of the first leaver to trigger a collective departure of all fish computed as the proportion of CRE that were followed by a CDE.

organisation of the leaders over successive departures. To do so, we computed the conditional entropy $H(l_t|l_{t-1})$ normalized for the group size for all sequences of leaders that informs us on the probability to correctly predict the leader of the departure t knowing the identity of the leader of the previous departure $t-1$. While no shoal shows a strongly predictable turnover of leaders, the succession of leaders was proportionally less predictable in smaller shoals than in large ones (Kruskal-Wallis test, $\chi^2=22.94$, $df = 4$, $p < 0.001$). Indeed, the distributions of the conditional entropy were shifted towards lower values as the shoal size increased (Fig. 2.4B). However, the particularly low values observed for 10 fish probably results from the small number of collective departures observed for some shoals (2.3D), reducing the potential to observe repeated measures.

These first quantifications at the collective level showed that the leadership during collective departures in zebrafish is shared among the shoal members without a strong temporal organisation. However, they also reveal an inter-shoal variability in addition to a shoal size effect. To highlight this variability, we computed the proportion of collective departures initiated by each fish for each shoal. As expected by the computation of the entropy $H(L)$, we observed a continuum from a homogeneous distribution of the initiation between the shoal members to more heterogeneous ones with some fish having a higher tendency to start a departure than others (Fig. 2.5). Therefore, we studied the individual characteristics of the fish to determine the key factors that influence their propensity to initiate a collective departure.

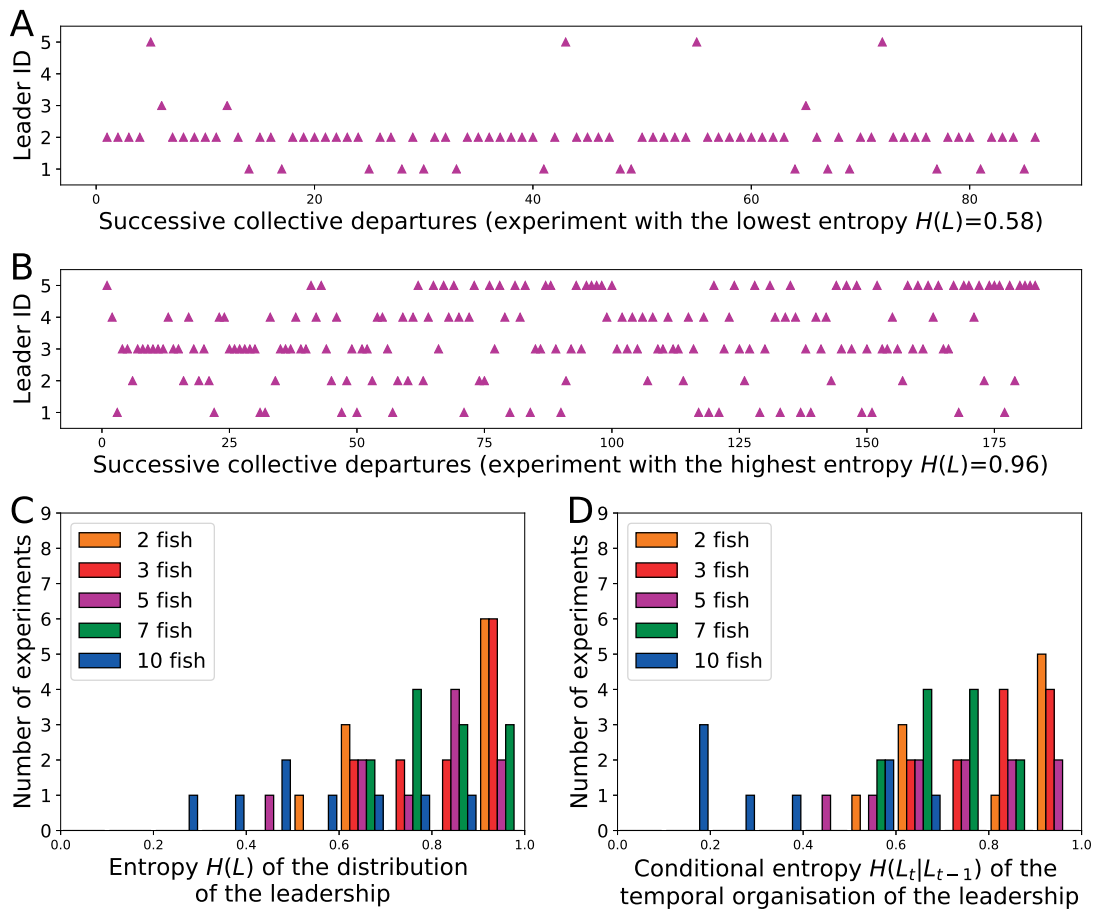


Figure 2.4: Distribution of the leadership between the shoal members. Sequence of leaders observed for the experiments with the most heterogeneous (A) and the most homogeneous (B) distribution of leadership in shoals of five zebrafish. Entropy (C) and conditional entropy (D) computed for all experiments with shoals of 2, 3, 5, 7 and 10 zebrafish.

2.3.2 Success and profile of the leaders

First, we studied the link between the temperament of the fish and their propensity to initiate collective departures. We determine the boldness of the fish by quantifying the number of times that a fish was the first one to exit a room, independently of its success to be followed by the other shoal members (defined as an *attempt*). For each shoal, we analyzed the potential correlation between the number of attempts and the number of initiations made by each fish. A linear regression showed that the number of initiations is linearly correlated to the number of attempts performed by the fish and that the coefficient of this correlation (i.e. the success rate) depends on the shoal size (Fig 2.6A). For shoal of two fish, 96% of the attempts made by an individual resulted in a collective departure of the dyad. In accordance with the results observed at the shoal level (Fig 2.3C), the success rate for each fish decreases when the population increases: 93% for 3 fish, 82% for 5 fish, 74% for 7 fish and 44% for 10 fish. To determine the factors that significantly influence the number of departures initiated by each fish, we

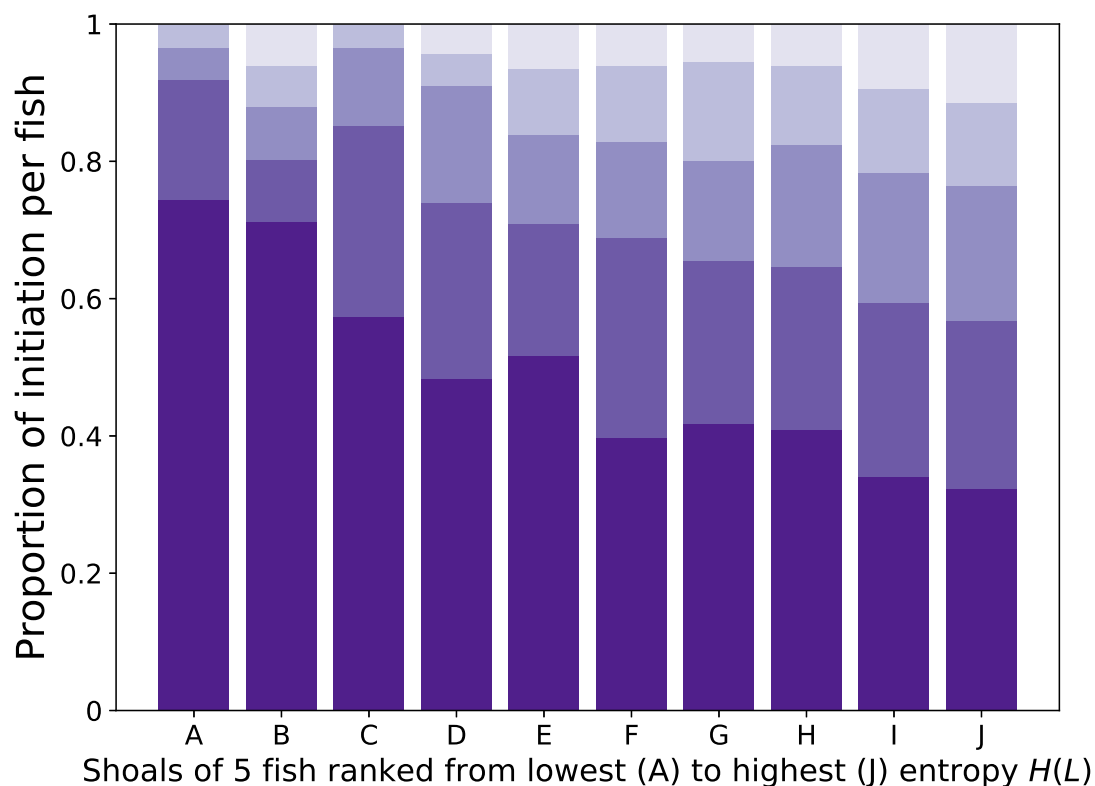


Figure 2.5: Proportion of departures initiated by each fish in shoals of five zebrafish. The shoals are ranked according to their entropy $H(L)$ from the most heterogeneous distribution (left) to the most homogeneous distribution (right). In each shoal, the fish are identified with different colors and ranked from the highest proportion of initiation (bottom) to the lowest proportion of initiation (top).

designed the GLM $Initiations \sim shoal\ size + attempts$. As expected, the shoal size ($p < 0.0001$) as well as the number of attempts made by the fish ($p < 0.0001$) significantly impact the leading success of the fish (Null deviance = 11532.4 with 269 df, Residual deviance = 1880.6 with 267 df). Thus, the attempts made by the fish were significantly more successful in small shoals than in larger ones.

Then, we analysed the success of the fish by comparing their intra-shoal proportion of initiated departures with the intra-shoal proportion of attempts that they made. A linear relationship would imply that all fish shared the same success rate, independently of the number of attempts that they made while a wider dispersion or a non-linear relationship would highlight that some fish have a higher success rate than others. As shown in Fig 2.6B, the two proportions are linearly correlated for all shoal sizes with a slope ≈ 1 , revealing an equal success rate for all shoal members. This conclusion is supported by the model $Proportion\ of\ initiations \sim shoal\ size + proportion\ of\ attempts$ identifying only the intra-shoal proportion of attempts ($p < 0.0001$) made by a fish as a significant predictor of the proportion of collective departures that it initiated with no influence of the shoal size ($p = 0.648$) on its success (Null deviance = 7269.78 with 269

df, Residual deviance = 521.27 with 267 df). Thus, the larger number of departures initiated by some fish is not related to a higher influence on other group members or a better success rate but on a higher tendency to exit the resting sites.

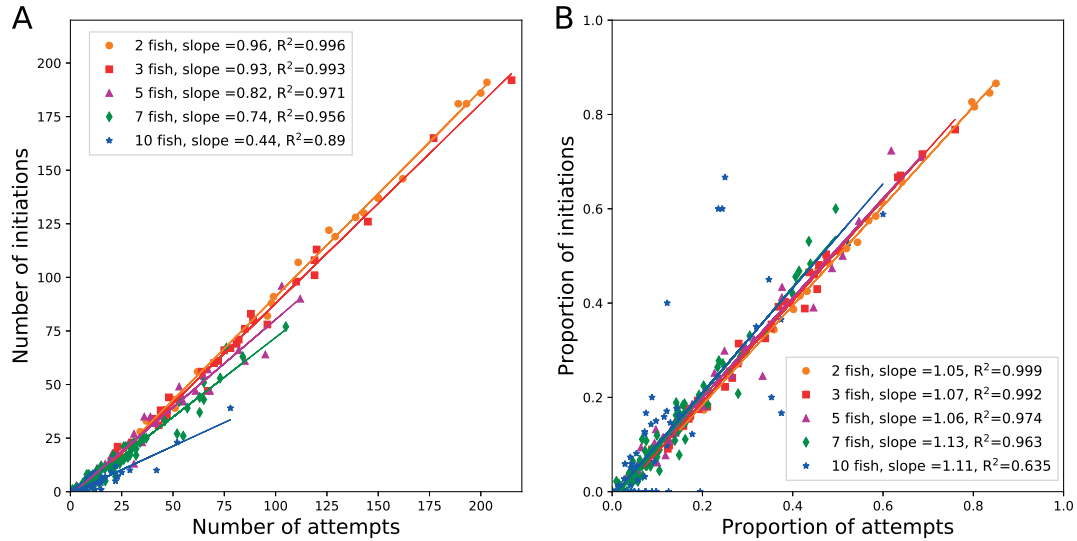


Figure 2.6: Success rate of the fish. (A) Total number of collective departures initiated as a function of the total number of attempts for each fish. The number of initiations is directly proportional to the number of attempts but the success rate of the initiations decreases for larger group sizes. (B) Proportion of attempts made by the fish in relation to the proportion of departures initiated. For each group size, the success rate is identical for all fish in the shoal.

Finally, we looked at a potential link between the motion characteristics of the fish and the number of collective departures that they have initiated. To do so, we measured the average linear speed of all individuals and also compared their intra-group ranking for the number of initiations with their intra-group ranking for the linear speed using the Kendall's τ coefficient. For small shoals (two and three fish), we found no correlation between the average speed of the fish and their tendency to lead a departure (Figs 2.7A-B for global comparisons and & F-G for intra-group comparisons). On the contrary, there was a significant positive correlation between the average speed of the fish and the number of initiations that they performed for shoals of 5, 7 and 10 fish (Figs 2.7C-E). Indeed, for the majority of the shoals (8 out of 10 for 5 fish; 8 out of 12 for 7 fish and 4 out of 8 for 10 fish) the individual that initiated the largest number of collective departures was also the one with the highest average speed (Figs 2.7H-J). Thus, the initiation of collective movements is related to the motility of the fish but only for large shoals.

2.4 Discussion

The coordinated movements of fish shoals are often reported as a collective process in which each fish can potentially lead a departure. Here, we confirmed that the initiation

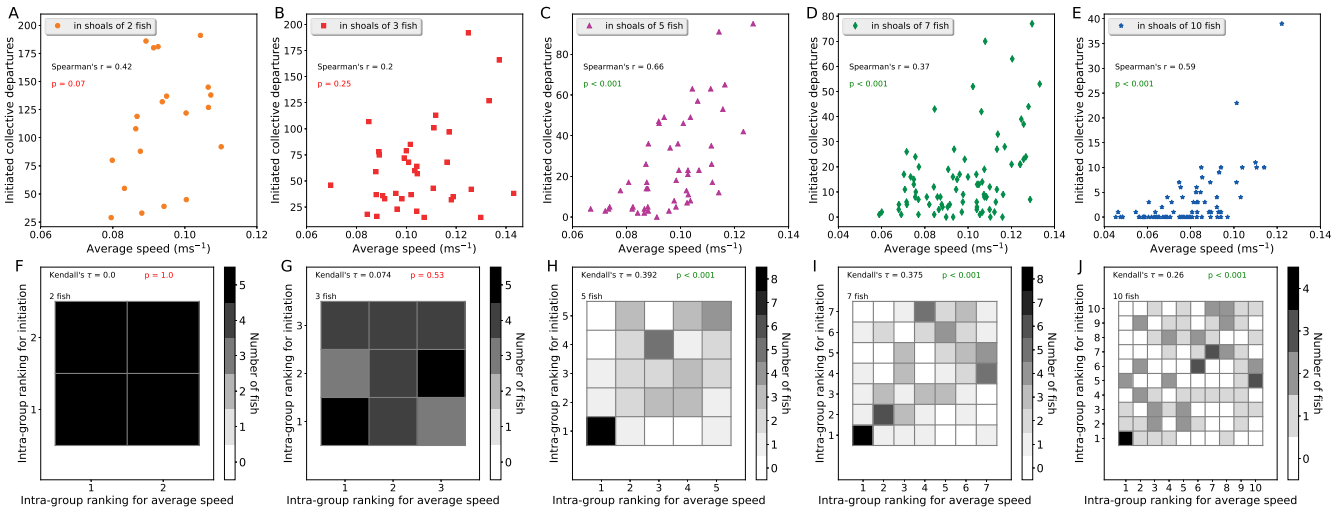


Figure 2.7: Motility of the fish and leadership. (A-E) Proportion of attempt made by each fish according to its average speed. For small group sizes (2 or 3 fish), the absolute value of the mean linear speed is not a good predictor of the number of attempts performed by a fish (Spearman's correlation) but for larger group size (5, 7 and 10 fish), the linear speed of a fish is statistically correlated with the number of attempts. (F-J) Distribution of the fish according to their intra-group ranking of the number of collective departures that they initiated and their intra-group ranking for the average speed. By taking into account their ranking inside the group, the relationship is statistically significant as soon as the groups is formed by at least five fish.

of collective departures is a distributed process among the shoal members. However, our results also showed that the sharing of the leadership across the different groups was a continuum from a homogeneously distributed leadership to strongly asymmetrical distributions. A similar diversity was observed in groups of four zebrafish during foraging [181]: in two groups out of four, the order of arrival was consistent over successive trials while the fish in the two other groups showed a random arrival order. In our experiments, dyads showed the most egalitarian situations but also the strongest monopolization of leadership with one fish performing up to 85% of the initiations of its shoal. A similar result was observed in trios with some shoals sharing equally the leadership between all members and other groups with a disproportionate number of initiations led by the same fish (up to 75% for one group). As the shoal size increases, almost all shoals showed a heterogeneous distribution of the leadership between the fish even if we did not observe a clear monopolization of the initiations of collective departures in these shoals. A similar effect of group size on leader-followers interaction was evidenced in minnows [177]. In this latter study, 6 out of 9 dyads displayed a clear leader-follower relation, 2 showed an equally shared leadership and 1 was formed by fish that did not interact with each other. The author concluded that one fish leads the other in groups of two but that this behaviour is not observed for larger groups.

Stronger asymmetries are more likely to be observed in small group sizes but an unbalanced distribution is almost always present in groups of a dozen of individuals. Such

outcome can be the result of sampling of a continuous distribution for an individual characteristic that influences the probability to lead the group. Indeed, as we add more individuals, there is a higher probability that at least two of them significantly differ from each other, leading to an unshared decision-making process but by the same time, the average difference between individuals tends to stabilize to a limit value. On the contrary, as only two fish are forming a dyad, there is a probability that these fish are either almost identical, resulting in a homogeneous leadership, or on the contrary strongly different, leading to a heterogeneous leadership, with a continuum of possibilities between these extrema. For example, the boldest individual of a dyad tend to take the lead more often than the shier one in pairs of sticklebacks, this tendency being amplified for greater difference in boldness between the fish [98].

Incidentally, one should be cautious when subdividing the tested population into binary behavioural classes and then performing experiments with pairs of opposed individuals. Indeed, such classification may lead to a misrepresentation of the social organisation observed in free-living groups by forming only asymmetrical pairs. This effect was already mentioned by [201] that concluded that a significant relation between boldness and leadership in golden shiners only when they classified the fish into the binary *leaders* or *non-leaders* classes. Thus, social species in which the initiation of specific behaviours is related to individual characteristics rather than to a particular hierarchical position, are more likely to display a whole range of social structures from despotic to egalitarian groups without any behavioural changes but only due to sampling effects.

Thanks to the individual tracking of repeated departures, we also highlighted that the heterogeneous distribution of leadership was not the result of a higher success rate of some individuals that could have a higher tendency to be followed. On the contrary, the number of successful departures initiated by the fish was linearly correlated to their number of attempts. In addition, we showed that the initiation process was not temporarily organised as the identity of the fish that led a departure does not provide information on the identity of the fish leading the next one. A similar result was also observed in other fish species like Damselfish in which collective departures from one spot to another was mainly led by fish that performed a higher number of attempts [3]. In sticklebacks, shy individuals tend to be more responsive to the departure of another shy fish while bold individuals are less sensible to the personality of the initiator [165]. Nevertheless, bold individuals are more likely to be observed as leader simply because they try to initiate more collective movements.

While the linear relation between the number of attempts and initiations was observed for all group sizes in our experiments, the success rate of the attempts drops from $\approx 90\%$ in dyads to only $\approx 20\%$ in groups of ten fish. The majority of attempts led to a temporary fission of the shoal into subgroups for this larger group size. In our experimental setup, the subgroups always reassembled after a short period of time. However, in natural conditions where the fish are not restrained to a small environment, those splitting events

could lead to a permanent fission of the group. Indeed, zebrafish form shoals of a few to dozens of individuals in their habitat [174]. The size of the shoals observed in nature can be driven by a trade-off between the advantages (e.g. detection of predators and potential food sources) and disadvantages (e.g. larger groups are more easily spotted by predators, increased inter-individual competition for food) of being in groups. According to our results, the intermediate shoal sizes observed in zebrafish could be maintained by a strong cohesion in small groups but a loose organisation of larger ones making them more prone to splitting.

Our results also highlighted that the motility of the fish was a predictor of its tendency to initiate collective departures for larger groups. Indeed, the intra-group ranking of a fish for the average speed was correlated to its intra-group ranking for the number of led departures for shoals of five, seven and ten fish. In those shoals, the leaders of collective movements do not seem to occupy a particular hierarchical status in the group but are generally the most mobile individuals. A similar result was predicted by a theoretical analysis on the emergence of leadership in simulated zebrafish [262]. This study showed that an informed individual moving in a specific direction is more likely to be followed by a group of naive individuals when it moves just faster than the naive group. In Damselfish *Dascyllus aruanus*, the initiator of a collective movement also displays a higher level of activity than their group members before the departure [3]. A similar result was observed during pigeon flocking: birds with the highest ground speed tend to lead the flock more often than others [186]. A favored direction, a higher level of activity or a higher average speed can lead an individual to occupy the front position of the group more often than other individuals. As the direction of the group is mainly decided by the front individuals (at least in shoals of fish [33]), these inter-individual behavioural differences lead to a heterogeneously distributed leadership in the shoal.

The present study demonstrated that any fish could potentially lead the collective movements of its shoal, with the same success rate for all shoal members. Such democratic organisation may enhance the transfer of information between shoal members. Indeed, the fish did not differ in their needs or in their knowledge about the environment in the experiences described in this paper. However, in a natural context, some fish may differ in the level of information that they have about environmental opportunities or threats. In this context, it seems more adaptive to adopt a collective decision-making process that allows any group member to initiate a collective decision or movement. Nevertheless, we also showed that even if the process allows any individual to act as a leader, some individuals do so more often than others. This variation in the tendency to initiate a collective movement or to follow other shoal members could tend to stabilize over time and lead to specialized roles. Indeed, if initiation is linked to individual personality traits such as boldness, individuals could repeatedly experience the same role in decision making process (i.e. initiator or follower). This division of labour in decision-making may be advantageous as some member may become more incline to propose new

directions of movement that can be evaluated and then approved or rejected by other group members.

This may be a primitive division of labour in collective information processing and decision-making. Initiators may be bolder individuals that react more promptly and less carefully to their environment while followers may be more cautious in their decision. This balance between bold initiators and shy followers may prove to be advantageous for group living species, as initiators will bring possibilities and followers may help to select the best options proposed as well as maintain social cohesion. Indeed, it has been shown that the presence of naive followers in fish may maintain the social cohesion of their shoal when some shoal members have conflicting information about the direction to adopt [54]. This process may be particularly adaptive for collective decision-making in groups that share the same interests as each individual is able to express an opinion but also rely on the approval of the majority of the group members.

Part II

Collective behaviour model of the zebrafish

Collective motion has been intensively studied in physics, biology and computer sciences. In the early-developed models, individuals move along the resulting vector computed as the sum of attraction/alignment/repulsion vectors describing the interactions with congeners according to their distance. Although these rules are sufficient to produce coherent and aligned groups travelling in the same direction, they can result in biological incoherent behaviour. Particularly, this mechanism does not allow an individual to choose one of two equally distant stimuli. Moreover, a fish will always respond in the same way when facing identical situations since the classical approach generally relies on a deterministic, albeit noisy, behaviour of the individuals. However, new studies revealed that multiple species do not exhibit such highly ordering of individuals and show stochastic behaviour in binary choices.

In this context, my colleagues developed a stochastic model [49] based on a probability distribution function to move in targeted directions rather than on a summation of influential vectors as it is classically assumed. This model defines the probability for fish to move in all possible directions by a circular probability density function. Each stimulus perceived by the fish locally increases the probability for the focal fish to move in its direction. This model can spontaneously produce transitions from consensus (an individual follows the direction of its congeners) to choice (an individual choose between congeners). Experimental results of zebrafish (alone or in groups of 10) swimming in both bounded homogeneous and heterogeneous environments have been used to calibrate it. This model is novel in three aspects: (i) it is a perception based model that explicitly take into account information gathering and processing, (ii) it is a hybrid modelling approach including behaviour based stochasticity leading to biased random walks and self-propelled particles properties, (iii) it takes into account a bounded and inhomogeneous environment closer to natural conditions while most models are designed for homogeneous unbounded environments.

The Chapter 3 presents a new model derived from this one and adapted to the two room arena, which is a complex and narrow environment. This new model is segmented into spatial zones that each corresponds to different behavioural patterns. The model parameters are automatically fit for each zone using a multi-objective evolutionary algorithm to match the behaviours exhibited by experimental fish. We evaluate how the resulting calibrated model compares to the experimental data. This new model is multi-contextual and takes account of narrow space that previous models could not handle.

This work is based on:

Chemtob Y, Cazenille L, Halloy J. Modelling context dependent collective behaviours of zebrafish in bounded fragmented environment.

I wrote the model, based on Bertrand Collignon previous work. The transcription into computer code and the calibration system were mainly carried out by Leo Cazenille, with

some adaptations by myself. I developed and implemented the data analysis scripts. This document was written mainly by me, with the help of José Halloy and Leo Cazenille.

Chapter 3

Modelling context-dependent collective behaviours of zebrafish in bounded and structured environment

Abstract

Modelling animal collective behaviours and in particular of fish has been fruitful during the last two decades. Important progress has been made thanks to mathematical models based on experimental data and empirically validated hypotheses. However, most models consider a simplified homogeneous environment without borders to focus on inter-individual interactions. Obviously, animals do also take into account others elements in their environments like tank walls or structured space. Furthermore, most models focus on aligned individuals in cohesive group behaviours. Thus, it would be interesting to develop mathematical models for collective behaviours that describe more complex dynamics. Hopefully, these models can describe a group of fully autonomous individuals in bounded and structured environments. Here, we consider the case of small zebrafish groups in a structured tank. The tank is composed of two rooms linked by a corridor. This tank can be used to study how fish groups explore their environment and make transitions between the two rooms. We propose an agent-based model that takes into account the modulation of the collective behaviours depending on the location in the environment and the social context. The model also reproduces the fission and fusion dynamics of the group. The model, based on intrinsic stochastic individuals reacting to visual perception, can automatically be calibrated with experimental data by evolutionary computation.

3.1 Introduction

Collective movements are one of the most frequently observed phenomena, whether in insects, fish or birds. Still, this remains a challenge to study despite the interest of researchers from various fields, including biology, mathematics, physics and computer science. To better understand this phenomenon, many mathematical models have been developed to simulate the collective movement of agents inspired by birds, mammals or fish.

From the 1960s to the present day, many models have succeeded in reproducing collective movements using general rules of attraction, repulsion and alignment [146]. However, these works are only weakly linked to biological data. Thanks to the development of methods for tracking and identifying individuals during experiments, more accurate data on collective movements are now available [9, 31, 163, 169, 215, 247]. Several models have been designed to reproduce several behavioural mechanics extracted from biological data [9, 15, 38, 86, 109, 261].

In previous article [49], a stochastic model has been developed based on a probability distribution function to move in targeted directions rather than on a summation of influential vectors. This model defines the probability for fish to move in all potential directions by a circular probability density function. Each stimulus perceived by the fish locally increases the probability for the focal fish to move in this direction. While most models are designed for homogeneous unbounded environments, it takes into account a bounded and inhomogeneous environment closer to natural conditions. This model makes the link between fish visual perception and motor response.

Even so, the environment described in this model is a large square aquarium, which remains a fairly simple environment. In many studies, researchers use more complex and constrained environments in order to be able to observe certain collective behaviours more accurately [26, 27, 98, 164, 167, 205, 252]. In previous studies a constrained environment, a two rooms arena with a corridor, has been used to highlight decision-making and leadership in the zebrafish [48, 211]. Principally, it has been observed that fish produce different behaviour in the different areas of the set-up, with slower speeds and higher occupancy in the rooms and lower residence time and higher speed in the corridor. To successfully simulate the behaviour of the zebrafish in such an environment, the previously produced model [49] can not be used without adaptation [43].

In this context, we have developed a new agent-based, stochastic and context-dependent model capable of simulating the collective behaviour of zebrafish in a complex and structured environment. In this study, the environment is a maze composed of two rooms connected by a corridor and the model is able to take into account the different behaviours produced by fish in each zone of the arena. This model is calibrated according to our experimental observations, but because of the different zones taken into account,

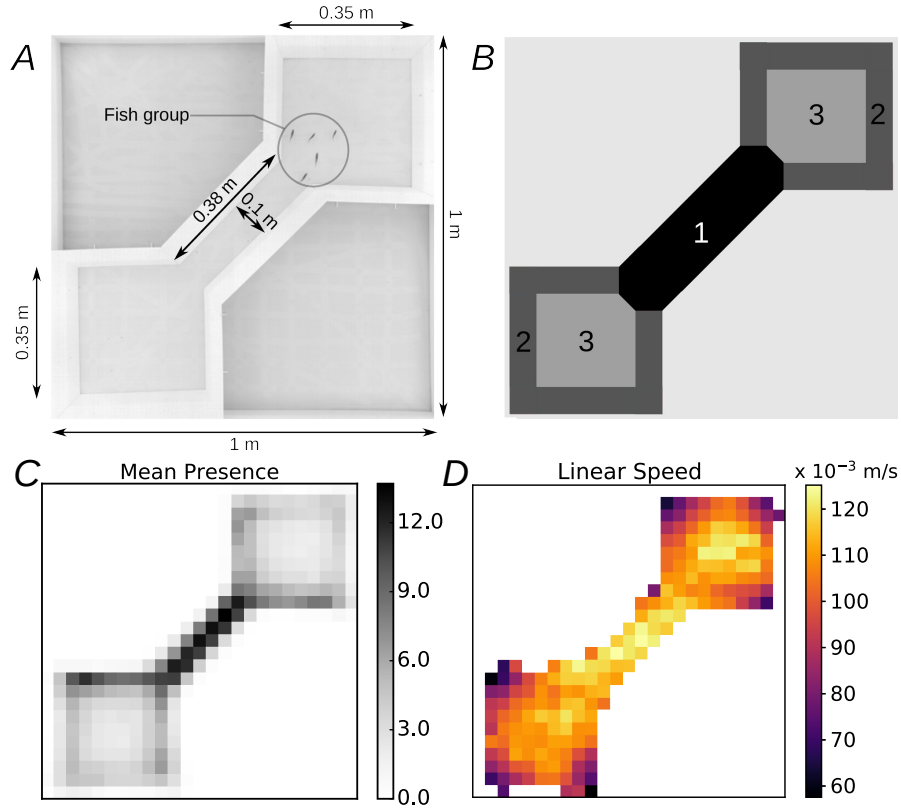


Figure 3.1: Experimental set-up. (A) Experimental arena composed of a tank containing two square rooms (350×350 mm at floor level) connected by a corridor (380×100 mm at floor level). The fish tend to swim from one room to the other, either in small groups, or individually. This set-up is used to study the zebrafish collective dynamics. (B) Positions of the three different zones corresponding to different types of behaviours: in the corridor (zone 1), in the centre of each room (zone 2) and near of the walls of each room (zone 3). (C) Probabilities of presence and (D) Linear Speed of 8 group of five zebrafish swimming in the experimental arena for 30 minutes. Fish are mainly observed along the wall of the room with a slow speed, and in the corridor with a superior speed. They are less in the centre in the room, but higher speed values are measured.

the number of parameters to be optimised is too high to be fitted empirically. To this end, we used in this article an evolutionary algorithm to optimise each parameter of the model. Then, we compare simulated agents trajectories to the biological experiments using the distributions of several metrics as references.

3.2 Results

3.2.1 Zebrafish context-dependent behaviours

Fish behave differently in complex environments depending on their spatial position. Based on the set-up (figure 3.1 A) described in our various experiments [41–44, 48, 211], we want to identify zones of the structured set-up where fish display different behaviours.

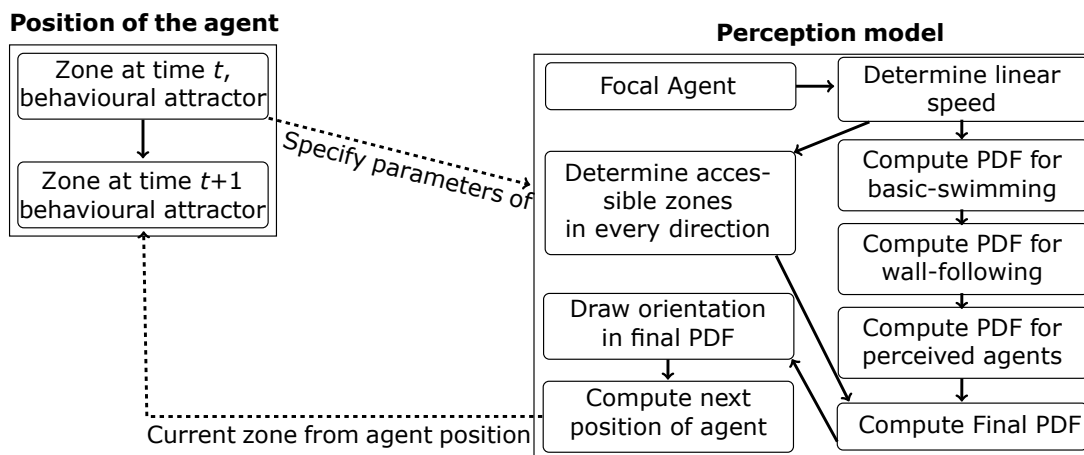


Figure 3.2: Multilevel model used to describe fish behaviour. The agents display different behavioural attractors depending on the zone where they are situated. Thus, according to the agent spatial position, the physical features of the zone drive them towards a specific behavioural attractor. A behavioural attractor corresponds to a set of behavioural patterns adapted to the zone where they are located. It can correspond to different parameters sets for the same behaviour kind.

In a constrained system of two rooms connected by a corridor, previous works [48, 211] have shown that fish have a different dynamic depending on the area. For instance, groups of fish remain mainly in the rooms while frequently passing through the corridor [211]. They perform collective departures from one room to the other [48].

Figures 3.1 highlights such variation in behaviour between zones. The spatial distribution (figure 3.1C) shows a stronger presence near the walls of the rooms and in the corridor. The high presence in the corridor is explained by the high number of transitions and the width of the area, as the residence times in this part are in the order of a few seconds [211]. The spatial representation of linear speeds (figure 3.1D) also shows strong variation. Speeds are high in the corridor, confirming the rapid transitions in this area. Speeds are also high in the centre of the rooms, unlike the areas near the walls which show lower speeds. We therefore identify three zones of the structured set-up with different fish behaviours (figure 3.1 B) : near the walls, at the centre of the rooms, and inside the corridor.

We have empirically selected the distributions of speed, distance to walls, subgroup size and inter-individual distance in the different zones of the set-up as markers of zebrafish behaviour (figures 3.5&3.6 in blue). The last two are representative of the group cohesion while the rest of the distributions represent the swimming metrics.

The distribution of subgroup sizes (figure 3.5) is particularly interesting because it highlights a stronger cohesion in the corridor than in the rest of the areas.

We measured the differences between the zones for each of these distributions with a Friedman test. All distributions were significantly different from one zone to another

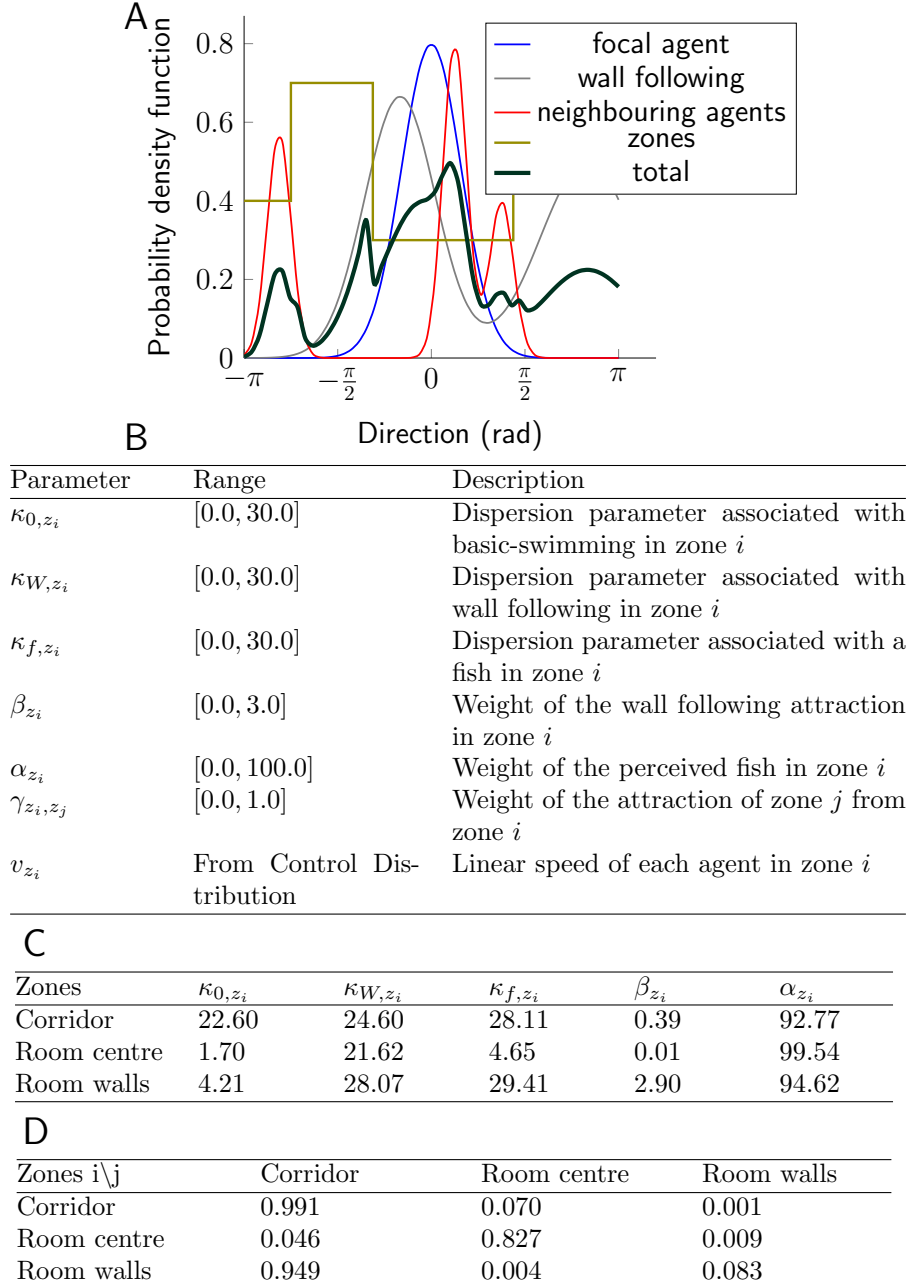


Figure 3.3: **Panel A** Computation of the PDFs functions used by the model. One function corresponds to the focal fish; another corresponds to the perceived neighbouring agents. The final PDF is a weighted sum of these functions, with a normalisation factor γ_{z_1,z_2} corresponding to the affinity between the zones z_1 (origin) and z_2 (destination). The direction taken by an agent is drawn randomly from the resulting PDF by inverse transform sampling. **Panel B** Table of model parameters for each agent. The zone z_i corresponds to the zone where the agent is situated at time t , and z_j to the zone where the agent would be at time $t + 1$. The linear speed distributions of the agents are the same as the ones observed in the Control experiments, and they are not optimised. The other parameters in the table are optimised. **Panel C** Table of parameters values for each zone z_i . **Panel D** Table of parameters values γ_{z_i,z_j} corresponding to the affinity between the zones z_i (origin) and z_j (destination).

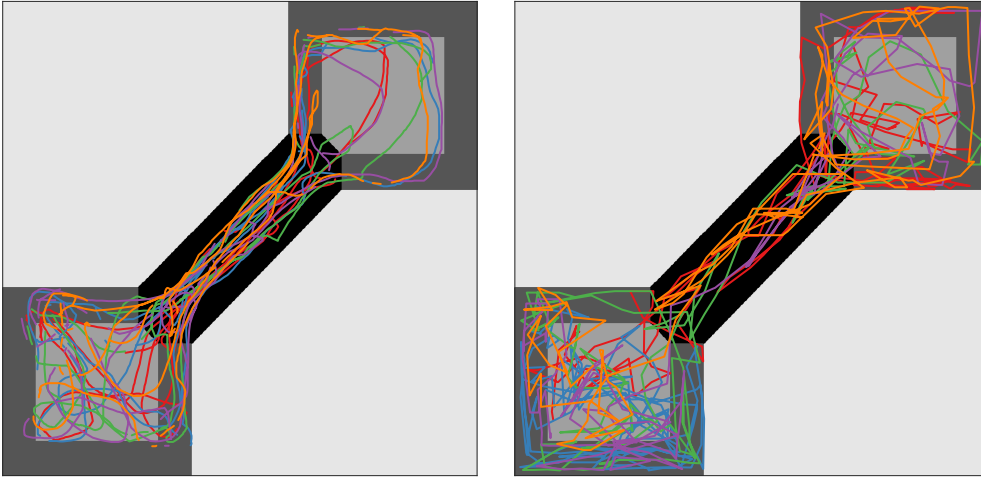


Figure 3.4: Examples of trajectories over a duration of one minute (Left reference, Right simulation). In this example, the model simulates transitions between the rooms while maintaining the cohesion of the group.

(pvalues $\ll 0.01$) except for the subgroup size between the borders and centres of the rooms (pvalue = 0.07) and all the zones of the interindividual distance of 2 fish (pvalue = 0.15). This choice of these distributions is therefore relevant in the context of a dependent context model.

3.2.2 Multilevel and context-dependent stochastic model

The model developed in this article describes the individual and collective behaviours of fish. It is stochastic, multi-level and context-dependant. Zones of different behaviour are take into account and thus allows context-dependent behaviours. Our model describes individual choices close to action selection and collective behaviours at the same time. This model is inspired by a previous model [49] as it makes the link between fish visual perception (of congeners and walls) and motor response (*i.e.*: trajectories of the agents). However, it is also capable of expressing a variability in agents behaviours when they occupy specific zones of the arena. Table 3.3B lists the model parameters.

The agents update their position vector X_i with a velocity vector V_i :

$$X_i(t + \delta t) = X_i(t) + V_i(t)\delta t \quad (3.1)$$

$$V_i(t + \delta t) = v_i(t + \delta t)\Theta_i(t + \delta t) \quad (3.2)$$

The model computes a circular probability distribution function (PDF) [49] corresponding to the probability of the agent to move in a specific direction (Θ_i). This PDF is as a mixture of von Mises distributions, an equivalent to the Gaussian distribution in circular probability. The computation of this PDF involves the calculation of three different PDF functions: the first one describing agent behaviour when no stimuli is present, the

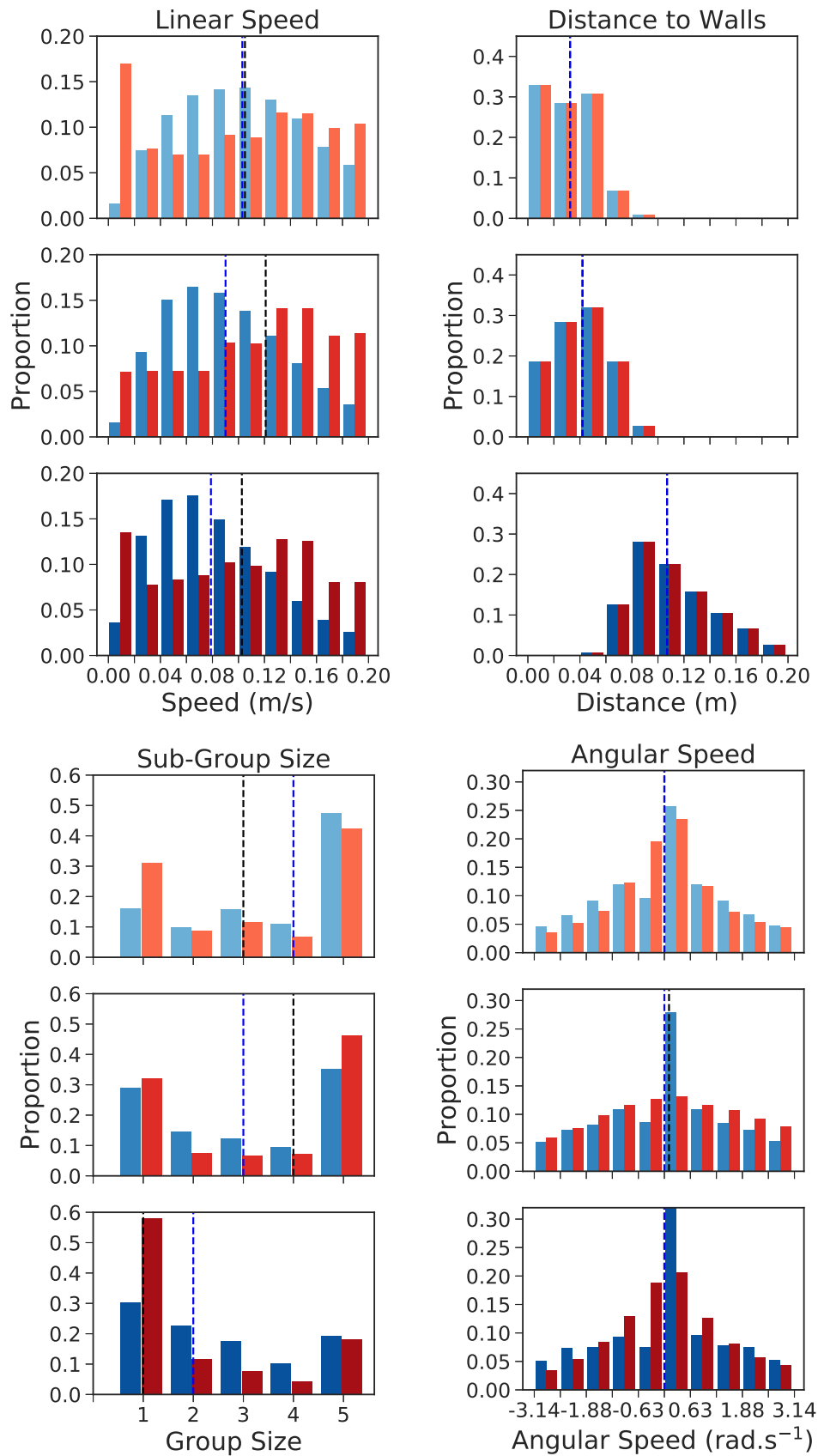


Figure 3.5: Speed density, distance to walls and subgroup size distributions for the zebrafish over the span of an hour in the three different zones of the maze (from top to bottom : corridor, border of rooms and centre of rooms. Reference data in blue and simulation in orange). All distributions are significantly different from each zones (pvalues $\ll 0.01$) except for the subroupe size between borders and centres of the rooms (pvalue = 0.07)

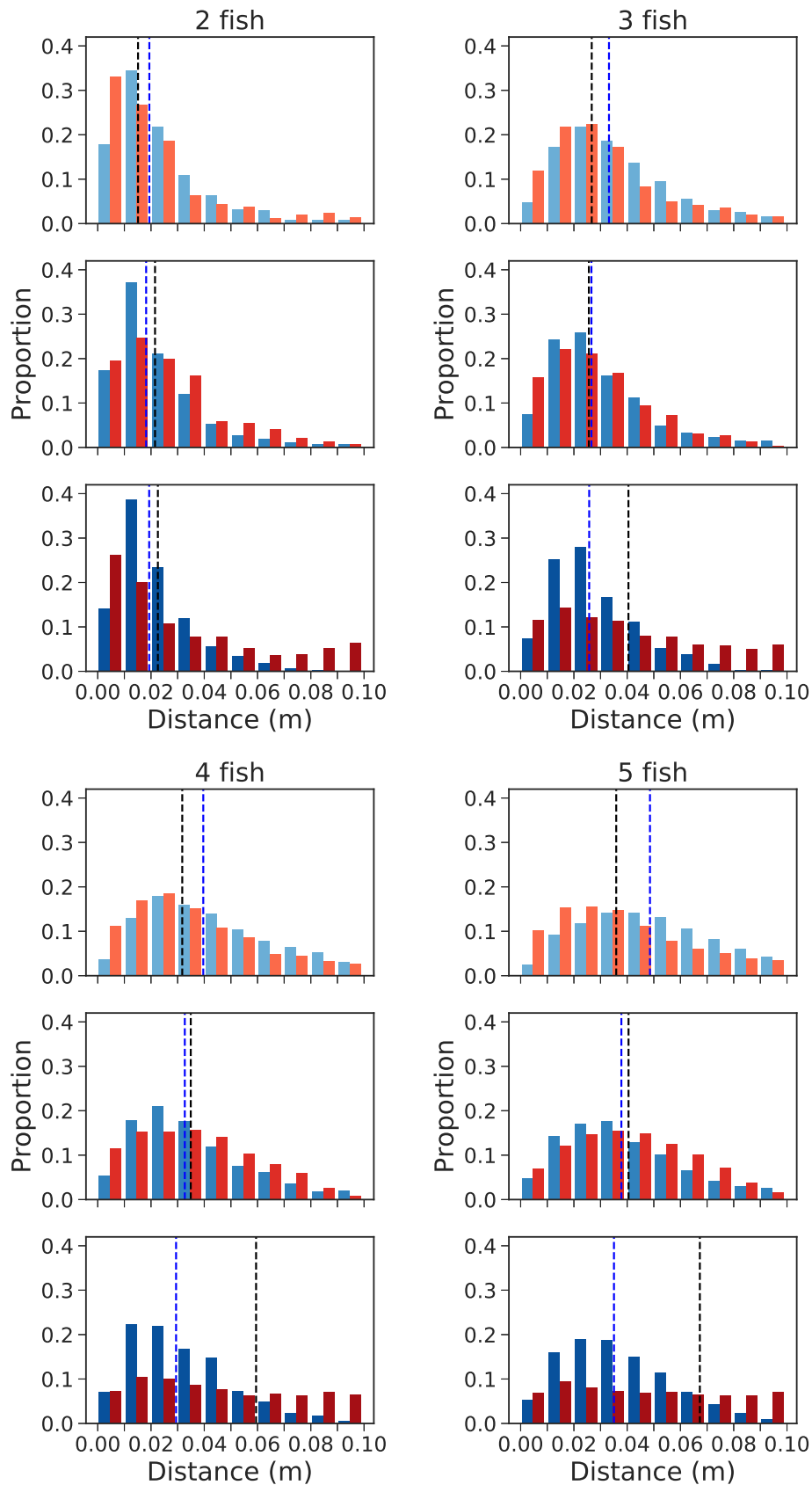


Figure 3.6: Interindividual distance between the zebrafish over the span of an hour in the three different zones of the maze (from top to bottom : corridor, border of rooms and centre of rooms. Reference data in blue and simulation in orange). All distribution are significantly different from each zones (p values $\ll 0.01$) except for the interindividual distance for groups of 2 individuals (p value = 0.15)

second one implement the wall-following behaviour, and the third one characterising agent behaviour when conspecifics are perceived by the agent. The attraction of agents towards target zones is represented by the parameter γ_{z_1, z_2} , used as a multiplicative term of the final PDF.

The PDF capturing agent behaviour when no stimuli is present is given by:

$$f_{0, z_j}(\theta) = \frac{\exp(\kappa_{0, z_j} \cos(\theta))}{2\pi I_0(\kappa_{0, z_j})} \quad (3.3)$$

for an agent situated in zone z_j , and with I_0 the modified Bessel function of first kind of order zero.

The wall-following behaviour is implemented by increasing the probabilities of moving towards either side of the closest wall. This is achieved by using the following PDF:

$$f_{W, z_j, w}(\theta) = \frac{1}{2} \sum_{k=1}^2 \frac{(\mu_{w_k} - \pi) \exp(\kappa_{W, z_j} \cos(\theta - \mu_{w_k}))}{2\pi I_0(\kappa_{W, z_j})} \quad (3.4)$$

with μ_{w_k} the two possible directions along the considered wall. The factor $\mu_{w_k} - \pi$ increase the probability to go in the direction close to the fish one.

Once the PDFs of the basic swim have been computed, we must calculate the influences of the other agents. In this model, the perceived fish are represented in three dimensions in order to be able to calculate the solid angle they represent in the visual field of the focal fish. The calculation of the solid angle instead of the angle allows a better discrimination of the different distance and positions of the perceived fish [49]. The probability of the focal fish to orient towards a perceived fish is given by a von Mises distribution clustered around the fish position:

$$f_{F, z_j}(\theta) = \sum_{i=1}^n \frac{A_{f_i}}{A_{T_f}} \frac{\exp(\kappa_{f, z_j} \cos(\theta - \mu_{f_i}))}{2\pi I_0(\kappa_{f, z_j})} \quad (3.5)$$

with μ_{f_i} the direction towards the perceived agent, $A_{T_f} = \sum_{i=1}^{n_f} A_{f_i}$ the sum of the solid angles A_{f_i} captured by each agent and n_f the number of perceived agents.

The final PDF $f(\theta)$ is computed as follow:

$$f_{z_j, z_k}(\theta) = \gamma_{z_j, z_k} \frac{f_{0, z_j}(\theta) + \beta_{z_j} f_{W, z_j}(\theta) + \alpha_{z_j} A_{T_f} f_{F, z_j}(\theta)}{1 + \beta_{z_j} + \alpha_{z_j} A_{T_f}} \quad (3.6)$$

with α_{z_j} and β_{z_j} the parameters weighting the influence of the perceived fish and the wall-following.

Figure 3.3A describes how the final PDF is computed and how it is used to determine the agents next positions.

Unreachable areas of the PDF (e.g. the walls) are attributed a probability of 0. Then, we numerically compute the cumulative distribution function (CDF) corresponding to this custom PDF $f(\theta)$ by performing a cumulative trapezoidal numerical integration of the PDF in the interval $[-\pi, \pi]$. Finally, the model draws a random direction Θ_i in this distribution by inverse transform sampling. The position of the fish is then updated according to this direction and his velocity with equations 3.1 and 3.2.

3.2.3 Numerical simulations and experimental results

The interpretation of the calibrated parameter values (figure 3.3) allows us to better understand the rules of behaviour. By comparing the parameter values between the corridor and the zone near the walls, we can deduce that wall following is less important in the corridor as the β value is much lower than near the walls in the rooms. Similarly, the low κ_0 value in this area indicates a lower attraction to conspecifics than in the corridor. Finally, in the centre of the rooms, the low values of κ_0 , κ_W and β made it difficult to interpret the influences in this zone.

We defined a similarity measure (see 3.4.6) to compare the experimental results and numerical simulations. We used the distribution of zones occupation, polarisation and subgroup size for the similarity scores. Those scores are used as references for the automatic calibration of the model parameters.

The fitness function value between the best-performing individuals and the Control case is 0.670 with similarity scores of 0.769 for the subgroup size, 0.498 for the transition probabilities, and 0.787 for the polarisation distributions. The score of other distributions is also calculated but is not used for the calibration: 0.982 for the zones occupation and 0.726 for the inter-individual distance.

By comparing the distributions of simulations and control experiments in figure 3.5, we can see that the angular speed distributions and the distance to the wall are correctly respected. Looking in detail at the distributions of subgroup sizes, it can be seen that if the proportion of groups of five individuals (the whole group) is well simulated, intermediate sizes differed from the reality. In particular, the proportion of isolated individuals is over-represented in the corridor and in the centre of the rooms. In figure 3.6, the interindividual distance distributions also show higher distances in the simulations than in the control experiments for the zone representing the centre of the rooms. However, the rest of the distributions are accurate.

In the trajectory example in figure 3.4, we can see a correct simulation of the transitions between the rooms. However, the trajectories seem less fluid and more subject to U-turns than in real fish.

3.3 Discussion

Collective behavioural models often focus on collective movement in a homogeneous and unbounded environment. We present here a model capable of simulating the behaviour of several agents in a constrained environment by expressing different behaviour depending on the spatial context. This model is based on previous work [49] presenting a behavioural model in a simple bounded environment. Here we have added the consideration of the spatial context. We have performed a series of experiments, and combined with the knowledge acquired in similar works [48, 211], we observed that agents behave differently depending on the type of environment. Based on those biological observations, we segment our environment into several characteristic zones, each corresponding to different types of agent behaviour. Due to these different zones, we obtain a large number of parameters to calibrate. To solve this problem, we use a multi-objective optimisation methodology to automatically calibrate this model. Compared to the model developed by Collignon *et al.* [49], wall following has also evolved. Previously, it was modelled by a new PDF replacing the basic-swimming PDF when the agent was near it. In the environment presented here, the omnipresence of walls has forced us to re-think this mechanism by integrating it as an influence in the same way as the attraction towards another agent.

We compared our simulations to our experiments and observed that this biomimetic multilevel and context-dependent model reproduces the collective behaviour of zebrafish. It maintains group cohesion while producing satisfactory transitions between the different zones.

However, some aspects could be further improved. The calibration of the model is performed on a relatively small number of metrics that have been empirically selected. Also, even if the calibration is automatic, the parameters limits are defined manually. Similarly, the areas selected in this study were defined empirically. If the model does not simulate agents with a fixed speed, it draws its speeds randomly according to a distribution calibrated on fish experiments. The model could be improved by taking into account the speed according to previous movements and speeds. Other studies [38] have, for example, produced models describing burst-and-coast dynamics present in several fish species, including zebrafish. Finally, the model could take into account variations in individual behaviour within the same group to highlight decision-making and leadership [48]. This could allow more fluid trajectories to be obtained while maintaining cohesion, as an agent with ‘bold’ parameters could drive a group of ‘shy’ agents without necessarily seeking to turn around when several individuals are behind him. Similarly, the model could be improved by more accurately simulating the fish reaction time. Here, the fish reaction is correlated to the time step used in the simulation and arbitrarily set to 1/3s. We could imagine a model where the time step would be adaptive to the social context

and would take into account the temporality of fish reaction as it could be shown in Harpaz *et al.* [99].

In addition to understanding the interactions between fish, the approach used to produce our model allows us to simply adapt to the problem of a constrained environment and could be implemented in robotic agents used in ethological experiments. It has been shown that the use of biomimetic behaviour in a robot agent increases its ability to be integrated [43] and the use of such a model could allow a robot to be driven in a constrained environment.

3.4 Materials and Methods

3.4.1 Ethic statement

The experiments reported in this study were performed in accordance with the recommendations and guidelines of the Buffon Ethical Committee (registered to the French National Ethical Committee for Animal Experiments #40) after submission to the state ethical board for animal experiments.

3.4.2 Animals and housing

The fish were reared in housing facilities ZebTEC and fed two times a day (Special Diets Services SDS-400 Scientific Fish Food). We kept the fish under laboratory conditions, 27 °C, 500 μ S salinity with a 10:14 day:night light cycle. The water pH was maintained at 7 and the nitrite concentration (NO²⁻) was below 0.3 mg/l. All zebrafish observed in this study were 6-12 months old at the time of the experiments.

3.4.3 Experimental set-up

We observed groups of zebrafish swimming in an arena consisting of two square rooms connected by a corridor starting at one corner of each room placed in a 100 cm x 100 cm x 30 cm experimental tank (figure 3.1A). The walls of the arena were made of white opaque PMMA. The water depth was kept at 60 mm during the experiments. One lamp (400W) was placed on the floor at each edge of the tank which is 60 cm above the floor to provide indirect lightning. The whole set-up was confined behind white sheets to isolate experiments and homogenise luminosity. An overhead acA2040-25gm monochrome GigE CCD camera (Basler AG, Germany) and equipped with low distortion lenses CF12.5HA-1 (Fujinon, Tokyo, Japan) was mounted 1.60m above the water surface to record the experiments at a resolution of 2048 \times 2048 pixels and at 15 frames per second.

3.4.4 Experimental procedure

We observed 8 groups of five adult laboratory wild-type zebrafish (*Danio rerio* AB strain) during one hour for a total of 8 experiments. Before the trials, the fish were placed with a hand net in a cylindrical arena (20 cm diameter) in one of the two rooms randomly. Half of the experiments started in the upper room and the other half in the lower room. Following a 5 minutes acclimatisation period, the fish were released and able to swim in the experimental arena. After one hour, the fish were caught by a hand net and replaced in the rearing facilities. All the experiment was recorded by the camera.

3.4.5 Data analysis

The videos were analysed offline by the idTracker software [181]. This multi-tracking software extracts specific characteristics of each individual and uses them to identify each fish without tagging throughout the video. This method avoids error propagation and is able to successfully solve crossing, superposition and occlusion problems. We obtained the coordinates $P(x, y, t)$ of all fish at each time step $\Delta t = 1/15s$. With these coordinates, we built the trajectories of each fish and computed their position in the arena and their instantaneous speed v_t computed as the distance between $P(x, y, t - 1)$ and $P(x, y, t + 1)$ divided by two time steps. We compute the probability of presence (figure 3.1C&D) and from both information, we built three zones (figure 3.1B): the corridor, the centre of each room and near of the walls of each room. Position of the wall where found from the video and consider as multiple lines. The distance to the walls have been calculate as the smallest distance between the lines and fish positions. Unlike more cohesive species, zebrafish have a very dynamic collective behaviour in which the group will frequently split into sub-groups before quickly reforming. We identify sub-groups of agents using a clustering algorithm that uses only the position of each individual to detect the sub-groups in each frame.

3.4.6 Implementation and numerical simulations

We define a similarity measure (ranging from 0.0 to 1.0) to compare two experiments (e_1 and e_2), and define it as:

$$S(e_1, e_2) = \sqrt[3]{I(O_{e_1}, O_{e_2})I(T_{e_1}, T_{e_2})I(D_{e_1}, D_{e_2})} \quad (3.7)$$

O_e is the distribution of sub-groups sizes in zone e , T_e the transition probabilities from zone e to the others, and D_e the distribution of the polarisation between agents in zone e . The similarity measure $S(e_1, e_2)$ corresponds to the geometric mean of these three

features. The function $I(P, Q)$ is defined as such:

$$I(P, Q) = 1 - H(P, Q) \quad (3.8)$$

The $H(P, Q)$ function is the Hellinger distance between two histograms [66]. It is often used as a metric to compare distributions [41–43]. It is defined as:

$$H(P, Q) = \frac{1}{\sqrt{2}} \sqrt{\sum_{i=1}^d (\sqrt{p_i} - \sqrt{q_i})^2} \quad (3.9)$$

We use the NSGA-II [64] multi-objective algorithm with three objectives to maximise, as it was done in Cazenille *et al.* [41]. The first objective is a performance objective corresponding to the $S(e_1, e_2)$ function. We also consider two other objectives used to guide the evolutionary process: one that promotes genotypic diversity [162] (defined by the mean euclidean distance of the genome of an individual to the genomes of the other individuals of the current population), the other encouraging behavioural diversity (defined by the euclidean distance between the O_e , T_e and D_e scores of an individual). We use populations of 50 individuals (approximately twice the number of dimensions of the problem) and 200 generations. The linear speed v_i of the agents is not optimised, and is randomly drawn from the instantaneous speed distribution measured in the control experiment.

Part III

Integration of robotic fish into zebrafish groups

Animals recognise and interact with each other through mechanisms that have been the focus of behavioural research for a long time. Animal communication can be based on simple signals [243] and it is therefore possible to interact with animals via artefacts specially designed to specifically generate relevant signals for social behaviour. First of all, for each lure used, only one specific behaviour could be studied. Consequently, these devices remain limited to simple interactions and are unable to adapt their behaviours to the animal responses. But, thanks to advances in technology, these simple tools have been replaced by robotic devices, now more advanced and affordable, capable of sending stimuli to the animal, detecting the response and adapting its behaviour to it. Each element of the robot behaviour can be controlled individually and their abilities allow them to interact with groups of animals and not be limited to interactions from one robot to one animal.

As part of the European project ASSISibf, a new robot has been developed to study the collective behaviour of zebrafish [17, 19, 24]. It consists of a mobile robot moving below the aquarium tank and coupled magnetically with a lure module moving inside the aquarium. The small dimensions of the mobile robot and the fact that it is controlled using wireless link, offer the possibility of multi-robot experiments. The robot is powered using conductive plates that are installed on the experimental set-up to allow long-term experiments.

In Chapter 4, we study the parameters necessary for the integration of a robot into a social group of zebrafish. Our hypothesis is that the robotic lure not only needs to have a biomimetic visual appearance, but also needs to be driven by a biomimetic behavioural model, in order to be socially accepted. We describe a robotic behavioural model inspired from a previously developed model of fish behaviour [49]. We validate this model experimentally, and compare it to experiments where the robot is not driven by a biomimetic model. We complement this study by investigating the effect of lure visual appearance on social integration. We show that both robot behaviour and lure appearance are relevant for a robotic lure to be socially integrated into a group of fish. Our results confirm that both aspects need to be designed to be biomimetic.

This work is based on the publication [43]:

Cazenille L, Collignon B, **Chemtob Y**, Bonnet F, Gribovskiy A, Mondada F, Bredeche N, Halloy J. How mimetic should a robotic fish be to socially integrate into zebrafish groups? *Bioinspiration & biomimetics*. 2018

The experimental set-up was designed and built by Bertrand Collignon, Axel Seguret, Leo Cazenille, José Halloy and myself. The FishBot robot was developed at the EPFL, by Frank Bonnet and Francesco Mondada. The experimental arena was built by Axel Seguret and myself. The control and tracking system was jointly developed by Frank Bonnet, Alexey Gribovskiy and Leo Cazenille. The experiments were carried out by

Bertrand Collignon and myself. This document was written mainly by Leo Cazenille, with the help of José Halloy and Nicolas Bredeche.

In Chapter 5, in order to improve the biomimetic behaviour of the robot, we implement in it the biomimetic multi-level and context-dependent model from Chapter 3. We show experimentally that this model allows good social integration of fish and robots in a structured environment.

This work is based on the publication [41]:

Cazenille L, **Chemtob Y**, Bonnet F, Gribovskiy A, Mondada F, Bredeche N, Halloy J. Automated calibration of a biomimetic space-dependent model for zebrafish and robot collective behaviour in a structured environment. In Conference on biomimetic and biohybrid systems 2017

The experimental set-up was designed and built by Bertrand Collignon, Axel Seguret, Leo Cazenille, José Halloy and myself. The FishBot robot was developed at the EPFL, by Frank Bonnet and Francesco Mondada. The experimental arena was built by Axel Seguret and myself. The control and tracking system was jointly developed by Frank Bonnet, Alexey Gribovskiy and Leo Cazenille. I carried out all the experiments. This document was written mainly by Leo Cazenille, with the help of José Halloy and Nicolas Bredeche.

Finally the Chapter 6 developed the use of a continuous real-time calibration method for the multi-level behavioural model. In the previous Chapter, the model has been calibrated on experimental data from a set of experiments with only fish. In this Chapter, while the robot moves along the fish, the model is calibrated using an evolutionary algorithm to correspond to the observed fish behaviour in real-time. The calibrated model is implemented on the robot every minute with each new update. This method allows to cope with changes of dynamics in fish behaviour as well as taking the individual behavioural variability into account. This real-time calibration methodology can optimise the robot behaviours during the experiments.

This work is based on the publication [42]:

Cazenille L, **Chemtob Y**, Bonnet F, Gribovskiy A, Mondada F, Bredeche N, Halloy J. How to blend a robot within a group of zebrafish: Achieving social acceptance through real-time calibration of a multi-level behavioural model. In Conference on Biomimetic and Biohybrid Systems 2018

The experimental set-up was designed and built by Bertrand Collignon, Axel Seguret, Leo Cazenille, José Halloy and myself. The FishBot robot was developed at the EPFL,

by Frank Bonnet and Francesco Mondada. The experimental arena was built by Axel Seguret and myself. The control and tracking system was jointly developed by Frank Bonnet, Alexey Gribovskiy and Leo Cazenille. I carried out all the experiments. This document was written mainly by Leo Cazenille, with the help of José Halloy and Nicolas Bredeche.

Chapter 4

How mimetic should a robotic fish be to socially integrate into zebrafish groups ?

Abstract

Biomimetic robots are promising tools in animal behavioural studies. If they are socially integrated in a group of animals, they can produce calibrated social stimuli to test the animal responses. However, the design of such social robots is challenging as it involves both a luring capability including appropriate robot behaviours, and the acceptance of the robots by the animals as social companions. Here, we investigate the integration of a biomimetic robot driven by biomimetic behavioural models into a group of zebrafish (*Danio rerio*). The robot behaviours are based on a stochastic model linking zebrafish visual perception to individual behaviour and calibrated experimentally to correspond to the behaviour of zebrafish. We show that our robot can be integrated into a group of zebrafish, mimic their behaviour and exhibit similar collective dynamics compared to fish-only groups. This study shows that an autonomous biomimetic robot was enhanced by a biomimetic behavioural model so that it can socially integrate into groups of fish.

4.1 Introduction

Robots are used in ethology and behavioural studies to untangle the multimodal modes of interactions and communication between animals. They can be biomimetic mechanical artefacts that are teleoperated in order to trigger a response from the studied animals. This allows testing various hypothesis on the type of signal used by animals for social interactions [125, 180]. In those studies the human is still in the loop of interactions between the artefact and the animal. This simplifies the design, the implementation and the control of the robot. Alternatively, since the pioneer work of the robot sheepdog and

ducks [249], an increasing number of studies [120] have used fully autonomous robots to interact with animals. These robots are programmed to induce reproducible stimuli (possibly embedded in the social context), in order to observe the response of the studied animals during repetitive, sustained and long-lasting social interactions. In this case, the human is not in the experimental loop, and doesn't even need to be present during the experiments. Examples of autonomous robots used in ethology include cows [52], drosophila flies [260] or fish [77]. Different strategies exist to build robot and animal interactions:

(i) First, the artificial systems do not copy any feature of the animal but send cues that the animal responds to. It can for example make use of supra-normal stimuli [243]. The cues can also be abiotic repellent signals such as threats or pain (like electric shocks), or can be attractant such as food or any other attracting chemicals or features. The devices can be carried by animals that are somehow tele-operated by these devices [52]. We call this approach *non-biomimetic* as the aim of the design of the robots or devices is not to mimic a feature of the studied animal. Indeed, being biomimetic is not a necessity to interact with animals.

(ii) Second, the artificial agent acts as a different animal species such as a sheepdog. The robot can be biomimetic but to another species like a dog for the sheep. This can be a special biomimetic case of inter-species interactions [230] if the robot copies the sheepdog-like animal in its features and behaviours and pertains also to the next category below. But this approach can also be non biomimetic and fall back to the first approach, the robot being just an alien agent interacting with the animals [249].

(iii) Third, the artificial agent is mimicking the animal, luring it as if being the same animal species and using similar signals and behaviours [97]. We call this approach *biomimetic* and it is the approach developed in this study.

However, the biomimetic parameter and feature space are very large. The challenge is to choose the most relevant features like some specific visual resemblance, olfactory signature, behavioural similarity. This approach needs metrics to compare each selected features or set of features to select the most relevant ones to avoid the elusive task of building an artificial animal in all its features. We define *social integration* as being part of a group displaying biomimetic features compared with the animals. The *social integration* (and its quantification using these metrics) of a robot in a group of fish is the main contribution developed in this study.

Several studies (see Tab. 4.1) have used robots to influence or to control the behaviour of fish, either alone or in a shoal. Most studies involve a robot driven by simple and non-adaptive behaviours (termed *fixed pattern* in Tab. 4.1), and open-loop social interactions. Closed-loop interactions occur when the robot influences the behaviour of the fish, and the fish influence the behaviour of the robot. The study on the electric fish (*Mormyrus rume*) opens interesting perspectives where a physical communication signal is used by

both the animals and the robots in a closed loop [68]. The electric fish study is closer to the work done on cockroaches where a chemical signal was used [97]. However in [68], the fish robot system represents a breakthrough where a modulated signal is used to achieve a close-loop communication, in this case electric.

Often the robotic systems are composed of two parts. The first part is a robot actuating the second part that is an artefact mimicking some fish features (see Tab. 4.1). This second part acts as a lure to the fish. This lure can be designed to look like a conspecific. In general, the term "robot" is used loosely to mean the whole system or only the actuating first part. Here, we refer to the second part as "the lure" because it carries the bio-mimetic body features.

Type of robots	Study	Robot behaviour	Social Interaction	Number of fish	Type of lure
Fixed to mobile arm	Phamduy et al. 2014 [187]	Fixed pattern	open-loop	Single	Biomimetic
	Polverino et al. 2013 [191]	Fixed pattern	open-loop	Shoal	Biomimetic
	Polverino & Porfiri 2013 [193]	Fixed pattern	open-loop	Shoal	Larger Size
	Abaid et al. 2012 [1]	Fixed pattern	open-loop	Single	Biomimetic
	Butail et al. 2014 [35]	Fixed pattern	open-loop	Single	Biomimetic
	Ladu et al. 2015 [136]	Fixed pattern	open-loop	Single	Biomimetic
	Ladu et al. 2015 [137]	Fixed pattern	open-loop	Single	Biomimetic
	Polverino et al. 2012 [190]	Fixed pattern	open-loop	Shoal	Larger Size
	Spinello et al. 2013 [227]	Fixed pattern	open-loop	Single	Larger Size
	Polverino & Porfiri 2013 [192]	Fixed pattern	open-loop	Single	Biomimetic
	Ruberto et al. 2016 [206]	Fixed pattern	open-loop	Single	Biomimetic
	Bartolini et al. 2016 [10]	Fixed pattern	open-loop	Single	Biomimetic (several lures on a robot)
	Kruusmaa et al. 2016 [134]	Fixed pattern	open-loop	Shoal	Biomimetic (shape only)
Donati et al. 2016 [68]	Fixed pattern, communication by electric signals and tail beats	closed-loop	Shoal	Biomimetic (shape and communication)	
Self-propelled	Abaid et al. 2013 [2]	Fixed pattern	open-loop	Single	Larger Size
	Butail et al. 2013 [34]	Fixed pattern	open-loop	Shoal	Larger Size
	Butail et al. 2014 [36]	Fixed pattern	open-loop	Shoal	Larger Size
Moved by a mobile robot	Faria et al. 2010 [77]	Fixed pattern	open-loop	Single	Biomimetic
	Swain et al. 2012 [240]	Follow the centroid	closed-loop	Shoal	Biomimetic
	Landgraf et al. 2014 [139]	Follow the centroid or Recruitment	closed-loop	Shoal	Biomimetic
	Landgraf et al. 2016 [138]	Follow the centroid or Recruitment	closed-loop	Shoal	Biomimetic

Table 4.1: Recent research using robots to study fish behaviour. We classified the studies according to the technique used to move the lure, the behaviours of the lure, the number of fish swimming with the robot and the shape of the lure. The robots used in these studies can be classified into three categories: First, by using a fish-lure fixed to a mobile arm. This allows to test the reaction of one (or a few) fish to lures that differ by one characteristic, *e.g.* colour, size, tail-beating frequency. Second, by using a self-propelled aquatic robot that swim in the water with the animals. Such robots are fully autonomous and are a first step towards robots that can be used out of the lab in natural conditions. Third, by using a fish-lure linked through magnets to a mobile robot that moves under the tank. This allows the decoupling of the stimuli shown to the fish (the mock-up fish in the water) and the robot responsible for mobility. By doing so, it is possible to develop a biomimetic lure that has the same size and aspect as a real fish but also capable of reproducing their movement patterns. Four types of lures are used in the literature: *biomimetic* lures are designed to look like (in term of shape and texture) a fish and are of similar size of a fish; *biomimetic (several lures)* involves several lures actuated by a single robot; *biomimetic (shape only)* lures have a biomimetic shape, but not a biomimetic texture; *large sized* lures are not biomimetic, and are (far) larger than a fish. In [68], the lure is equipped with electric sensors and actuators, which enables the lure to communicate with a shoal of electric fish and to create closed-loop interactions between fish and robot.

The studies [138, 139, 240] describe experiments where there is a closed-loop of social interactions and adaptive robotic behaviour. Additionally, [138] presents experiments where the robot was driven by a biomimetic behaviour; but it was not adaptive (with respect to the fish behaviour) and only followed fixed-patterns of behaviour. However, in these studies, the integration of the robot in fish groups is not quantified and not established. Indeed, this kind of controller implies that the robot is more a *follower* than a real group-member (*i.e.* integrated into the group) making its own decisions. Simply following the centroid of the group is not biomimetic as fish do not exhibit this kind of behaviour, and such controller does not allow the robot to initiate action but forces it to simply follow fish. In this regard, the embodiment of biomimetic behavioural models could lead to a better integration of artificial agents in animal groups and could allow the robots to influence the collective decision of the mixed group by giving specific preferences to the robot by tuning parameter values of the model [97]. While such controllers have been said to be developed in [139], no experiments have been analysed and reported in the literature yet. Moreover, social integration can only be measured on long-lasting experiments, as it allows to test robot social integration across a large set of social and environmental contexts. While such long-lasting experiments were described in [139] (30 minutes experiments), the non-adaptive biomimetic experiments in [138] were relatively shorter (five minutes experiments).

In this study, we ask the following question: can we quantify and discriminate between sets of biomimetic features needed for a robotic fish to be socially integrated into a group of fish as an autonomous member of the group? We make the hypothesis that this robot must be designed not only to be biomimetic at the level of the lure morphology and other physical aspects but also at the level of its social behaviours.

Here, we propose to implement, in a closed-loop of social interaction, a multi-level and context-dependent biomimetic behavioural model as a controller of a small mobile robot moving a biomimetic fish-lure in a closed tank with a shoal of zebrafish.

We observe ten different groups of four zebrafish and one fish-robot moving in a two-patch square tank. For each trial, we track and identify all agents. Contrary to more cohesive species, the zebrafish often tend to have a very dynamical collective behaviour, with short-lived sub-groups of individuals. Because such splitting influences their behaviour as it changes the social context, we use a simple clustering method to identify sub-groups of agents. Then, we analyse the collective dynamics of the population, and the propensity of the robot to be integrated into the fish group. These results are then compared with data obtained by observing five zebrafish swimming in the same conditions without the fish-robot. In order to compare the experiments with fish and a robot to the control experiments with only fish, we define a similarity measure quantifying the social integration. This measure is based on several metrics characterising the impact

of the chosen biomimetic features: the linear speed distribution of the agents, the inter-individual distances distributions of agents in a sub-group, the distribution of sub-group size.

4.2 Materials and Methods

4.2.1 Ethics statement

The experiments performed in this study were conducted under the authorization of the Buffon Ethical Committee (registered to the French National Ethical Committee for Animal Experiments #40) after submission to the French state ethical board for animal experiments.

4.2.2 Experimental set-up

We use ten groups of five adults wild-type AB zebrafish (*Danio rerio*) in our experiments.

Our experimental set-up (Fig. 4.1A) is equipped with two environmental patches (rooms) linked by a corridor (see Fig. 4.2A). The geometry of the setup is designed to study collective transitions between patches allowing to quantify the group cohesion and collective decision-making as in [48, 211]. Thus the robot has to be socially integrated to be part of the collective transitions between the rooms. The floor of the aquarium is covered with a sheet of teflon to provide a smooth surface for the motion of the fish-lure. An overhead camera (Fig. 4.1A) captures video frames of experiments. Then, our control and tracking software (called **CATS2** [18]) is able to track the positions of the agents (fish and robots), and to control the robots. A system that controls the robots of a mixed-society containing zebrafish must cope with their fast reaction time and sudden movements. Our system is designed to handle low latencies (less than 70ms, as we work in 15 frames per seconds), both at the tracking and at the control levels.

The control of fish lure motion is done through events that are sent from the control software and that contain the parameters for the locomotion.

4.2.3 FishBot and fish lures

We consider two kinds of fish lures: a biomimetic lure, and a non-biomimetic lure. The biomimetic lure was designed to mimic the visual appearance of a zebrafish as close as possible (Fig. 4.1B) using the methodology presented in [20], based on a 3D scan of a zebrafish. The lure was also covered with a decal to have the similar color patterns as

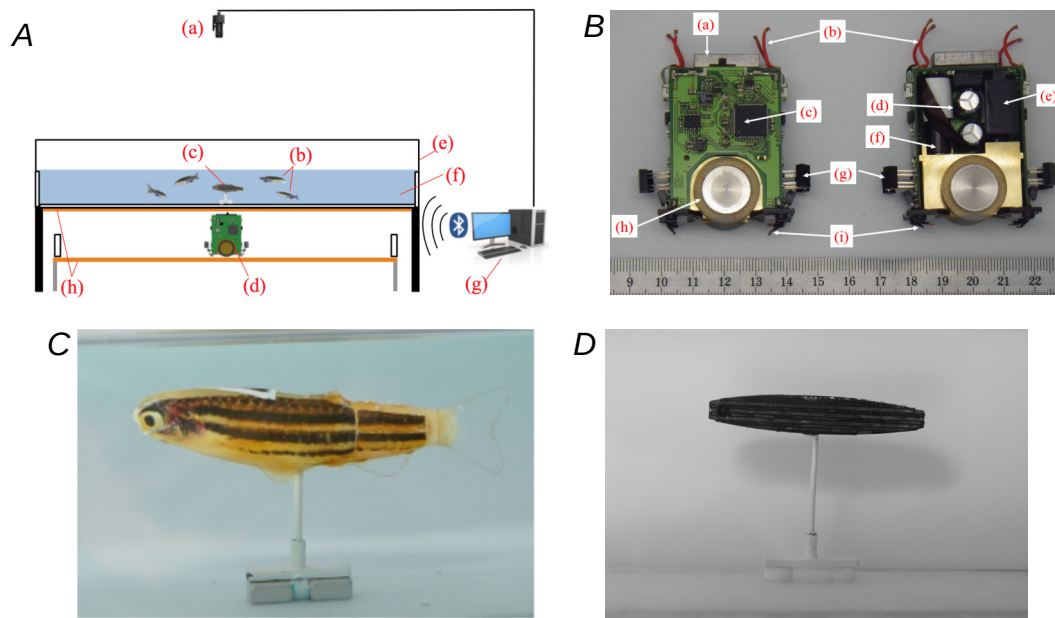


Figure 4.1: **Panel A:** Experimental set-up used during the experiments [17,19,24]. (a) Camera used to track the lure and the zebrafish. (b) Zebrafish. (c) Fish-lure inside the aquarium linked to the mobile robot through magnetic coupling. (d) FishBot moving under the aquarium. (e) Aquarium of $1000 \times 1000 \times 250$ mm. (f) Water layer of 60 mm depth. (g) The computer that processes the camera frames and remotely controls the robots via Bluetooth. (h) Conductive plates to power the mobile robot. **Panel B:** Description of the FishBot [17,19,24], the robot used for mimicking fish motion patterns. a) Magnets to magnetically couple FishBot with the lure module. b) Electric brushes to retrieve the power from the positive conductive plate. c) Microcontroller dspic33f128. d) Supercaps that store power if the contact with the plates is lost. e) Bluetooth antenna. f) Maxon DC motor. g) Infrared Proximity sensors. h) Wheel i) Electric brushes to retrieve the power from the Ground connected conductive plate. **Panel C:** biomimetic lure used during the **C1,C2,C3** experiments (see Table 4.3) [17, 19, 24]. **Panel D:** Non-biomimetic lure used in the **C4** experiment.

the zebrafish (the methodology is described in Supplementary Information). The non-biomimetic lure is a zebrafish-sized 3D printed black ellipsoid (of $4.5 \times 0.5 \times 0.8$ cm). The lures are linked by magnetic coupling to a mobile robot moving below the experimental tank.

We use the miniature mobile robot "FishBot" described in [17,19,24] that can achieve the required speeds and accelerations in order to reproduce the fish displacement under water. The robot is continuously powered as described in Figs. 4.1A and 4.1C and controlled with a wireless bluetooth link, therefore it is possible to achieve long duration experiments in closed-loop (described in Supplementary Information).

4.2.4 Data analysis

Zebrafish tend to form short-lived sub-groups of individuals (especially in fragmented environments [211]), which often translate to different sub-group behaviour, as sub-group size changes the social context. In particular, fish do not behave the same way when they are alone as when they are in a group (for instance, they have different distributions of linear and angular speeds). As such, we identify sub-groups of agents in each frame of our experimental videos. We use a clustering algorithm that uses only the position of each individual to detect the sub-groups in each frame of our experimental videos. This algorithm is described in Supplementary S1.

Then, by using the tracked positions of agents (fish and robot) and the information describing sub-groups membership, we compute several statistics of individual and collective behaviour: the density of presence in the arena, the distributions of linear speeds in each room, the inter-individual distances in sub-groups of size two to five individuals, and the distribution of the number of agents in sub-groups.

4.2.5 Quantifying social integration

To quantify the social integration of the robot into the group of fish, we compare the results of the experiments with a robot with the corresponding results from the biological reference case. We define a similarity measure to compare the results of all experiments, taking into account the distribution of linear speeds, the distribution of inter-individual distances and the distribution of sub-groups size. We postulate that this similarity measure translates directly to the capabilities of the robot to socially integrate into the group of fish. We term this similarity measure the *social integration index* ($I(Ci)$ for the Ci experiment), and define it as:

$$I(Ci) = \frac{I_{\text{linSpeedRooms}}(Ci) + I_{\text{linSpeedCorridor}}(Ci) + I_{\text{interindivSG2}}(Ci) + \dots + I_{\text{interindivSG5}}(Ci) + I_{\text{SGSize}}(Ci)}{7} \quad (4.1)$$

$$I_a(Ci) = 1 - H(C0_a, Ci_a) \quad (4.2)$$

The *social integration index* $I(Ci)$ of the experiment Ci has a value between 0.0 and 1.0. A value of 0.0 corresponds to the absence of social integration of the robot, and a value of 1.0 corresponds to a social integration of the robot comparable to that of a fish.

The vectors $I_{\text{linSpeedRooms}}(Ci)$, $I_{\text{linSpeedCorridor}}(Ci)$, $I_{\text{interindivSG2}}(Ci)$, \dots , $I_{\text{interindivSG5}}(Ci)$ and $I_{\text{SGSize}}(Ci)$ correspond respectively to the histograms of the distributions of linear

speeds in the rooms, linear speeds in the corridor, inter-individual distances in sub-groups of sizes two to five individuals and to the size of sub-groups, for experiment *Ci*. These histograms are presented in Figs. 4.6, 4.7 4.8, and in Supplementary Information. We hypothesise that these are the most relevant features to identify the social integration of the robot into the group of fish. First, the speed distributions show the ability of the robot to follow the fish at the same speed as the fish. Second, the inter-individual distance distributions show the capability of the robot to be at the same distance to the fish as a fish would be towards its neighbours. Lastly, the distribution of sub-group sizes shows that sub-group dynamics in experiments with a robot are similar to those in experiments with only fish.

The $H(P, Q)$ function is the Hellinger distance between two histograms. The Hellinger distance [66] is a divergence measure, similar to the Kullback-Leibler (KL) divergence. However, the Hellinger distance is symmetric and bounded, unlike the KL-divergence (and most other statistical distance metrics). It is often used as a metric to compare distributions, and is defined as:

$$H(P, Q) = \frac{1}{\sqrt{2}} \sqrt{\sum_{i=1}^d (\sqrt{p_i} - \sqrt{q_i})^2} \quad (4.3)$$

where p_i and q_i are respectively the i -th bin values of histograms P and Q ; d is the number of bins in both histograms P and Q .

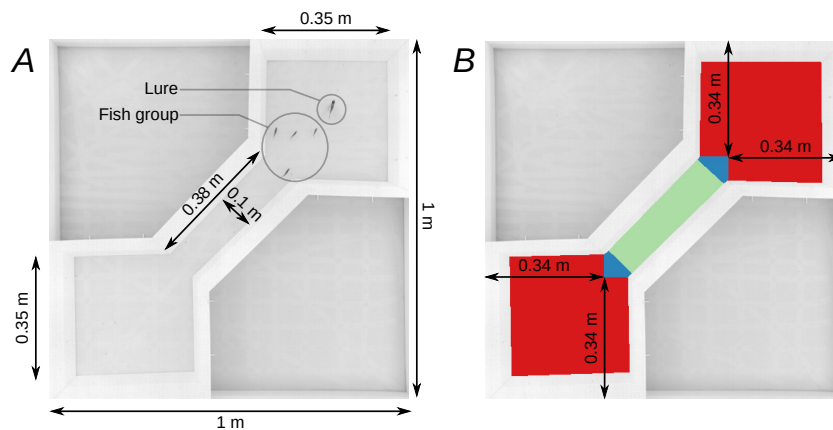


Figure 4.2: **Panel A:** Experimental arena composed of two linked square rooms used to study collective transitions and decision-making. Our biomimetic lures, described in Fig. 4.1B, can be integrated within the fish group and mimic their collective behaviours. **Panel B:** Colored zones of the arena corresponding to the three different types of behaviour of the robot. These behaviours are outlined in Table 4.2. When the robot is in the rooms (in red) or near the entrance of the corridor (in blue), it is driven by the biomimetic model presented in Sec. 4.3. This model is used to generate a new target position of the robot every $1/3$ s. If this target position is near the entrance of the corridor (in blue), the target position of the robot is not updated before 5 s are passed, to give the robot enough time to go to the entrance of the corridor. When the robot is in the corridor (green zone), it passes straight through the corridor, with a constant speed.

4.3 Multi-level approach for the robot behaviour

We present here a biomimetic model of fish behaviour, that can be implemented as a robotic controller. Our model is multi-contextual, to take into account the different behaviours exhibited by the fish in the different zones of our fragmented experimental set-up (with two rooms and a corridor, cf Fig. 4.2A). We design this model to be multi-level (Fig. 4.3).

The high-level control manages the biomimetic trajectories of the robot. We use different behavioural schemes to generate desired target positions of the robot depending on its position in the arena. These trajectories are calibrated to correspond to the analysed fish trajectories.

The low-level control corresponds to the movement patterns of the robot that are also important to facilitate social integration. It describes how the robot can move from its current position to the target positions provided by the high-level control system.

Control at both levels can be designed to be biomimetic. At the level of trajectories, zebrafish have a complex social behaviour and tend to form dynamic and short-lived groups. At the level of movement patterns, zebrafish move by successive bouts of tail-beats [261]. Each tail-beat can be modelled as a sequence of three steps: first, the tail-beat allows the fish to reorient itself towards its target position; second, the fish accelerates linearly by using the thrusting effect of the tail-beat; third, the tail-beating stops and the fish slides into water with a decreasing linear speed [19]. Here, we present a biomimetic multi-level model of these dynamics, taking into account both high-level (in Sec. 4.3.1) and low-level (in Sec. 4.3.2) controls.

Figure 4.2B and Table 4.2 summarise the different behaviours depending of the position of the robot, and how they are implemented in terms of high-level control (trajectories) and low-level control (movement patterns). A general description of this biomimetic model can be found in Fig. 4.3.

We designed our behavioural model to exhibit different behaviours depending on the spatial position of the robot in the arena. Indeed, we observed that zebrafish exhibit

Color in Fig.4.2B	Robot Current Zone	Target Zone	Trajectories	Movement patterns	Obstacle avoidance
Red	In Rooms or Near entrance	In Rooms	probabilistic (cf. Sec. 4.3.1), updated every 1/3 s	biomimetic (cf. Sec. 4.3.2)	Turn to avoid (cf. Sec. 4.3.2)
Blue	In Rooms or Near entrance	Near entrance	probabilistic (cf. Sec. 4.3.1), updated every 5 s	biomimetic (cf. Sec. 4.3.2)	Turn to avoid (cf. Sec. 4.3.2)
Green	Corridor	any	Pass-through (cf. Sec. 4.3.3)	Constant speed (cf. Sec. 4.3.4)	Braitenberg (cf. Sec. 4.3.4)

Table 4.2: Robot behaviours depending on the context

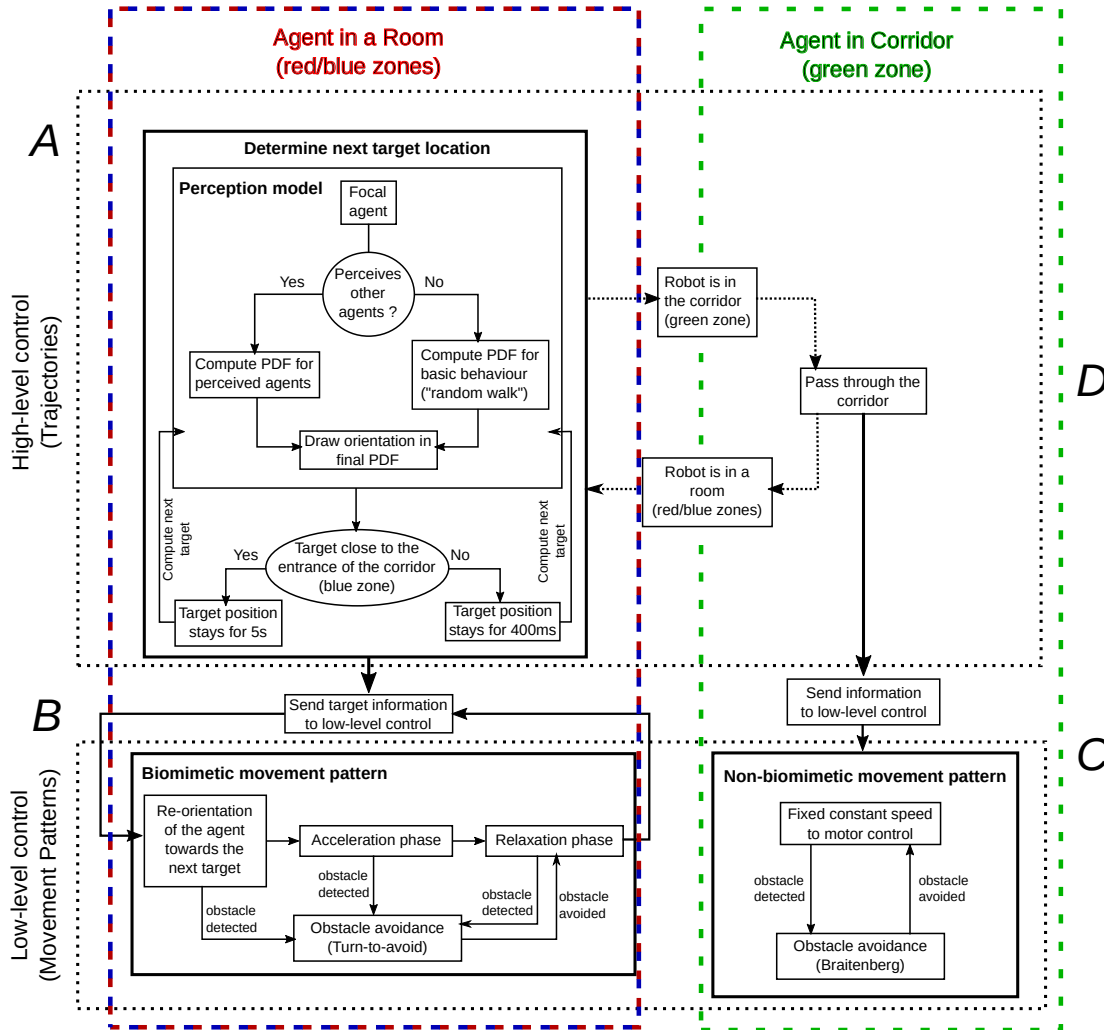


Figure 4.3: Multilevel behavioural model (here denoted **BM**) used as robot controller. Our robot control system is divided into two layers. The high-level control layer (in A and D) describes the trajectory of the robot in the arena and generates target positions. The low-level control layer (in B and C) handles the movement patterns of the robot to reach the target positions derived from the high-level control layer. The robot has different behaviours depending on whether it is in the rooms (in A and B, represented in red and blue in Fig. 4.2B) or in the corridor (in C and D, in green in Fig. 4.2B). In the rooms, the high-level (in A) and low-level (in B) controls are biomimetic. In the corridor, the high-level (in D) and low-level controls (in C) are not biomimetic, and drive the robot to transit from one room to the other through the corridor. These behaviours are summarised in Table 4.2.

different behaviours when they are in the corridor compared to when they are in a room [211] (cf Supplementary Information Figs. S2, S3, S4, S5). When they are in the corridor, they tend to go from one of the entrances of the corridor to the other room, with a different speed distribution. The fish only transit in the corridor in a rather straight manner.

When the robot is in the rooms (in red) or near the entrance of the corridor (in blue), it is driven by a biomimetic probabilistic behavioural model (Fig. 4.3A), and described

below (cf Sec. 4.3.1). This model is used to generate new target positions of the robot every 1/3 s (this time step was chosen to correspond to the tail beat frequency of the zebrafish of $\sim 2.5\text{Hz}$, as in [49]). The low-level controller of the robot makes it follow and reach these target positions with fish-like biomimetic movement patterns (Fig. 4.3B, cf Sec. 4.3.2). If a target position of the robot is near the entrance of the corridor (in blue), it is not updated before 5 s are passed, to give the robot enough time to reach the entrance of the corridor. When the robot is in the corridor (in green), it passes straight through the corridor with a constant speed.

At present, while the robot can pass through the corridor from one room to the other without wall-collision, it can be difficult for the robot to perform U-turns in the corridor (*i.e.* turn around in the corridor and go back to the room the robot was previously situated) without colliding into the walls due to the narrowness of the corridor. As such, when the robot enters the corridor, it is driven by a *pass-through* behaviour to transit from one room to the other in a straight line, without possibility of U-turn (Fig. 4.3D). We do not use the biomimetic probabilistic behavioural model (Fig. 4.3A) in this case, as it would have a small probability of generating target positions toward the room where the robot was previously situated, thus driving the robot to perform an U-Turn.

Additionally, the teflon sheet that we put on the floor of the aquarium is not perfectly plane (as it is difficult to glue this layer to the aquarium), which can slightly influence the movement of the robot due to the magnetic coupling between the robot and the lure (sliding on the teflon layer). This is especially the case when the robot is moving at high speed, or when it performs strong accelerations, for instance when it is driven by the biomimetic low-level controller (Fig. 4.3B). This is why we use a *constant speed* behaviour (without strong acceleration, Fig. 4.3C) when the robot is passing through the corridor.

4.3.1 High-level biomimetic behavioural model in the rooms

To define the trajectories of the robot in the rooms (red and blue zones in Fig. 4.2B, Fig. 4.3A), we developed a biomimetic behavioural model inspired from the probabilistic model in [49], which used a gaussian mixture agent-based approach to describe zebrafish behaviour in a shoal. However, the model in [49] was designed to model zebrafish behaviour in an empty square arena. We modified this model to be used as a robotic controller, and to handle our arena with two rooms and a corridor.

In this model, the agents update their position vector X_i with a velocity vector V_i :

$$X_i(t + \delta t) = X_i(t) + V_i(t)\delta t \quad (4.4)$$

$$V_i(t + \delta t) = v_i(t + \delta t)\Theta_i(t + \delta t) \quad (4.5)$$

The speed v_i is drawn from the speed distribution of the fish in the rooms (cf Supplementary Information Fig. S4, experiment C0). The orientation Θ_i is drawn from the probability density function (PDF) computed as a mixture distribution of *von Mises* distributions centred on the stimuli perceived by the focal agent. In this study, we only take into account the influence of other agents. The attraction towards the walls is not considered yet as it would put the robots too close to the walls, increasing greatly the number of collisions between them.

The PDF $f_0(\theta)$ for an agent to move in each potential direction θ in a bounded environment without perceptible stimulus is given by:

$$f_0(\theta) = \frac{\exp(\kappa_0 \cos(\theta))}{2\pi I_0(\kappa_0)} \quad (4.6)$$

with κ_0 a dispersion parameter associated with movements not influenced by stimulus, and I_0 the modified Bessel function of first kind of order zero, defined as:

$$I_0(\kappa) = \sum_{k=0}^{\infty} \frac{(\kappa/2)^{2k}}{k! \Gamma(k+1)} \quad (4.7)$$

where $\Gamma(z)$ is the gamma function:

$$\Gamma(z) = \int_0^{\infty} x^{z-1} e^{-x} dx \quad (4.8)$$

The model computes a PDF for the focal agent to move according to other agents (congeners). The probability of the focal agent to orient towards a perceived agent is given by a von Mises distribution clustered around this agent:

$$f_{f_i} = \frac{\exp(\kappa_f \cos(\theta - \mu_{f_i}))}{2\pi I_0(\kappa_f)} \quad (4.9)$$

with θ the potential direction of movement of the agent, μ_{f_i} the location of the perceived fish i and κ_f a dispersion parameter associated with agent attraction.

The model computes a weighted sum of all distributions f_{f_i} for all agents i , resulting in a PDF $f_F(\theta)$ defined as:

$$f_F(\theta) = \sum_{i=1}^{n_f} \frac{A_{f_i}}{A_{T_f}} \frac{\exp(\kappa_f \cos(\theta - \mu_{f_i}))}{2\pi I_0(\kappa_f)} \quad (4.10)$$

$$A_{T_f} = \sum_{i=1}^{n_f} A_{f_i} \quad (4.11)$$

with A_{T_f} the sum of solid angles A_{f_i} captured by each perceived agent i and n_f the number of perceived agents.

Finally, we calculate a weighted sum of the PDFs to obtain the global probability distribution function $f(\theta)$ of the focal fish to move towards a given direction. This global PDF is different from [49], as we removed the attraction to the walls, and is computed as follow:

$$f(\theta) = \frac{f_0(\theta) + \alpha_0 A_{T_f} f_F(\theta)}{1 + \alpha_0 A_{T_f}} \quad (4.12)$$

with α_0 a parameter weighting the influence of the perceived agents. The parameters $\kappa_0 = 6.3$ and $\kappa_f = 20$ are the same as in [49].

There is a delay (< 500 ms) between the time when a new target position is computed and the time when the robot actually reaches this position. This can have an adverse effect on the desired fish-following behaviour of the model, when the robot is part of a sub-group and is moving alongside its neighbours. Indeed, the target-following system (low-level control) must compromise between efficiency (the capability of reaching the target with a low latency) and biomimetism (fish-like movement, and fish-like speed distribution). To mitigate this effect, we selected a different value of α_0 than in [49]: we consider $\alpha_0 = n_A * 1000/n_D$ with $n_A = 5$ the total number of agents in the experiment, and n_D the number of detected agents in the current frame. This increases the tendency of the robot to follow fish groups. The number of detected agents is used to normalise the computation of α_0 as the model only takes into account the agents that are actually detected by the tracking system, which introduces a bias.

Then, we numerically compute the cumulative distribution function (CDF) corresponding to this custom PDF $f(\theta)$ by performing a cumulative trapezoidal numerical integration of the PDF in the interval $[-\pi, \pi]$ (as described in [49]). Finally, the model draws a random direction Θ_i in this distribution by inverse transform sampling. The position of the fish is then updated according to this direction and his velocity with equations 4.4 and 4.5. If the target position of the robot is in an unreachable area (*e.g.* the walls), we draw another random direction from the CDF.

4.3.2 Low-level biomimetic movement patterns in the rooms

In the rooms (red and blue zones in Fig. 4.2B), the robot is following trajectories drawn by the high-level biomimetic model described in Sec. 4.3.1. The low-level robot controller (Fig. 4.3B) is programmed to follow the target position computed using the high-level behavioural model. When the target is near the entrance of the corridor (blue zone of Fig. 4.2B), it remains there for 5s to leave enough time for the robot to reach the entrance of the corridor, and then pass through it to the next rooms. We choose the relatively long duration of 5s to prevent the high-level model to generate a new target not situated in the corridor before the robot is able to reach the entrance of the corridor, as it could lead to experiments where the robot never go through the corridor.

To accurately mimics the movement patterns of the zebrafish inside the two rooms, we used the low-level robot controller designed in [19]. At each control steps, the desired target position generated by the model described in Sec. 4.3.1 is compared with the current robot position and orientation. The difference in orientation is sent to the robot, that executes a step machine composed of three steps: First, the wheels are controlled in position to reorient the robot towards the desired target (Re-orientation phase). Second, the robot accelerates to 0.7 m.s^{-2} (Acceleration phase) and third, the speed of the robot is set to a constant speed of 8.5 cm.s^{-1} (Relaxation phase). The robot will keep this speed until the next control step. This mimics the typical locomotion of zebrafish in open areas as demonstrated in [19].

We use a simple collision avoidance scheme (termed *Turn to avoid*), where the robot stops when it is too close to a wall and then turns at a reduced speed (5 cm.s^{-1}) for 3.5 s before going back to its normal behaviour.

4.3.3 Robot trajectories in the corridor

When the robot is in the corridor (green zone in Fig. 4.2B), it is programmed to transit in a straight line towards the other room (Fig. 4.3D), with a constant speed of 12 cm.s^{-1} if the robot is not in collision with a wall. This speed is reduced to 5 cm.s^{-1} when the robot is too close to the walls, *e.g.* after a collision. The resulting mean speed of the robot is still lower than the one of the fish (cf Supplementary Information). We could not increase the speed of the robot to match the mean speed of the fish in the corridor, as it would increase the probability of crashing against a wall.

4.3.4 Biomimetic movement patterns in the corridor

Inside the corridor (green zone in Fig. 4.2B), the zebrafish usually have a constant speed as they are stressed due to the reduced size of the environment. Therefore, we implemented a controller (Fig. 4.3C) for the robot to maintain a constant linear speed of 9.4 cm.s^{-1} inside the corridor while the rotating speed is controlled using a PID (Proportional-Integral-Derivative) controller for the robot to reach the opposite room.

To mitigate the effect of eventual collisions of the robot with the walls, the low-level controller of the robot implements a *Braitenberg*-based obstacle avoidance scheme [30]. While this *Braitenberg* obstacle avoidance scheme is more efficient than the *Turn to avoid* scheme used in the rooms, it only works when the robot is moving with a constant speed. This is why we did not use it in the rooms, where the robot is driven by the biomimetic movement patterns (described in Sec. 4.3.4).

Label	Experiment	Number of agents	Robot Trajectories in the rooms	Robot Movement pattern in the rooms	Lure
C0	wild-type zebrafish only	5 fish + 0 robot	-	-	-
C1	Biomimetic model with Biomimetic lure	4 fish + 1 robot	Biomimetic (cf Sec. 4.3.1)	Biomimetic (cf Sec. 4.3.2)	Biomimetic
C2	Random walk with Biomimetic lure	4 fish + 1 robot	Random Walk	Biomimetic (cf Sec. 4.3.2)	Biomimetic
C3	Biomimetic model with Constant Speed	4 fish + 1 robot	Biomimetic (cf Sec. 4.3.1)	Constant Speed	Biomimetic
C4	Biomimetic model with non-biomimetic lure	4 fish + 1 robot	Biomimetic (cf Sec. 4.3.1)	Biomimetic (cf Sec. 4.3.2)	Non-biomimetic

Table 4.3: Analysed experiments. The **C0** experiment is the biological reference case, involving a group of five wild-type zebrafish and no robot. The **C1** experiment involves four wild-type zebrafish and one robot driven by the multi-level biomimetic model presented in Sec. 4.3. These experiments are performed in the set-up described in Fig. 4.1A with the arena in Fig. 4.2B after a 30 minutes period of acclimatization. Each experiment is repeated ten times and lasts 30 minutes. The **C2, C3** and **C4** experiments are control experiments based on the **C1** experiment. The **C2** experiment involves a robot driven by a random walk behaviour, to assess to impact of biomimetic trajectories on social integration. In the **C3** experiment, the robot moves with a constant speed along its trajectory instead of moving using biomimetic movement patterns. The **C4** experiment is performed with a non-biomimetic lure.

4.4 Results

Our goal is to socially integrate a robot driven by a multi-level biomimetic behaviour into a group of laboratory wild-type zebrafish. The experiments are done in a two connected room aquarium that is designed to study social cohesion, collective departures and decision-making [48, 211]. We consider five different experimental conditions summarised in Table 4.3. Each one of the five experimental conditions is composed of ten repetitions of 30 minutes lasting trials. Each experimental condition has thus been tested for five hours in total. We tested ten different fish groups per experimental conditions, i.e. 40 different fish with the robot. We compare these five experiments by using the similarity measure described in Sec. 4.2.5.

4.4.1 Individual trajectories

We rebuild the trajectories of each individual (fish and robot) by using the individual tracking of the agents (cf Sec. 4.2.4). Figure 4.4 presents examples (of 1 minute) of such trajectories for experiments C0 and C1 (in Panel B, the robot trajectories are in black). The fish tend to follow walls rather than staying in the center of the rooms.

Even though we did not implement any wall following behaviour into the high-level part of the BM model (Fig. 4.3A, see Sec. 4.3.1), the robot still exhibits a wall following behaviour as it tends to be attracted to fish. The trajectories of the robot appear qualitatively biomimetic. We further quantify the mixed group in the following sections.

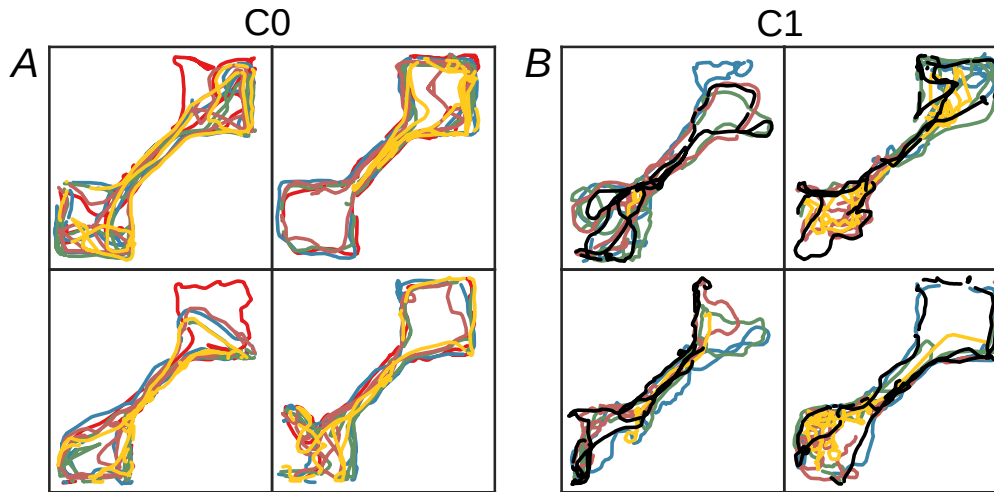


Figure 4.4: Examples of trajectories of the agents in experiments **C0** (**Panel A**) and in experiment **C1** (**Panel B**). Each subplot corresponds to 1-minute sections of a 30-minutes experiment. In **Panel B**, the robot trajectories are in black.

4.4.2 Group clustering and social cohesion

We compute the mean inter-individual distances between each pair of agents (fish and robots) of a sub-group for all experiments. Three characteristic cases were considered: all sub-groups (i.e. sub-groups of two to five individuals) in Fig. 4.6, sub-groups of two individuals in Fig. 4.7, and sub-groups of all (five) individuals in Fig. 4.8. For all three cases, the distributions of inter-individual distances of the C1 and C3 experiments are the closest to the ones of the C0 experiment.

We compute the mean fraction of non-isolated individuals in each experiment (Fig. 4.5A). In experiments with a robot, C1 (mean of 78.1%, std of 0.050) is the closest to C0 (mean of 83.3%, std of 0.037), followed by C3 (mean of 75.9%, std of 0.049), C4 (mean of 74.4%, std of 0.622), and C2 (mean of 67.4%, std of 0.062). The robot tends to be isolated slightly more often than the fish. When the robot follows our biomimetic model, it increases its capability to be in a sub-group (experiments C1 and C3). The type of movement pattern and lure also affect the capability of the robot to be in a sub-group (experiments C2 and C4).

Figure 4.5B presents the mean fraction of the population either isolated ("sub-group" of size 1) or in a sub-group with two to five individuals. Agents in the experiments with the robot (C1, C2, C3, C4) have a higher probability to be isolated and a lower probability to be in sub-groups of five individuals (whole population). Experiments C1 and C3 provide the results that are the most similar to C0. Indeed, while the robot can integrate into the group of fish, especially when driven by a biomimetic model, its behaviour could still be improved to be closer to a fish.

	C1	C2	C3	C4
Linear speed in the rooms	0.706	0.739	0.708	0.739
Linear speed in the corridor	0.812	0.694	0.608	0.637
Inter-indiv. distances in SG of 2 indiv.	0.894	0.893	0.780	0.524
Inter-indiv. distances in SG of 3 indiv.	0.927	0.897	0.972	0.885
Inter-indiv. distances in SG of 4 indiv.	0.915	0.837	0.953	0.798
Inter-indiv. distances in SG of 5 indiv.	0.866	0.759	0.861	0.706
Distribution of SG size	0.899	0.854	0.875	0.848
Social integration index (Mean score)	0.860	0.810	0.822	0.734

Table 4.4: Social integration indexes for all experiments. Higher values correspond to better integration of the robot in the group of fish. Results in bold correspond to the highest values for each feature. The **C1** experiment involves 4 wild-type zebrafish and one robot driven by the multi-level biomimetic model presented in Sec. 4.3. The **C2,C3** and **C4** experiments are control experiments based on the **C1** experiment. The **C2** experiment involves a robot driven by a random walk behaviour, to assess to impact of biomimetic trajectories on social integration. In the **C3** experiment, the robot moves in a constant speed along its trajectory, instead of biomimetic movement patterns. The **C4** is performed with a non-biomimetic lure.

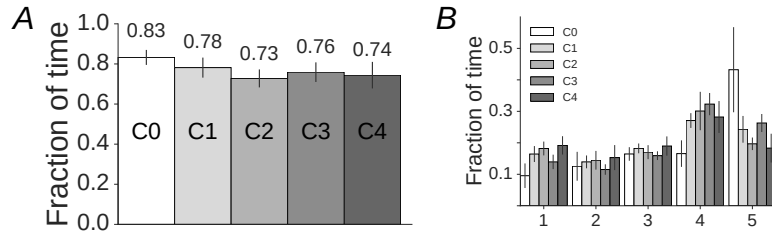


Figure 4.5: Panel A: Mean fraction of non-isolated individuals in all experiments. The distributions differ significantly (Two-sample Kolmogorov-Smirnov test, with p-values < 0.05 ; cf Sec. 4.2.4). **Panel B:** Mean fraction of the population in a sub-group of one (isolated individuals) to five (whole population) individuals. The distributions differ significantly (Two-sample Kolmogorov-Smirnov test, with p-values < 0.05 ; cf Sec. 4.2.4). Results are obtained in ten trials of 30 minutes experiments using groups of wild-type zebrafish moving in the set-up described in Fig. 4.2A.

4.4.3 Quantifying social integration

Table 4.4 presents the resulting *social integration index* and the associated $I_a(C_i)$ values for all experiments with a robot (**C1**, **C2**, **C3**, **C4**). The experiment with the highest number of biomimetic characteristics, **C1**, has the highest integration index: 0.860. Experiment C3 has the second highest integration index: 0.822, followed by C2 (0.810) and C4 (0.734). It shows the veracity of our hypothesis: a robotic fish has higher social integration capability into a group of fish if it is designed to be biomimetic on three key aspects: the morphology of the lure, the type of trajectories, and the type of movement patterns. Here, we show that the morphology of the lure has the highest impact on social integration, followed by the type of trajectory of the robot and by the type of movement patterns of the robot, with differences of social integration indexes (mean score) with C1 of respectively 0.126, 0.050 and 0.038. While C1 has the highest social integration

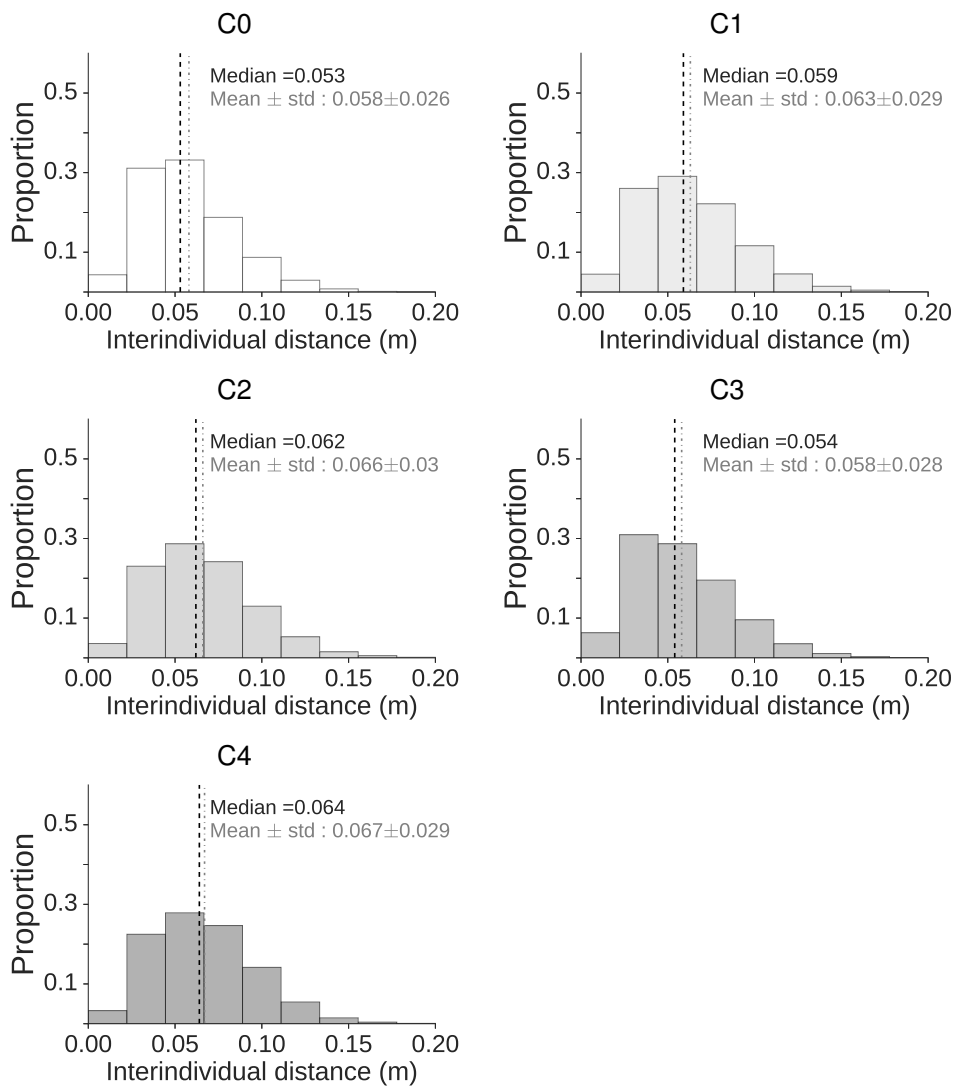


Figure 4.6: Mean inter-individual distances between each pair of agents (fish and robot) in all sub-groups. Results are obtained in 30 minutes experiments using groups of wild-type zebrafish moving in the set-up described in Fig. 4.2A. Each experiment is reiterated ten times. All distributions of inter-individual distances differ significantly (Two-sample Kolmogorov-Smirnov test, with p-values < 0.05; cf Sec. 4.2.4).

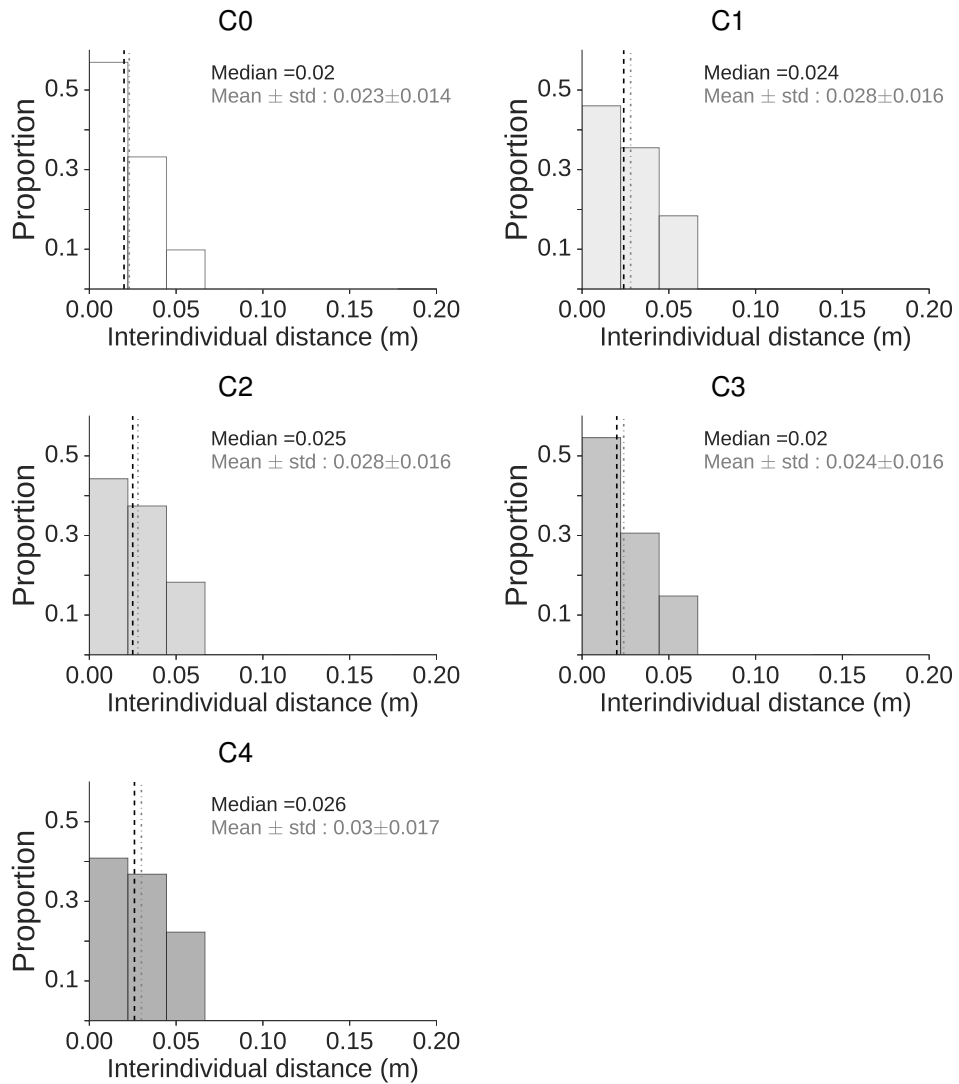


Figure 4.7: Mean inter-individual distances between each pair of agents (fish and robot) in sub-groups of only two individuals (pairs). Results are obtained in 30 minutes experiments using groups of wild-type zebrafish moving in the set-up described in Fig. 4.2A. Each experiment is reiterated ten times. All distributions of inter-individual distances differ significantly (Two-sample Kolmogorov-Smirnov test, with p-values < 0.05 ; cf Sec. 4.2.4).

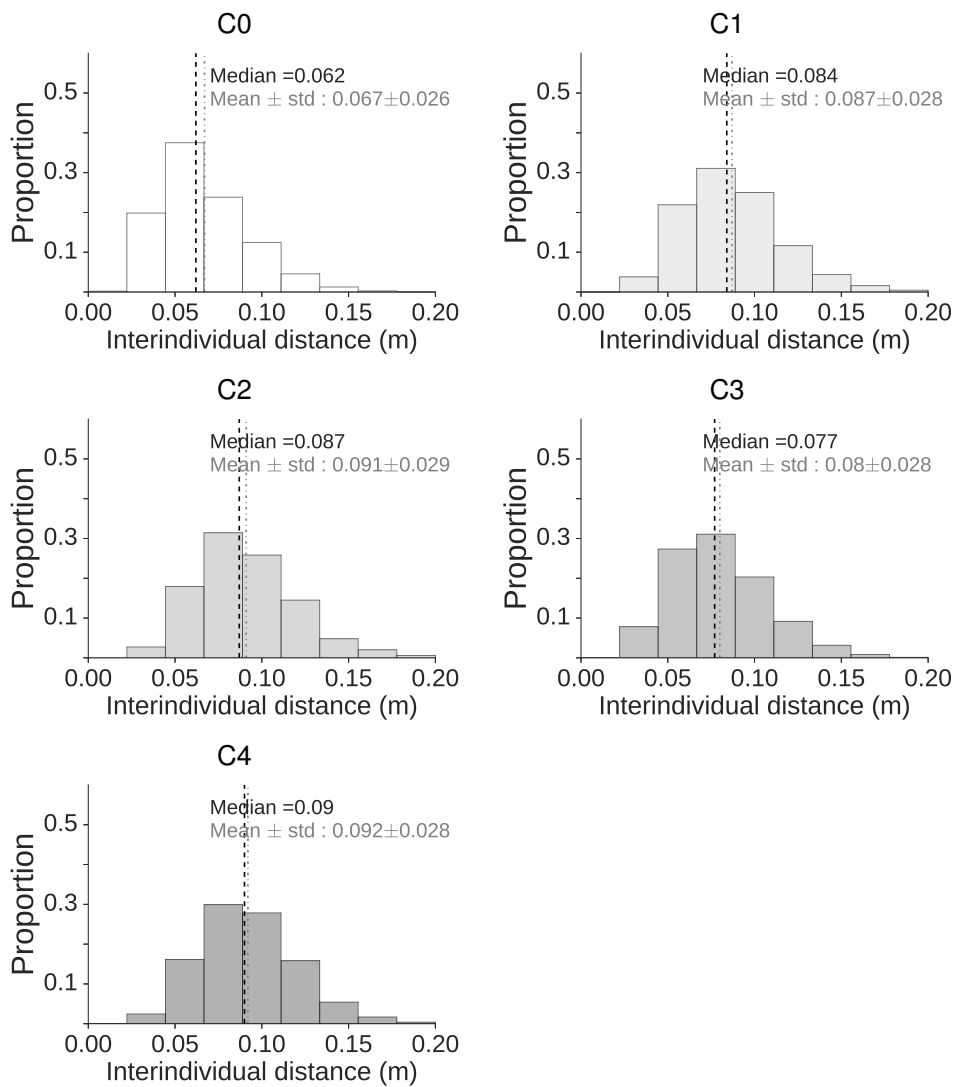


Figure 4.8: Mean inter-individual distances between each pair of agents (fish and robot) in sub-groups of five individuals (entire population). Results are obtained in 30 minutes experiments using groups of wild-type zebrafish moving in the set-up described in Fig. 4.2A. Each experiment is reiterated ten times. All distributions of inter-individual distances differ significantly (Two-sample Kolmogorov-Smirnov test, with p-values < 0.05; cf Sec. 4.2.4).

index, C2 and C4 have the highest score of linear speed in the rooms, and C3 has the highest score in inter-individual distances in sub-groups of three to four individuals.

4.5 Conclusions

In this study, we present a biomimetic model of fish behaviour in a fragmented environment. This model is multi-contextual spatially (square rooms and corridor), multi-level (collective dynamics, collective departures, trajectories, movement patterns) and probabilistic. Then, we describe a methodology to create a closed-loop of social integration between a shoal of zebrafish and a biomimetic fish-lure moved by a robot driven by this biomimetic behavioural model. We use a similarity measure as a metric to quantify the capability of this lure to be socially integrated in the shoal of fish and the biomimetism of the behaviour of the robot.

While previous studies of the literature (Tab. 4.1) showed that a robotic fish needs to be biomimetic in order to be socially integrated in a group of fish, most efforts were made on finding attractive biomimetic lure morphologies, with no or few considerations ([138, 139, 240]) on the effects of robot behaviour (trajectories and movement patterns) on social integration. Both [139] and [138] present experiments involving a closed-loop of interaction between a group of fish and a robot driven by a controller inspired from fish behaviour and magnetically coupled with a biomimetic lure. However, in these studies, the social integration of the robot is not quantified and the robot is more a follower or initiator following fixed patterns of behaviour rather than an entity capable of being integrated with the group of fish and initiating its own decisions during trials lasting 30 minutes.

The biomimetic model of fish behaviour we present can effectively socially integrate a group of wild-type zebrafish.

This problem is challenging because zebrafish present loose social organisation [211]. Indeed this fish species does not form stable schooling patterns (*i.e.* fish aligned and swimming together in the same direction) but form shoals (tendency to form irregular groups). Moreover, it is difficult to model zebrafish collective behaviour and translate it directly into a robotic controller. Here we consider complex social behaviour in a designed set-up to study social cohesion and collective departures. This fragmented set-up induce more elaborate behaviours than simpler round or square empty tanks. Such environment requires to develop context dependent collective behaviour models taking into account spatial context and social effects.

In this study, we compare mixed groups of one robot and four fish to groups of five fish and no robot. It allows us to quantify the social integration of the robot *i.e.* if the robot belongs to the group, like another fish, for trials lasting for 30 minutes each and during

five hours in total. We present metrics that quantify the distance between a mixed and a pure fish group. According to these metrics, the closer we are to a pure fish group the more the robot is socially integrated.

We analyse the impact of both lure morphology and robot behaviour aspects on social integration and show that a robotic fish driven by a biomimetic behavioural model is more akin to be socially accepted by the fish compared to a robotic fish driven by a simple non-biomimetic behavioural model. We assess the importance of biomimetism on different aspects of the robot design: the morphology of the lure, the type of trajectories of the robot, and the type of movement patterns exhibited by the robot, its decision making capabilities depending on the context. All three aspects are shown to be relevant to facilitate the social integration of the robot. We present results showing that both the lure and the behaviour of the robot are important in the design of robots able to socially integrate a group of fish. We show that the morphology of the lure is the feature with the highest impact, followed by the type of robot trajectories and the type of robot movement patterns.

Additionally, we include a behavioural analysis of the fish group and of the mixed-group of fish and robot. This analysis takes into account the tendency of fish to gather in short-lived and dynamic sub-groups, that can exhibit different behaviour depending on sub-group size and composition. While studies in the literature established the existence of these sub-group dynamics in fish [211], no method was described to identify them. We present an algorithm to identify sub-groups of fish based on their spatial proximity. The results of this sub-group analysis show that fish exhibit different behaviours depending on the size of the sub-group they are part of, and of their position in the arena. In particular, fish behave differently when alone compared to when in a group. We showed that the inter-individual distances of fish in a sub-groups is dependant of the size of the sub-group. When they are in the corridor, they tend to pass through quickly from one room to the other.

The social integration of the robot into the groups of fish could still be improved by refining the behavioural model. The model could be further calibrated to take into account more aspects of the fish collective behaviour in this complex environment. The robot behaviour could be closer to the fish behaviour that depends on the size of its sub-group and to its spatial position in the set-up. Our model does not yet take into account explicitly the attraction of the agents towards the walls, like the model in [49]. This was mainly motivated by current technical difficulties as this would greatly increase the number of collisions between the robot and the walls. This problem will be addressed in a subsequent study. Robots can still be seen to be following walls in the colored trajectory presented in Fig. 4.4 because they are attracted to fish, which, in turn, tend to follow walls.

Future works will focus on automating the calibration of model parameters, by optimising them using evolutionary algorithms. Additionally, the social integration can be further quantified by adding other kind of analysis, and by using more behavioural features in the computation of the social integration indexes.

This study is a first step towards more complex biohybrid groups: instead of just focusing on integration, the robots could be used to control or to modulate the collective behaviour of the mixed group of fish and robots. Indeed taking advantage of specific social behaviours the robots could modulate the whole mixed-groups [97]. The number of robots necessary to have a good control of the mixed groups can also be optimised for example by evolutionary computation [40, 41]. This type of control would be based on natural animal behaviours thus reducing stress and not using coercion to get the target results [97, 120]. Indeed, in zebrafish groups all the fish can be leaders and induce groups transitions from one place to the other. For example, we have shown that the number of initiation is linearly proportional to the number of attempts performed [48]. This allows biomimetic robots to make use of the fish behavioural features to be capable of inducing collective departures and to modulate the spatial distribution of the groups. Such biohybrid social systems would allow us to modulate and to control group living animals.

Chapter 5

Automated calibration of a biomimetic space-dependent model for zebrafish and robot collective behaviour in a structured environment

Abstract

Bio-hybrid systems made of robots and animals can be useful tools both for biology and robotics. To socially integrate robots into animal groups the robots should behave in a biomimetic manner with close loop interactions between robots and animals. Behavioural zebrafish experiments show that their individual behaviours depend on social interactions producing collective behaviour and depend on their position in the environment. Based on those observations we build a multilevel model to describe the zebrafish collective behaviours in a structured environment. Here, we present this new model segmented in spatial zones that each corresponds to different behavioural patterns. We automatically fit the model parameters for each zone to experimental data using a multi-objective evolutionary algorithm. We then evaluate how the resulting calibrated model compares to the experimental data. The model is used to drive the behaviour of a robot that has to integrate socially in a group of zebrafish. We show experimentally that a biomimetic multilevel and context-dependent model allows good social integration of fish and robots in a structured environment.

5.1 Introduction

Robotics stands now as a convenient tool to study the animal behaviour. In recent ethological and animal behavioural studies, robots are used to induce specific and controlled stimuli and assess the response of the animals under scrutiny. This allows to test various hypotheses on the nature of the signals used by the animals for social interactions [125,179].

Autonomous robots interacting in real-time with animals [120] makes it possible to create social interactions between both of them. This has already been demonstrated by several authors for studying the behaviours of sheepdogs [249], cows [52] or drosophila [260] to cite a few.

In this paper, we focus on zebrafish (*Danio rerio*), and we describe a biomimetic model that can be implemented in a robotic lure and validated its acceptance by four zebrafish in a structured environment.

The main difficulty is to make the robotic lure behave in such a way that it is accepted by the animals as social companion, just as any other interacting fish would be. Beyond the scope of this paper, this is a first step to enable the modulation (though action) of the collective behaviours of the observed zebrafish [97].

Different approaches have been proposed to control the movement of fish-lures [43]. Most of them do not involve a closed loop of social interaction with the fish. This is often the case for lures fixed to a robotic arm that performs repeated movements, but also for studies with autonomous fish-lures. Closing the loop of social interactions requires a real-time tracking, or perception, of the agents (fish and robot), and a decision-making algorithm to control the robot behaviours. In most of the experiments reported in the literature, the robots driven with closed-loop control are programmed to follow the centroid of the fish group, to ensure that the robot will join and follow the group of fish. However, this type of controller implies that the robot is more a passive follower than a real group-member making its own decisions. The embodiment of bio-inspired models can lead to a better social integration of the artificial agents in animal groups and can allow the robots to influence the collective decision of the mixed group by giving specific preferences to the robot by tuning parameter values of the model [43,97].

We present a method to calibrate automatically a new behavioural zebrafish model by evolutionary parameters optimisation. This multilevel model describes collective behaviour in a structured environment in agreement with experimental observations. This model makes important extensions to our previous model for collective behaviour in a homogeneous environment [49]. The model takes into account a simple structured environment composed of two rooms and the fact that the fish adapt their behaviour to the zones where they are while performing collective behaviour. For such multilevel and

spatially dependent social behaviour model it is an issue to calibrate the model because it involves trade-offs between social tendencies (aggregation, group formation), and response to the environment (wall-following, zone occupation). We use an evolutionary algorithm (NSGA-II [64]) to optimise the parameters of this model so that the exhibited collective dynamics correspond to those observed in biological experiments. Then, we validate experimentally this model by implementing it as the controller of robots that are integrated in small fish groups.

5.2 Materials and Methods

5.2.1 Experimental set-up

We use the experimental set-up described in [18, 43, 48, 211], with the arena presented in [43, 211]. This set-up (Fig. 5.1A) consists of a white plexiglass arena (Fig. 5.1C) of $1000 \times 1000 \times 100$ mm, that is composed of two rooms linked by a corridor. To validate experimentally our calibrated model, we use a robot developed by the EPFL [17–19, 24] for the ASSISI project [210]. This robot is powered by two conductive plates under the aquarium. An overhead camera captures frames that are then processed for tracking and control purposes (see Fig. 5.1A).

All trials have a duration of $15min$. We tracked the positions of the agents by using the idTracker software [181]. Using this software, we obtain the positions $P(x, y, t)$ of all agents at each time step $\Delta t = 1/15$ s for all experiments, and build the trajectories of each agent. The experiments performed in this study were conducted under the authorisation of the Buffon Ethical Committee (registered to the French National Ethical Committee for Animal Experiments #40) after submission to the French state ethical board for animal experiments.

5.2.2 Behavioural model

Most of the fish collective behaviour models do not take into account the environment i.e. the walls or the structure of the tanks because they only focus on the social interactions [146, 237].

However, zebrafish show context-dependent behaviours when they are in a structured environment. Depending on their spatial position in the environment they adapt their individual behavioural pattern. Moreover, because they are a gregarious species they also take into account the position and the behaviours of the other fish and can aggregate or start collective behaviours. As many animal species, zebrafish display strong thigmotaxis and follow walls or edges. We show that they adapt their behaviour in three different zones of the structured set-up: first the zone when they are close to the

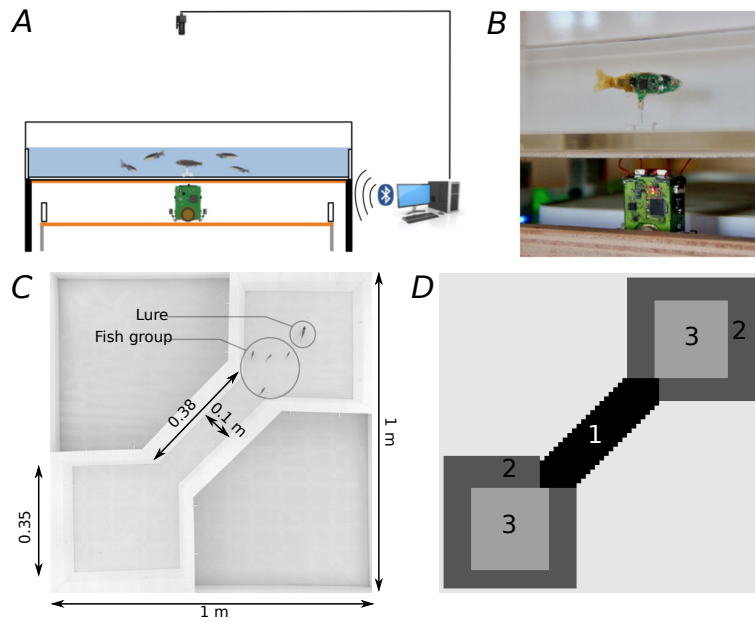


Figure 5.1: **Panel A:** Experimental set-up used during the experiments [17–19,24,43]. **Panel B:** FishBot [17, 19, 24]: the robot used for mimicking fish motion patterns, with the biomimetic lure used during the reference experiments. This robot was developed by the EPFL for the ASSISI project [210]. **Panel C:** Experimental arena composed of a tank containing two square rooms (350×350 mm at floor level) connected by a corridor (380×100 mm at floor level). The fish tend to swim from one room to the other, either in small groups, or individually. This set-up is used to study the zebrafish collective dynamics. **Panel D:** Positions of the three different zones corresponding to different types of behaviours: in the corridor (zone 1), in the center of each room (zone 2), and near of the walls of each room (zone 3).

walls, second the zone when they are in the centre of the rooms and third when they use the corridor to change room. We take into account this spatial and context-dependent behaviours.

Each zone corresponds to a behavioural attractor. When the individuals are in one of the three zones they adapt their behaviour and perform specific behavioural patterns. In the zone near the walls they perform mainly thigmotaxis (wall following), in the centre of the room they explore, in the corridor they transit from one room to the other. At the same time they also take into account the behaviour of the other fish as they also do collective behaviour such as collective departures from the rooms. The other fish can be in any of the other zones and thus can also induce behavioural attractor switching of their companions.

We extend the biomimetic hybrid model [43, 49] using microscopic and macroscopic information [40, 41]. This new model (described in Fig. 5.2) takes into account zones that correspond to different behavioural attractors and thus allows context-dependent behaviours. The individual can switch from one behavioural attractor to the other and at the same time perform collective behaviour. Our model describes individual choices

close to action selection and collective behaviours at the same time. It is a step towards modelling action selection in the context of collective behaviours.

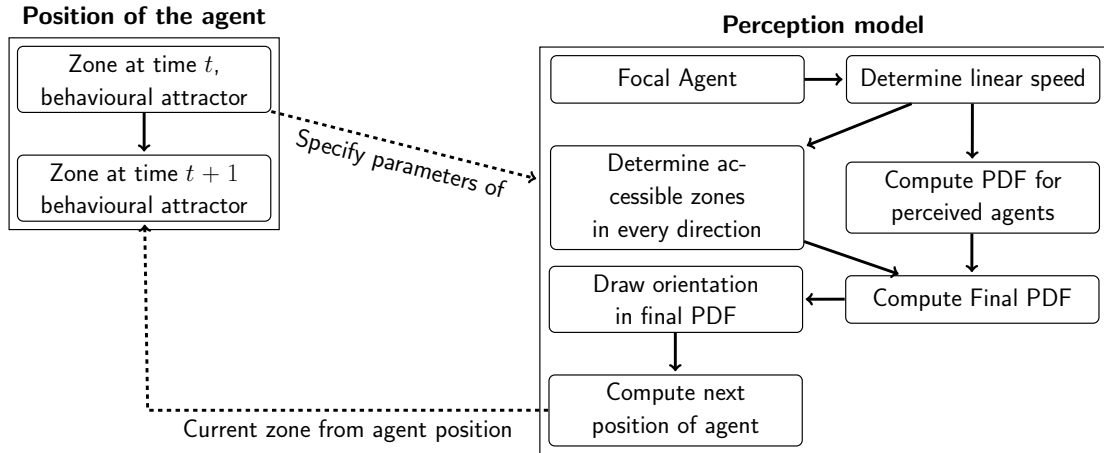


Figure 5.2: Multilevel model used to describe fish behaviour. The agents display different behavioural attractors depending on the zone where they are situated. Thus, according to the agent spatial position, the physical features of the zone drive them towards a specific behavioural attractor. A behavioural attractor corresponds to a set of behavioural patterns adapted to the zone where they are located. It can correspond to different parameters sets for the same behaviour kind.

We present a multi-level and multi-agent biomimetic model, inspired from [43, 49] that describes the individual and collective behaviours of fish. As in [49], this model makes the link between fish visual perception (of congeners and walls) and motor response (*i.e.*: trajectories of the agents). However, it is also capable of expressing a variability in agents behaviours when they occupy specific zones of the arena (behavioural attractors). Table 5.3B lists the model parameters.

In this model, the agents update their position vector X_i with a velocity vector V_i :

$$X_i(t + \delta t) = X_i(t) + V_i(t)\delta t \quad (5.1)$$

$$V_i(t + \delta t) = v_i(t + \delta t)\Theta_i(t + \delta t) \quad (5.2)$$

The model computes a circular probability distribution function (PDF) [49] corresponding to the probability of the agent to move in a specific direction (Θ_i). This PDF is as a mixture of von Mises distributions, an equivalent to the Gaussian distribution in circular probability. The computation of this PDF involves the calculation of two other PDF functions: the first one describing agent behaviour when no stimuli is present, and the second one characterising agent behaviour when conspecifics are perceived by the agent.

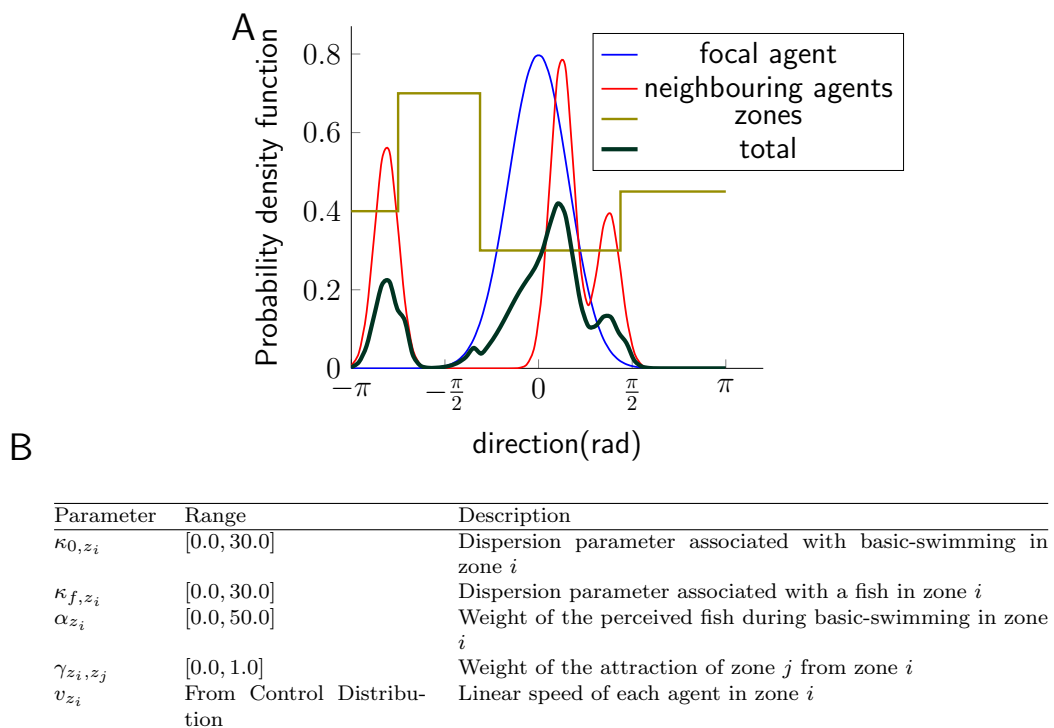


Figure 5.3: Panel A Computation of the PDFs functions used by the model. One function corresponds to the focal fish; another corresponds to the perceived neighbouring agents. The final PDF is a weighted sum of these functions, with a normalisation factor γ_{z_1,z_2} corresponding to the affinity between the zones z_1 (origin) and z_2 (destination). The direction taken by an agent is drawn randomly from the resulting PDF by inverse transform sampling. **Panel B** Table of model parameters for each agent. The zone z_i corresponds to the zone where the agent is situated at time t , and z_j to the zone where the agent would be at time $t + 1$. The linear speed distributions of the agents are the same as the ones observed in the Control experiments, and they are not optimised. The other parameters in the table are optimised.

The PDF capturing agent behaviour when no stimuli is present is given by:

$$f_{0,z_j}(\theta) = \frac{\exp(\kappa_{0,z_j} \cos(\theta))}{2\pi I_0(\kappa_{0,z_j})} \quad (5.3)$$

for an agent situated in zone z_j , and with I_0 the modified Bessel function of first kind of order zero. When the agent is situated in a zone close to a wall (zones 1 and 2 of Fig. 5.1D), we implement a wall-following behaviour, by increasing the probabilities of moving towards either side of the closest wall. This is achieved by using the following PDF:

$$f_{0,z_j,w}(\theta) = \frac{1}{2} \sum_{k=1}^2 \frac{\exp(\kappa_{0,z_j} \cos(\theta - \mu_{w_k}))}{2\pi I_0(\kappa_{0,z_j})} \quad (5.4)$$

with μ_{w_k} the two possible directions along the considered wall.

Examples of agents trajectories are found in Fig. 5.5B. The probability of the focal fish to orient towards a perceived fish is given by a von Mises distribution clustered around

the fish position:

$$f_{F,z_j}(\theta) = \sum_{i=1}^n \frac{A_{f_i}}{A_{T_f}} \frac{\exp(\kappa_{f,z_j} \cos(\theta - \mu_{f_i}))}{2\pi I_0(\kappa_{f,z_j})} \quad (5.5)$$

with μ_{f_i} the direction towards the perceived agent, $A_{T_f} = \sum_{i=1}^{n_f} A_{f_i}$ the sum of the solid angles A_{f_i} captured by each agent and n_f the number of perceived agents.

The final PDF $f(\theta)$ is computed as follow:

$$f_{z_j,z_k}(\theta) = \gamma_{z_j,z_k} \frac{f_{0,z_j}(\theta) + \alpha_{z_j} A_{T_f} f_{F,z_j}(\theta)}{1 + \alpha_{z_j} A_{T_f}} \quad (5.6)$$

The parameter γ_{z_1,z_2} , used as a multiplicative term of the final PDF, modulates the attraction of agents towards target zones. Figure 5.3A describes how the final PDF is computed and how it is used to determine the agents next positions.

Unreachable areas of the PDF (e.g. the walls) are attributed a probability of 0. Then, we numerically compute the cumulative distribution function (CDF) corresponding to this custom PDF $f(\theta)$ by performing a cumulative trapezoidal numerical integration of the PDF in the interval $[-\pi, \pi]$. Finally, the model draws a random direction Θ_i in this distribution by inverse transform sampling. The position of the fish is then updated according to this direction and his velocity with equations 5.1 and 5.2.

5.3 Results

We consider four cases. We define the **Control** results as obtained from biological experiments with five zebrafish in the experimental set-up described in Sec. 5.2.1. The **Sim-MonoObj** and **Sim-MultiObj** results are defined to correspond to the model in simulation with five agents, calibrated respectively using mono-objective or multi-objective optimisation. The **Biohybrid** results are obtained from experiments with four zebrafish and one robot driven by the model using the best optimised parameters.

5.3.1 Optimisation of model parameters

We define a similarity measure (ranging from 0.0 to 1.0) to compare two experiments (e_1 and e_2), and define it as:

$$S(e_1, e_2) = \sqrt[3]{I(O_{e_1}, O_{e_2})I(T_{e_1}, T_{e_2})I(D_{e_1}, D_{e_2})} \quad (5.7)$$

with O_e the distribution of zones occupation, T_e the transition probabilities from zone e to the others, and D_e the distribution of inter-individual distances of all agents in zone e . The similarity measure $S(e_1, e_2)$ corresponds to the geometric mean of these three

features. The function $I(P, Q)$ is defined as such:

$$I(P, Q) = 1 - H(P, Q) \quad (5.8)$$

The $H(P, Q)$ function is the Hellinger distance between two histograms [66]. It is defined as:

$$H(P, Q) = \frac{1}{\sqrt{2}} \sqrt{\sum_{i=1}^d (\sqrt{p_i} - \sqrt{q_i})^2} \quad (5.9)$$

We consider two optimization methods. In the **Sim-MonoObj** case, we use the CMA-ES [8] mono-objective optimisation algorithm, with the task of maximising the $S_{(e_1, e_2)}$ function. In the **Sim-MultiObj** case, we use the NSGA-II [64] multi-objective algorithm with three objectives to maximise. The first objective is a performance objective corresponding to the $S_{(e_1, e_2)}$ function. We also consider two other objectives used to guide the evolutionary process: one that promotes genotypic diversity [162] (defined by the mean euclidean distance of the genome of an individual to the genomes of the other individuals of the current population), the other encouraging behavioural diversity (defined by the euclidean distance between the O_e , T_e and D_e scores of an individual). In both methods, we use populations of 60 individuals (approximately twice the number of dimensions of the problem) and 300 generations. The **Sim-MonoObj** stabilises around the 50-th generation. The **Sim-MultiObj** stabilises around the 250-th generation. The linear speed v_i of the agents is not optimized, and is randomly drawn from the instantaneous speed distribution measured in the control experiment. It should be noted that evolutionary algorithms do not over-fit (as it is an optimization process), even if we use the same data (trajectories) for both training and testing.

5.3.2 Robot implementation

The robot is driven by the model described in Section 5.2.2, after calibration. Robotic trials have a duration of 15 minutes, and are repeated 10 times. They involve one robot and four zebrafish. Every 333ms, we integrate the tracked positions of the four fish into the model, and compute the target position of a fifth agent. We then control the robot to follow this target position by using the biomimetic movement patterns described in [19, 43].

5.3.3 Model performance analysis and experimental validation

We assess the similarity between the results from the calibrated cases (**Sim-MonoObj**, **Sim-MultiObj** and **Biobrybrid**) and those of the **Control** case by using the similarity measure defined in Sec. 5.3.1. The similarity scores are shown in Table 5.1.

Using information about zones occupation and probabilities of transition from one zone to another, we define a finite state machine corresponding to the behavioural attractors dynamics of the entire agent population. The resulting finite state machines obtained from the **Control** and **Biohybrid** cases are shown in Fig. 5.4. The probability of presence of an agent in each part of the arena is presented in Fig. 5.5A. Examples of agents trajectories are found in Fig. 5.5B.

The best-performing individuals of the **Sim-MonoObj** and **Sim-MultiObj** cases display distributions of inter-individual distances that are relatively close to those of the **Control** case, which suggests that these models can convincingly exhibit fish tendency to aggregate. However, of the two cases performed in simulation, only **Sim-MultiObj** is capable of displaying zones dynamics (occupation of the zones, and transition probabilities from one zone to the others) similar to the **Control** case. This suggests that multi-objective optimisation is required to handle the conflicting dynamics present in fish collective behaviour.

The robot of the **Biohybrid** case is driven by a controller using our model with the parameters of the best-performing individual obtained in the **Sim-MultiObj**. The results of the **Biohybrid** case correspond to those of the **Sim-MultiObj** case. The ethogram of the **Biohybrid** case (cf Fig. 5.4) shows an increased preference for the centre of the rooms compared to the **Control** case. This could be explained by our current lower level robotic implementation of wall-following behaviour that could still be sub-optimal.

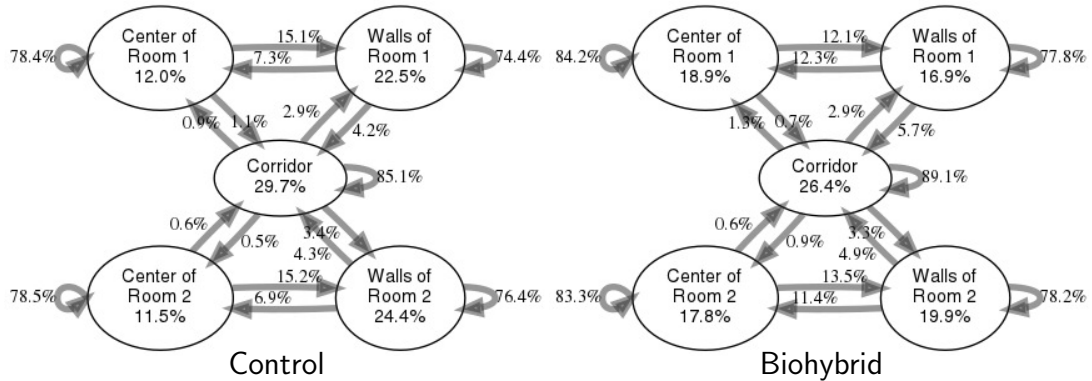


Figure 5.4: Ethogram as finite state machine corresponding to the behavioural attractors for all agents. Each zones drive the agents into the corresponding behavioural attractor. Thus, agents modulate their behaviour in each zone as if they enter into a specific behavioural state. Here we show the resulting transition probabilities obtained after optimisation and implementation as robotic controllers (biohybrid) based on the experimental observations (control). The number in each state corresponds to the proportion of time agent spend in this state. The numbers on the arrows correspond to the transition probabilities between zones with a time-step of $1/3s$.

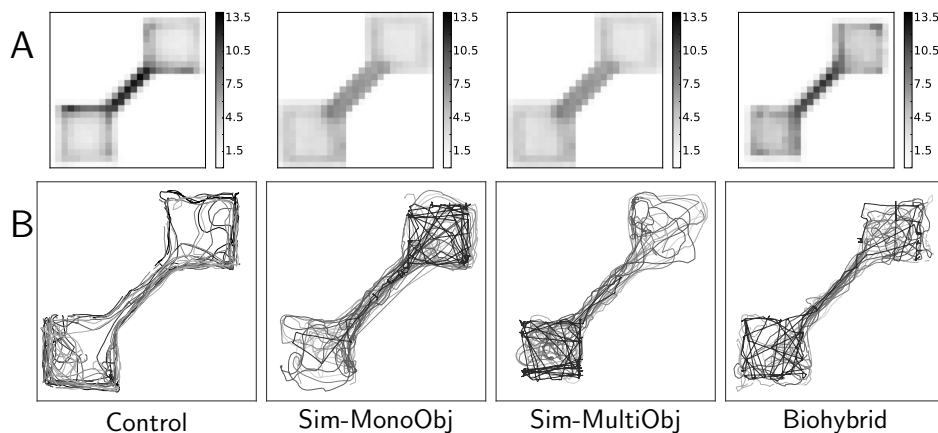


Figure 5.5: **Panel A** Probabilities of presence in each part of the arena, for all cases. **Panel B** Examples of trajectories over a duration of 2 minutes (1800 frames). In the Biohybrid case, the robot is in black.

	Sim-MonoObj	Sim-MultiObj	Biohybrid
Occupation	0.57	0.97	0.89
Transitions	0.76	0.81	0.88
Interindivid. Dists	0.90	0.87	0.89
Fitness	0.73	0.88	0.89

Table 5.1: Similarity scores between the best-performing individuals of the three calibrated cases and the **Control** case used as reference, as defined in Sec. 5.3.1. We consider three standard features to characterise the collective behaviour exhibited in each case. **Occupation** corresponds to the probability of presence of the agent in each zone. **Transitions** corresponds to the probabilities of an agent to transition from one zone to another. **Inter-individual distances** corresponds to the distribution of inter-individual distances between all agents in a specific zone. The fitness function is computed as the geometric mean of these scores.

5.4 Discussion and Conclusion

Collective behaviour models often focus on collective motion in homogeneous unbounded environment. Here we present a multi-level model that is space-dependent with individuals that behave in a context-dependent way. We make the hypothesis that the type of behaviour displayed by the agents depends on their position in the environment. This allows us to segment our environment into several characteristic zones, each corresponding to a particular behavioural attractor, matching different types of agent behaviour.

We present a methodology to calibrate this model to correspond to the collective dynamics exhibited by fish in the experiments. This calibration process is challenging, as it involves a trade-off between social tendencies (group formation), and response to the environment (wall-following, exploration). Moreover, our model encompasses the notion of behavioural attractors, allowing agents to exhibit several different behaviours depending on the context. Our methodology is able to cope with this trade-off by using multi-objective optimisation.

However, this calibration methodology could still be improved: the similarity measure we use to compare two cases only takes into account three aspects of collective behaviours corresponding to behavioural attractors, and aggregation dynamics. Other behavioural aspects could also be relevant at the level of collective dynamics and can be considered: *e.g.*: agent groups aspects, residence time in a zone, at the level of the individuals *e.g.*: agent trajectory aspects, curvature of trajectories, etc. Moreover, in relation to the environment *e.g.*: the distance of an agent to the nearest wall could also be taken into account. Alternatively, it would be possible to perform the calibration without defining a similarity measure explicitly, using a method similar to [144], by co-evolving simultaneously the parameters of the models and classifiers. These classifiers would be trained to identify whether or not the resulting behaviours of the optimised models are distinct from the behaviours from the reference experiments.

Here, we make the assumption that the behavioural attractors are linked to the position of the agent in their environment. This assumption could be relaxed, to handle ethograms with more complex classes of behaviours like behavioural attractors linked to agent group dynamics. Additionally, the idea that actions are selected and segmented by the fish is questionable. While our decomposition of fish behaviour in different behavioural attractors is convenient for modelling purpose and ease the implementation of a biomimetic robot controller by having a collection of discrete acts that it can perform, it is not determined that fish make this kind of decomposition into distinct elements (actions) [25]. Finally, we could apply our model in more complex set-up, involving large societies with a larger number of robots, and with a more complex topology.

Chapter 6

How to blend a robot within a group of zebrafish: Achieving social acceptance through real-time calibration of a multi-level behavioural model

Abstract

We have previously shown how to socially integrate a fish robot into a group of zebrafish thanks to biomimetic behavioural models. The models have to be calibrated on experimental data to present correct behavioural features. This calibration is essential to enhance the social integration of the robot into the group. When calibrated, the behavioural model of fish behaviour is implemented to drive a robot with closed-loop control of social interactions into a group of zebrafish. This approach can be useful to form mixed-groups, and study animal individual and collective behaviour by using biomimetic autonomous robots capable of responding to the animals in long-standing experiments. Here, we show a methodology for continuous real-time calibration and refinement of multi-level behavioural model. The real-time calibration, by an evolutionary algorithm, is based on simulation of the model to correspond to the observed fish behaviour in real-time. The calibrated model is updated on the robot and tested during the experiments. This method allows to cope with changes of dynamics in fish behaviour. Moreover, each fish presents individual behavioural differences. Thus, each trial is done with naive fish groups that display behavioural variability. This real-time calibration methodology can optimise the robot behaviours during the experiments. Our implementation of this methodology runs on three different computers that perform individual tracking, data-analysis, multi-objective evolutionary algorithms, simulation of the fish robot and adaptation of the robot behavioural models, all in real-time.

6.1 Introduction

The study of animal collective behaviour involves the search for the relevant signals and mechanisms used by the animals for social interactions [125, 179]. Robots can help ethologists to test various hypothesis on the nature of these signals by inducing specific and controlled stimuli to assess animal response.

Autonomous robots are capable to interact with animals and can serve as tools to study social dynamics [161]. This approach has already been used in studies to analyse the behaviour of ducks [249], drosophila [260], cockroaches [97], fish [14, 21, 43, 117, 121, 138, 139], bees [93, 140, 228] and birds [63, 91, 116].

Here, we socially integrate a behavioural biomimetic robotic lure into a group of four zebrafish (*Danio rerio*) moving in a structured environment and validate its acceptance by the animals. This problem is difficult because the robotic lure must be designed to be perceived as a social companion by the animals: it must, to a certain extent look like a fish, behave like a fish, be able to respond appropriately to environmental and social cues to close the loop of social interactions with the fish. Closing the loop of social interactions requires real-time individual perception and a decision-making algorithm to control the robot behaviours [43].

These aspects were investigated in [41, 43] through the use of biomimetic robotic fish lures driven by a calibrated biomimetic model to make the robot mimics expected fish behaviour. An evolutionary algorithm (NSGA-II [64]) was used to optimise the parameters of this model so that the resulting collective dynamics corresponded to those observed in biological experiments. This type of controller allowed the robot to be a real group-member making its own decisions rather than a passive follower.

However, the model calibration was done off-line and not during the ongoing experiments. As such, it could not take into account the changes in animal behaviour across experiments and the intrinsic behavioural differences between groups used in experiments.

Here, we tackle this problem by continuously refining and calibrating the biomimetic model driving the robot behaviours in real-time during the experiment by using on-line evolutionary algorithm (NSGA-II [64]). This task is computationally-intensive and requires three computers to deal with agent real-time tracking, robot control, real-time data-analysis, and model calibration. We test this methodology in a set of 10 experiments with four fish and one robot. In each case, the robot closed-loop behaviour becomes progressively socially integrated into the group of fish. This is the first step towards evolving mixed-group of animals and robot [43].

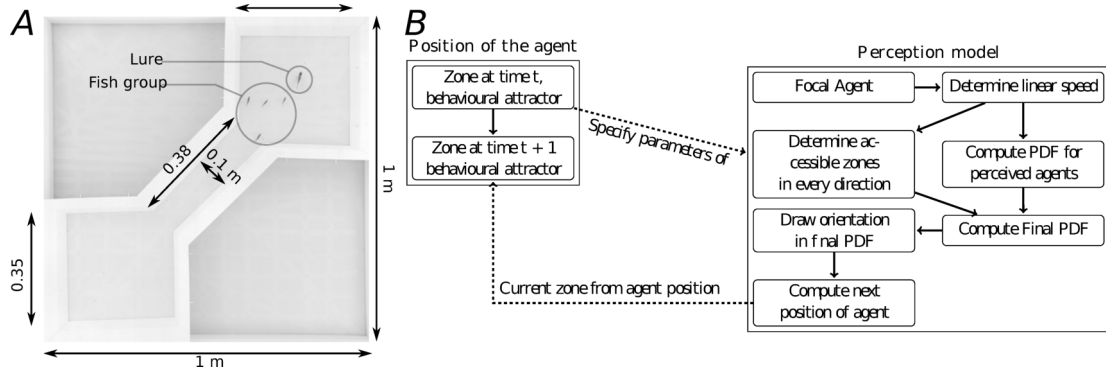


Figure 6.1: **A.** Experimental setup: a tank with two square rooms (350×350 mm at floor level) connected by a corridor (380×100 mm). This set-up is used to study zebrafish collective behaviours [41,43,49,211,212]. It is composed of three zones (corridor, center of the rooms, close to room walls) that correspond to three different behavioural attractors. **B.** Multilevel model for fish behaviour [41,43]. The agents behave differently depending on the zone where they are situated.

6.2 Materials and Methods

6.2.1 Experimental set-up

We use the experimental set-up from [18,41,43,48,211] (Fig. 6.2, "Control & tracking" part) with a white plexiglass arena (Fig. 6.1A) of $1000 \times 1000 \times 100$ mm composed of two rooms linked by a corridor. We use the FishBot robot [17–19], powered by two conductive plates under the aquarium, to interact with fish. An overhead camera captures frames (15 FPS, 500×500 px), that are then tracked to find the fish positions. A complementary fish-eye camera (15 FPS, 640×480 px) placed under the fish tank is used to track the position of the robot.

We used 10 groups of 4 adults wild-type AB zebrafish (*Danio rerio*) in ten 30-minute experiments as in [41,43,211]: 30 minutes is sufficient to capture the behaviour and dynamics of groups of 4 zebrafish. Fish are released in the aquarium after the lure is placed in the aquarium.

To ensure real-time adaptation, our methodology is computationally intensive, and uses three networked 32-core computers (Fig. 6.2). Computer 1 is used to track the agents in real-time and control the robot according to the behavioural model of Sec. 6.2.2. Computer 2 performs every 60s data-analysis on the tracked positions of agents from Computer 1, and estimates the biomimeticism of robot behaviour (which, in our case, can be viewed as a metric of social integration as defined in [43]). Computer 3 re-calibrates every 60s the behavioural model to correspond as close as possible to the behaviour of experimental fish (measured by Computer 2). The resulting calibrated parameter set is then sent to Computer 1 to serve as parameters of the robot controller model. It allows the robot to progressively mimics the behaviour of the fish and be socially accepted.

The experiments performed in this study were conducted under the authorisation of the Buffon Ethical Committee (registered to the French National Ethical Committee for Animal Experiments #40) after submission to the French state ethical board for animal experiments.

6.2.2 Behavioural model

We use the multi-level model from [41, 43] (inspired from [49]) that describes the individual and collective behaviours of fish (Fig. 6.1B). This model takes into account both social interactions and environmental cues (*i.e.* walls and structure of the tanks). It is stochastic, multi-level and context-dependent.

Fish behave differently depending on their spatial position. Namely, this model identify three zones of the structured set-up with different fish behaviours (Fig. 6.1A): when they are close to the walls, when they are in the centre of the rooms, and when they pass through the corridor. Near the walls, fish perform mainly thigmotaxis (wall following) while in room centre they exhibit exploratory behaviour. In the corridor, they tend to go in a straight line with increased speed to reach the subsequent room. Fish also react to social cues leading to collective behaviour such as collective departures from the rooms [48]. Very few models of fish collective behaviours take into account the presence of walls [38, 49].

The agents update their position vector X_i with a velocity vector V_i :

$$X_i(t + \delta t) = X_i(t) + V_i(t)\delta t \quad (6.1)$$

$$V_i(t + \delta t) = v_i(t + \delta t)\Theta_i(t + \delta t) \quad (6.2)$$

with v_i the linear speed of the i^{th} agent and Θ_i its orientation. The linear speed v_i of the agent is randomly drawn from the experimentally measured instantaneous speed distribution.

The orientation Θ_i is drawn from probability density function (PDF) computed as a mixture distribution of von Mises distributions centred on the stimuli perceived by the focal agent. It takes into account the influence of other agents and of the walls of the experimental arena. The resulting PDF is composed of the weighted sum of (i) a PDF taking into account the effect of the walls and (ii) a PDF describing the response to other agents. The parameter γ_{z_1, z_2} , used as a multiplicative term of the final PDF, modulates the attraction of agents towards target zones.

We numerically compute the cumulative distribution function (CDF) corresponding to this final PDF by performing a cumulative trapezoidal numerical integration of the PDF in the interval $[-\pi, \pi]$. Then, the model draws a random direction Θ_i in this distribution

by inverse transform sampling. The position of the fish is then updated according to this direction and his velocity.

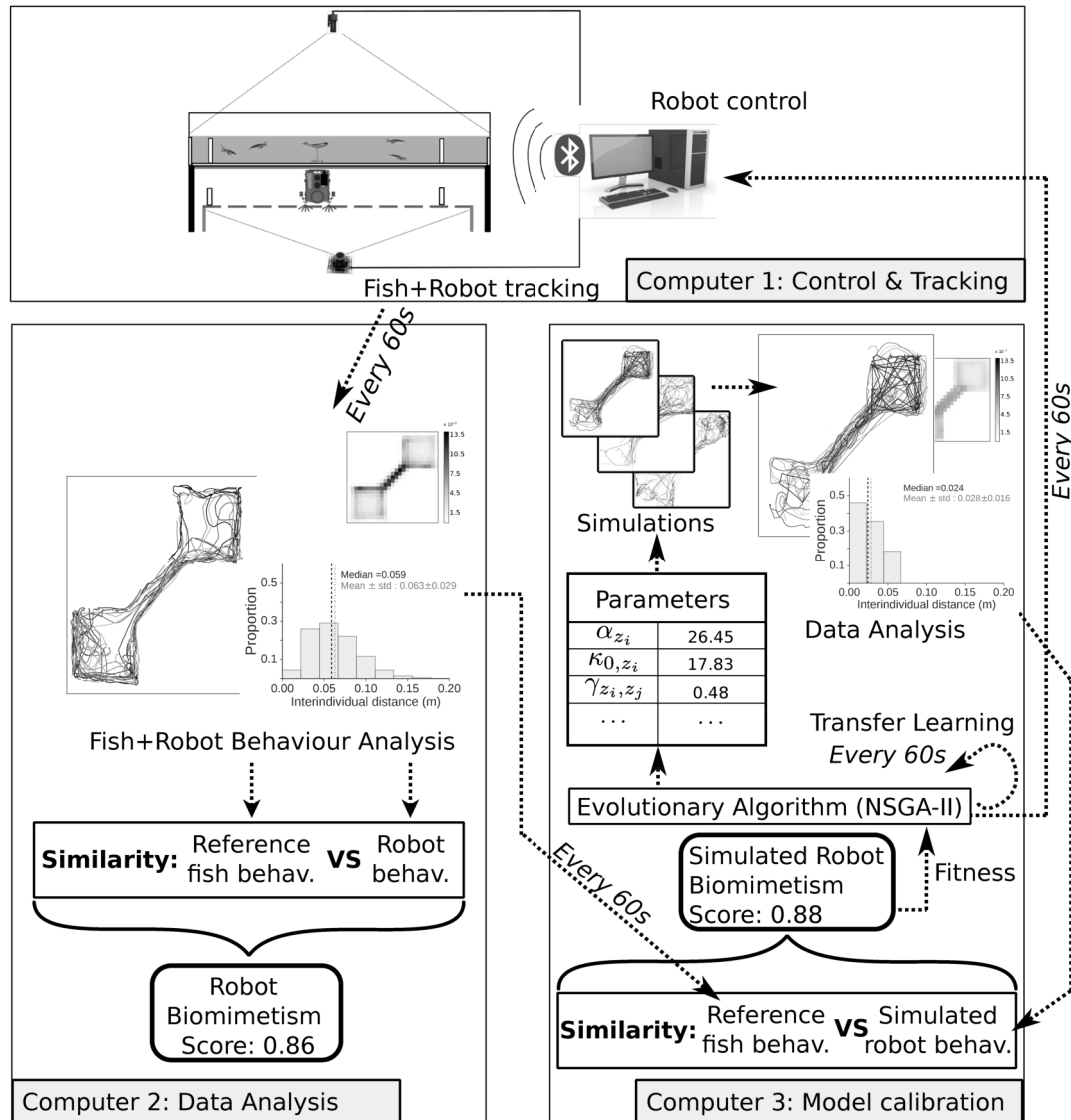


Figure 6.2: Workflow of our real-time calibration methodology. It involves extensive computation to be able to function in real-time, and thus is implemented over three 32-core computers. Computer 1 tracks the positions of fish and robot and is also running the robot controller. Computer 2 performs data-analysis of the fish and the robot behaviour using the data gathered by Computer 1 during 60s. Computer 3 calibrates the behavioural model (presented in Sec. 6.2.2) to be as close as possible to the observed behaviour of the fish as assessed by the data-analysis performed by Computer 2. It also uses the knowledge acquired during the previous calibration processes. The calibrated model is sent every 60s to Computer 1 to be used to drive the robot. The social acceptance of the robot behaviour is measured by Computer 2 with a distance metric of collective features.

6.2.3 Communication between computer nodes

We connect the three computers (Fig. 6.2) using the ZeroMQ distributed messaging protocol [104]: computers receiving messages act as ZeroMQ subscribers, and computers sending messages act as ZeroMQ publishers.

The tracked agent trajectories are compiled in the form of trajectory files, and sent every 60s from Computer 1 to Computer 2 through the `rsync` command line application (a process which usually only need 2s to 3s that is sufficient because the parameter update is every 60s). Then, Computer 1 send a ZeroMQ message to Computer 2 to acknowledge that the transfer is completed. Data-analysis scores from Computer 2 to Computer 3, and model parameters from Computer 3 to Computer 1 are sent every 60s through ZeroMQ messages.

6.2.4 Real-time tracking

We use the CATS framework [18] to track agents (fish and robot) in real-time, on Computer 1. Fish are tracked (but not identified) by using frames captured by the overhead camera (Fig. 6.2) through the Shi-Tomasi method [217] implemented in the OpenCV library [29]. In parallel, the robot is tracked through the video frames from the camera below the fish-tank by colour and contours detection [239]. Every 60s the positions of the agents are sent to Computer 2 for data-analysis.

6.2.5 Data-analysis

Every 60s, Computer 2 calculates the behavioural statistics using the tracked positions of agents (from computer 1) over the last 120s of the running experiment, for all three zones of the arena. For a zone e , these statistics are: the distribution of inter-individual distances between agents (D_e), the distribution of distances of agents to their nearest wall (W_e), the distribution of zones occupation (O_e), the transition probabilities from zone e to others (Te).

These statistics are computed either only on fish agents (**Control** case: e_c) or on fish and robotic agents (**Robot Social Integration** case: e_r). We define a similarity score (ranging from 0.0 to 1.0) to measure the biomimetism of robot behaviour compared to the **Control** case:

$$S(e_r, e_c) = \sqrt[4]{I(D_{e_r}, D_{e_c})I(W_{e_r}, W_{e_c})I(O_{e_r}, O_{e_c})I(T_{e_r}, T_{e_c})} \quad (6.3)$$

The function $I(P, Q)$ is defined as such: $I(P, Q) = 1 - H(P, Q)$. The $H(P, Q)$ function is the Hellinger distance between two histograms [66]. It is defined as: $H(P, Q) = \frac{1}{\sqrt{2}} \sqrt{\sum_{i=1}^d (\sqrt{P_i} - \sqrt{Q_i})^2}$ where P_i and Q_i are the bin frequencies.

Cazenille *et al.* [41, 43] demonstrated that robotic lures with biomimetic morphology and behaviour are be more socially integrated into the group of fish than non-biomimetic lures. As such, the biomimetism score defined earlier corresponds to the social acceptance of the robot by the fish.

When this statistics and scores are computed, they are dispatched to Computer 3 (by the ZeroMQ system described in Sec. 6.2.3) to guide the optimisation process.

6.2.6 Real-time optimisation of model parameters

We design a calibration methodology (Fig. 6.2) capable of optimising in real-time the parameters of the behavioural model from Sec. 6.2.2 to mimic as close as possible to the behaviour of experimental fish. The behavioural similarity is quantified as described in Sec. 6.2.5.

It is inspired from the off-line calibration methodology in [41] and uses the NSGA-II [64] multi-objective global optimiser (population of 60 individuals, 300 generations) with three objectives to maximise. We define a fitness with three objectives: the first objective is a performance objective corresponding to the $S_{(e_1, e_2)}$ function. Two other objectives are considered to guide the evolutionary process: one that promotes genotypic diversity [162] (defined by the mean euclidean distance of the genome of an individual to the genomes of the other individuals of the current population), the other encouraging behavioural diversity (defined by the euclidean distance between the D_e , W_e , O_e and T_e scores of an individual).

This process is performed and restarted every 60s on Computer 3 (starting 120s after the beginning of the experiment to gather data, Fig. 6.2) using data gathered during the last 120s. Every restart of the evolutionary algorithm keeps the last generation of individuals evolved during the previous round of evolution to bootstrap the current round of evolution, a system akin to transfer learning. On our 32-core computer, one generation is computed approximately every 4s, so around 15 generations are computed at every evolutionary round.

We do not optimise the linear speed v_i of the agents. It is randomly drawn from the experimental speed distribution. We use the NSGA-II implementation provided by the DEAP python library [81].

6.2.7 Robot implementation and control

The robot is driven by the model presented in Sec. 6.2.2 thanks to the CATS framework [18]. The model is calibrated every 60s using the methodology of Sec. 6.2.6, in experiments involving four fish and one robot. Every 200ms, the tracked positions of

the four fish are integrated into the model to compute the target position of a fifth agent. The robot is programmed to follow this target position by using the biomimetic movement patterns as in [19, 43].

6.3 Results

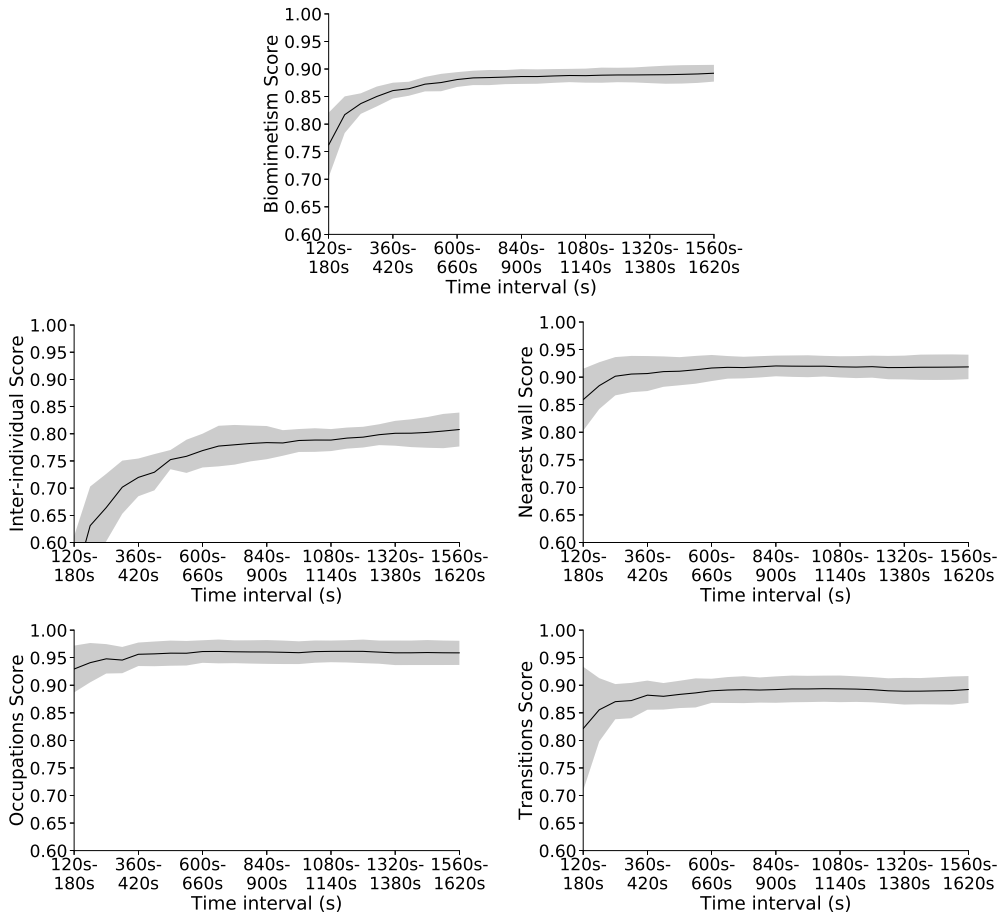


Figure 6.3: Similarity scores between the behaviour of the experimental fish and the behaviour of the best-performing individuals of the calibrated model at different time intervals of 10 different experiments. These scores are computed using data over 120s, and starting from the third time interval (120s to 180s) to ensure gathering enough experimental data. In these plots, lines correspond to the mean scores across the 10 experiments, and the grey translucent areas correspond to the standard deviation. We consider four behavioural features to characterise the behaviour exhibited in each time interval. **Inter-individual distances** corresponds to the similarity in distribution of inter-individual distances between all agents in a specific zone and measures the capabilities of the agents to aggregate. **Distances to nearest wall** corresponds to the similarity in distribution of agent distance to their nearest wall, and assess their capability to follow the walls. **Occupations** corresponds to the similarity in probability of presence of the agent in each zone. **Transitions** corresponds to the similarity in probabilities of an agent to transition from one zone to another. The **Biomimetic score** corresponds to the geometric mean of the other scores.

We assessed the evolution of the similarity scores (defined in Sec. 6.2.5) between robot behaviour and fish behaviour, across sliding windows of 120s intervals of a set of 10 trials each one lasting 30 minutes, starting in each trial from the second time interval (120s to 180s) to gather enough experimental data. These scores are compiled in Fig. 6.3. The variance for the 10 trials is plotted as a grey area around the curves and remains rather small.

From its initial value of about 0.610 (second time interval: 120s to 180s), the average fitness (mean scores) fastly converges to values around 0.850 starting from the fourth time interval (0.827 on 240s to 300s). This is also observed for similarity scores of transitions and of distances to nearest wall. This shows that both of these behavioural features can be effectively optimised through an online evolutionary algorithm process, and remain stables during the experiment.

The similarity score of zone occupation is particularly high at the beginning of the experiment, and is only slightly improved by our calibration methodology; this would suggests that room occupation is only slightly dependent of model parameters. This could be explained by a strong effect of room geometry (room size, and the general room configuration of the arena) over room occupation: rooms cover a larger area than the corridor. This could also be an effect of the aggregative behaviour exhibited by fish and by the model: the robot would follow the fish, which would tend to follow walls, thus explaining the relative invariance of the occupation score with respect to parameter values.

The variations in similarity score of inter-individual distances suggests changes of fish aggregative behaviour during the experiment. This could be explained by the fact that, while zebrafish tend to remain cohesive most of the time, they have the tendency of forming short-lived (a few seconds to a few minutes) sub-groups, especially when confronted to a fragmented environment [43].

6.4 Discussion and Conclusion

Animal-robot interaction studies employ simple robot behavioural model that are not adaptive or updated during the experiments. Often they are not biomimetic and do not close the interaction loop between the animals and the robots [43]. Here we present a methodology to calibrate in real-time a multi-level context-dependent biomimetic model of fish behaviour to drive the behaviour of a biomimetic robot into a group of zebrafish. The model parameters are continuously refined to accurately correspond to the collective dynamics exhibited by fish during the experiments. The real-time nature of this calibration process allows the robot to react to changes in observed fish dynamics and cope with uncertainties.

Animals can present significant inter-individual behavioural differences. They can present significant differences in terms of personalities typically bold and shy types [246]. In most of the experiments individuals are selected randomly from a stock. Consequently, each group trial present differences depending on the characteristics of the individuals. Currently, the models are calibrated on a set of averaged experimental data and are not optimised to take into account inter-individuals differences. We present here a method to adapt in real-time the models and that is thus capable to cope with this issue. This method can reduce significantly the number of experimental trials necessary to calibrate the model.

Our approach is computationally intensive and use three networked computers to handle in real-time the tracking, the robot control, the data analysis and the model calibration tasks.

Our methodology builds on the work presented in [41] by adding real-time capabilities to the calibration process. However, it also suffers from the same limitations. Namely, the model we calibrate must still be structurally defined empirically (*i.e.* defining behavioural attractors, zones of the environment, etc) with ethological *a-priori* knowledge about fish dynamics. The calibration process could also still be improved by taking into account additional behavioural metrics in the computation of similarity scores, either in term of collective dynamics (*e.g.* agent groups aspects, residence time in a zone), individual behaviours (*e.g.* agent trajectory aspects, curvature of trajectories). This could possibly be bypassed through the use of a calibration process without explicit similarity measure (*e.g.* GAN [89] or Turing Learning [144]). Our behavioural model could be revised to account for collective departures of agents from one room to the other, as described in biological studies [48].

Additionally, our methodology could make use of global optimisation techniques designed to minimise the number of evaluations before reaching convergence, like Bayesian Optimisation [58, 223]. This would reduce calibration computation costs, and possibly reduce the time needed to accurately calibrate the models.

Part IV

Modulating fish collective dynamics with robots

Collective behaviour based on self-organisation has been observed in populations of animals from insects to vertebrates. These findings have motivated engineers to investigate approaches to control autonomous multi-robot systems able to reproduce collective animal behaviours, and even to collectively interact with groups of animals.

In parallel of the work presented in Chapter 7, our colleagues at the EPFL in Lausanne have achieved closed loop interaction and modulation in a circular set-up [21] between the same robots as in Part III and zebrafish. In this study, they focused on the swimming direction of fish and in order to obtain clear reading, they used a constrained environment, a circular corridor. This set-up forces the fish to go either in a clockwise or a counter-clockwise direction. In experiment with zebrafish only, they move half of the time in each direction, which shows that this set-up is non-biased. In mixed group experiments, the robots were programmed to swim in only one direction and were able to influence the swimming direction of the fish. This result shows that robots are able to change the collective choices of group of social fish and modulate their behaviours.

In Chapter 7, we study the different strategies to modulate the behaviour of zebrafish in a more complex environment, the two rooms set-up. Unlike a more uniform environment such as the circular corridor, the two rooms environment requires that these strategies be based on the biomimetic behaviour developed in Part III. First we used the robot to highlight the mechanisms of leadership. Then we modulated the spatial distribution of zebrafish by extreme bold and shy strategies. Our results support the hypotheses made on the heterogeneity of leadership in zebrafish groups [48]. We also showed that shy strategies were more efficient to modulate the spatial distribution due to the high mobility of zebrafish.

This work is based on the publication [44]:

Chemtob Y, Cazenille L, Bonnet F, Gribovskiy A, Mondada F, Halloy J. Strategies to modulate zebrafish collective dynamics with a closed-loop biomimetic robotic system. *bioRxiv* 2019

The experimental set-up was designed and built by Bertrand Collignon, Axel Seguret, Leo Cazenille, José Halloy and myself. The FishBot robot was developed at the EPFL, by Frank Bonnet and Francesco Mondada. The experimental arena was built by Axel Seguret and myself. The control and tracking system was jointly developed by Frank Bonnet, Alexey Gribovskiy, Leo Cazenille. I carried out all the experiments. I developed and implemented the data analysis scripts. This paper was mainly written by me, with the help of José Halloy and Leo Cazenille.

Chapter 7

Strategies to modulate zebrafish collective dynamics with a closed-loop biomimetic robotic system

Abstract

The objective of this study is to integrate biomimetic robots into small groups of zebrafish and to modulate their collective behaviours. A possible approach is to have the robots behave like sheepdogs. In this case, the robots would behave like a different species than the fish and would present different relevant behaviours. In this study, we explore different strategies that use biomimetic zebrafish behaviours. In past work, we have shown that robots biomimicking zebrafish can be socially integrated into zebrafish groups. We have also shown that a fish-like robot can modulate the rotation choice of zebrafish groups in a circular set-up. Here, we further study the modulation capabilities of such robots in a more complex set-up. To do this, we exploit zebrafish social behaviours we identified in previous studies. We first modulate collective departure by replicating the leadership mechanisms with the robot in a set-up composed of two rooms connected by a corridor. Then, we test different behavioural strategies to drive the fish groups towards a predefined target room. To drive the biohybrid groups towards a predefined choice, they have to adopt some specific fish-like behaviours. The first strategy is based on a single robot using the initiation behaviour. In this case, the robot keeps trying to initiate a group transition towards the target room. The second strategy is based on two robots, one initiating and one staying in the target room as a social attractant. The third strategy is based on a single robot behaving like a zebrafish but staying in the target room as a social attractant. The fourth strategy uses two robots behaving like zebrafish but staying in the target room. We conclude that robots can modulate zebrafish group behaviour by adopting strategies based on existing fish behaviours. Under these conditions, robots enable the testing of hypotheses about the behaviours of fish.

7.1 Introduction

Current research in engineering and science is seeking to bridge the gap between living and artificial systems to design new technologies. Building on biomimetic approaches, a stream of this research trend aims to build biohybrid systems that harness the advantages of both living and artificial systems [194]. Some of these biohybrid systems comprise robots and animals producing collective behaviours [204]. Here, we focus on experiments with autonomous robots that interact socially in closed-loops with animals, that is, robots perceiving and reacting to animals and vice versa. A limited number of research studies deal with the difficult task of producing collective, closed-loop animal-robot behaviours, especially controlling entire biohybrid systems to perform predefined tasks.

For example, Vaughan *et al.* [249] showed that a robot can gather ducks by acting like a sheepdog. Halloy *et al.* [97] demonstrated that multiple robots socially integrated and interacting in a closed-loop with cockroaches could be used to control their decision-making mechanisms to reach predefined targets. Swain *et al.* [240] explored the interactions between a mobile robot and a shoal of Golden shiners by presenting the robot as a predator. Kawabata *et al.* [118] showed that a robot can modulate the behavioural reactions of a cricket. Kopman *et al.* [126] showed that fish adapt their behaviour to the tail-beating of a robotic lure and also that this response can be improved when the robotic tail beating is controlled, in closed-loop, according to the movement of the fish. Landgraf *et al.* [139] showed that a mobile robot can interact with a shoal of Guppies and make recruitment. Shi *et al.* [218] showed that a robotic rat can modulate the behaviour of several laboratory rats with or without direct interactions. Donati *et al.* [68] showed that a dummy attached to a robotic arm can socially interact with the electric fish *Mormyrus rume*. Landgraf *et al.* [138] showed that a mobile robot with realistic eye dummies and natural motion patterns significantly improved its acceptance level among a shoal of Guppies. Stefanec *et al.* [228] showed that static robots simulating the presence of individuals by controlling their temperatures could interact and attract young bees. Worm *et al.* [258] showed that a robot moving randomly can interact with the electric fish *Mormyrus rume* by displaying prerecorded electric organ discharges (EODs) in answer to the EODs made by the live fish. Cazenille *et al.* [42,43] showed that a mobile robot capable of biomimetic behaviours can successfully integrate a group of zebrafish and produce collective behaviours. Bonnet *et al.* [21] showed that such mobile robots can modulate the collective decision-making of a group of zebrafish in a circular set-up. Papaspyros *et al.* [173] improved the integration of the robot under similar conditions by using a biomimetic model to drive it. Quinn *et al.* [196] showed that rats can release robot-rats from restrainers as they do for other conspecifics and can discriminate between different behaviours the robots expressed. Kim *et al.* [121] used a robotic arm with a zebrafish lure moving in three dimensions in an isolated tank connected to the fish tank only visually to show that, if the system is in a closed-loop, some transfer learning can be observed. Bonnet *et al.* [23] showed that robotic fish and robotic bees

integrated with their respective conspecifics could produce cooperation between the fish and the bees, respectively, in real time and over long distances.

While these previous studies succeeded in modulating animal behaviour with robots in closed-loop, few used mobile robots on social animal groups, and even fewer used them with biomimetic trajectories. In studies involving fish, only Cazenille *et al.* [42,43] used a biomimetic model to drive a fish robot. However, this work did not modulate fish behaviour, as Bonnet *et al.* [21] and Papaspyros *et al.* [173] achieved in a simpler environment.

Here, we study the modulation capabilities of fish robots integrated into zebrafish (*Danio rerio*) groups in a complex environment. We aim to achieve this by reproducing specific behaviours of collective movement, such as the leadership described by Collignon *et al.* [48] and collective spatial distribution [211]. This allows us to validate hypotheses made about the collective behaviour of zebrafish and gain a better understanding of zebrafish group decision-making. Leadership is an essential element in collective decision-making, as it reflects the will of one or a minority of individuals to initiate the movement of the group in a new direction. We have implemented theoretical leadership mechanisms in the robot to make it initiate collective departure in groups of fish.

We test different biomimetic behavioural strategies to drive the fish groups towards a predefined *target room* in a set-up composed of two rooms connected by a corridor. We test the hypothesis that, to drive the biohybrid groups towards a predefined choice, the robots have to adopt some specific, fish-like behaviours. Several studies have shown the influence of personality in collective movements often described by the opposition of *bold* and *shy* behaviour [98,115,168,254]. Here, we test both a *bold* behaviour with a robot performing collective departures towards the target and a *shy* behaviour with a robot delaying collective departures from the target.

7.2 Materials and Methods

7.2.1 Ethics statement

All fish experiments were performed in accordance with the recommendations and guidelines of the Buffon Ethical Committee (registered to the French National Ethical Committee for Animal Experiments #40) after submission to the state ethical board for animal experiments.

7.2.2 Animals and housing

We used groups of four or five adult, wild-type AB zebrafish (*Danio rerio*) in our experiments for a total of 300 fish. The fish were 6–12 months old at the time of the

experiments with a body length of three to four centimeter. We kept the fish under laboratory conditions: 27 °C, 500 μ S salinity with a 10:14 day:night cycle. The fish were reared in ZebTEC housing facilities and fed twice a day (Special Diets Services SDS-400 Scientific Fish Food). The water pH level was maintained at 7, and nitrites (NO^{-2}) were below 0.3 mg l.

7.2.3 Experimental set-up

We used the set-up described in [18, 42, 48, 211] consisting of two rooms linked by a corridor ($1\,000 \times 1\,000 \times 100 \text{ mm}^3$) made of white plexiglass (figure 7.1B) placed in a $1\,200 \times 1\,200 \times 300 \text{ mm}^3$ experimental tank. The tank was filled with water up to a level of 60 mm. The whole set-up was exposed to diffused light and confined behind white sheets to isolate experiments and homogenise luminosity. We used the Fish-CASUs (Fish-Control-Actuator-Sensor-Unit) [17–19] to interact with the fish (figure 7.1A). They were composed of soft fish lures linked by magnetic coupling to mobile robots (FishBots) moving below the experimental tank. The fish lures had lengths of 4.5 cm that mimicked the morphology of the zebrafish by passively beating their tails when moving underwater. The floor of the aquarium was covered with a sheet of teflon to provide a smooth surface for the motion of the fish-lure. The FishBot robots were powered by two conductive plates, one glued to the bottom of the aquarium and one to the support on which the robot moved (figure 7.1C). An overhead acA2040-25gm monochrome GigE CCD camera (Basler AG, Germany) with a maximum resolution of $2\,048 \times 2\,048 \text{ px}$ and equipped with low distortion lenses CF12.5HA-1 (Fujinon, Tokyo, Japan) grabbed frames that were then processed to find the fish positions. A complementary fisheye camera (15 FPS, $640 \times 480 \text{ px}$) was placed under the fish tank and was used to track the position of the robot. The tracking of the agents and the control of the robot were done using the CATS (Control and Tracking Software) framework [18].

Each experiment was repeated 10 times and lasted 15 minutes. At the start of every trial, we let the fish occupy the set-up for 5 minutes with the robot not moving for acclimatisation.

7.2.4 Data analysis

In addition to the online tracker included in the CATS framework [18], we acquired the identity of each agent using idTracker software [181] after the experiments. We obtained the positions $P(x, y, t)$ of all agents at each time step $\Delta t = 1/15 \text{ s}$ for all experiments and built the trajectories of each agent. For each video, we manually selected which trajectory corresponded to the robot. Using the methodology developed in [48], we identified and detailed all collective departure. Using the agent positions, we also quantified the presence of fish in each part of the set-up.

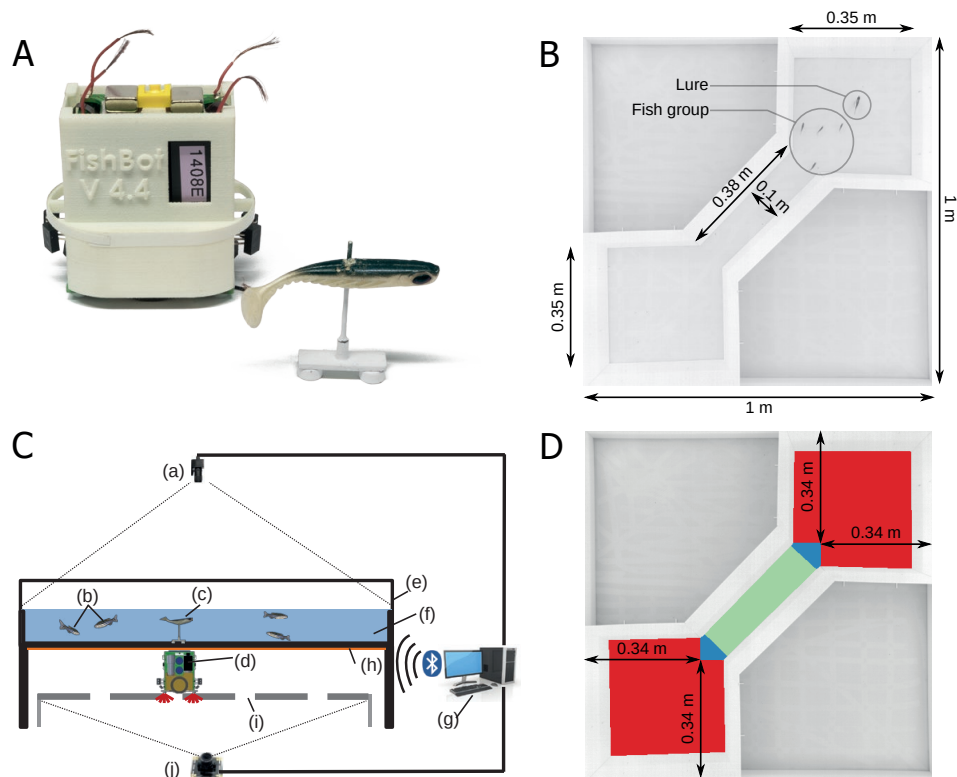


Figure 7.1: Experimental set-up. **A:** The FishBot [17,19,24] and a biomimetic lure used during the experiments. **B:** Experimental arena composed of two square rooms (350 mm x 350 mm at floor level) connected by a corridor (380 mm x 100 mm at floor level). **C:** Experimental set-up used during the experiments [17,19,24]. (a) Overhead camera used to track zebrafish. (b) Zebrafish. (c) Fish-lure. (d) FishBot moving under the aquarium and linked to the lure through magnetic coupling. (e) Fish tank of 1 000 x 1 000 x 250 mm. (f) Water layer 60 mm deep. (g) Computer processing camera frames and remote-controlling the robots via Bluetooth. (h) Conductive plate powering the mobile robot. (i) Perforated steel plate for powering the FishBot and to observe the FishBot LEDs from below. (j) 180 degree Fisheye camera to track the FishBot from below. **D:** Coloured zones of the arena corresponding to the three different types of behaviour of the robot [43]. When the robot is in one of the rooms (in red) or near the entrance of the corridor (in blue), it is driven by the biomimetic model presented in Sec. 7.2.5. When the robot is in the corridor (in green), it drives straight through the corridor at a constant speed.

7.2.5 Perception-based behavioural model

To control the FishBots, we used the multilevel behavioural model presented by Cazenille *et al.* [43], which is derived from the model developed by Collignon *et al.* [49] and can simulate the collective behaviour of zebrafish in a finite environment. This model relies on two levels of control which are biomimetic and context-dependent according to the different behaviour expressed by the fish and according to the geometry. The high level controller build the trajectories that the robot follow. In large environments, that is, the rooms in this set-up, the robot followed a perception model, describe in previous study [43] and bellow, while in narrow spaces, that is, the corridor, it followed a straight

line. The low level controller deal with the movement patterns and allows the robot to reach the new position, produced by the high level controller, by reorienting the robot, then performing acceleration and deceleration to reproduce the burst-and-coast swimming motion performed by the zebrafish [43].

This perception model (see [43]-III-A) is inspired by the probabilistic model described in [49]. The agents update their position vectors X_i with a velocity vector V_i :

$$X_i(t + \delta t) = X_i(t) + V_i(t)\delta t \quad (7.1)$$

$$V_i(t + \delta t) = v_i(t + \delta t)\Theta_i(t + \delta t) \quad (7.2)$$

where v_i is the linear speed of the i^{th} agent and Θ_i is its orientation. The linear speed v_i of the agent is randomly drawn from the experimentally measured instantaneous speed distribution.

The Θ_i orientation is sampled from a probability density function (PDF) calculated according to the direction of the different stimuli. This PDF is the combination of a first PDF of the potential directions of a fish with no perceptible stimulus and a PDF representing the attraction towards all fish perceived according to their distances. The final PDF is calculated at time t . The position of the fish and the robot is obtained in real time by the two cameras of the experimental system. The agent's position is updated according to this direction and linear speed.

7.3 Defining different robot behaviour modulation strategies

Figure 7.2 shows the description of the algorithm run by the robot for the initiation experiment E2. The goal was to influence the group of fish to move from one room to another. The robot initiated collective departure from the *starting room* towards the *target room*.

The robot had two phases in the *starting room*: when it waited for the group of fish and when it started a transition. When it waited, the robot stayed in the *starting room* using the biomimetic perception model described in Sec. 7.2.5. If three fish or more were detected in the *starting room*, the robot turned in the room over the course of one second, using the model to attract the fish.

After that, the robot reached a target put in the *target room* and wait for the fish using the perception model. Six seconds after the beginning of the transition, the controller checked the number of fish in the *target room*. The six seconds delay corresponded to the maximum delay observed for the fish to make a transition. If at least three fish

were detected in the room, the *target room* became the *starting room*, and the algorithm started again. If not, the robot went back to the *starting room*.

Ten trials were done with the robot following this behaviour over 15 minutes with four fish.

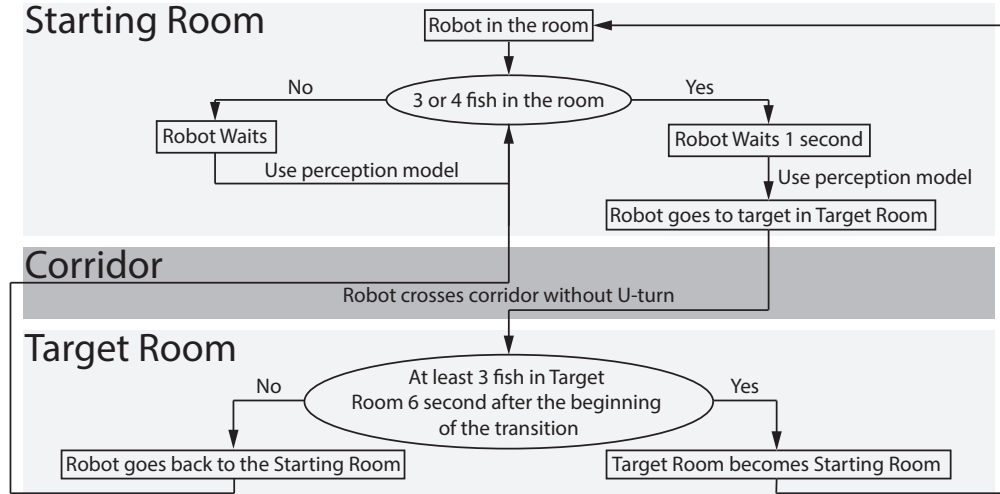


Figure 7.2: Initiation behavioural strategy of the robot. The behavioural model of the robot is a mix of biomimetic initiation behaviour and artificial behaviours developed to enhance the effect of the initiation strategy.

Label	Experiment	Number of agents	Robot strategy
E1	Reference AB zebrafish only	5 fish + 0 robot	–
E2	Initiation of transitions	4 fish + 1 robot	Biomimetic initiation without target room
E3	Modulation by initiation	4 fish + 1 robot	Biomimetic initiation to target room
E4	Modulation by attraction	4 fish + 1 robot	Fish behaviour & stay in target room
E5	Modulation by attraction	4 fish + 2 robots (R1,R2)	R1 & R2 stay in target room
E6	Mixed initiation and attraction	4 fish + 2 robots (R1,R2)	R1 biomimetic initiation & R2 stays in target room

Table 7.1: Strategies of modulation. The **E1** experiment is the biological reference case, 10 trials of five wild-type zebrafish groups and no robot. The **E2** experiment involves 10 trials of four wild-type zebrafish and one robot performing a maximum of collective departures. The **E3** experiment involves 10 trials of four wild-type zebrafish and one robot driven by the initiation strategy to modulate the presence of fish in the rooms. The **E4** experiment involves 10 trials with a robot driven by attraction behaviour and 4 zebrafish. The **E5** experiment is the same as **E4** but with two robots with fish-like behaviour. The **E6** experiment mixes these two strategies and uses two robots, one initiating and the other attracting.

Experiment E1 was the biological reference case, involving 10 groups of five wild-type zebrafish and no robot. In experiment E3, we used the initiation behaviour described above to modulate the presence of four fish between the two rooms with one robot. We wanted to attract the fish to one room more than the other, so the *target room* and the

starting room defined in the initiation behaviour were set at the beginning of each of the 10 trials and did not change during the trial. For experiments E4 and E5, either one or both robots used the following attraction behaviour on a group of four fish: the robot was driven by the biomimetic perception model but could only navigate inside the *target room*. The *target room* changed between each of the 10 trials and was selected randomly. This corresponded to the behaviour of a fish with a strong preference for one room and no exploration behaviour. Experiment E6 was a mix between experiments E3 and E4 with two robots and four fish. One robot used the initiation behaviour and the other the attraction behaviour. The *target room* was the same for the two robots and was selected randomly at the beginning of the 10 trials. All the trials lasted 15 minutes.

7.4 Results

7.4.1 Initiation of collective departure

In a previous study [48], we showed that zebrafish present a shared leadership: each fish has the same ability to initiate a collective movement, which our experiments demonstrated with the action of initiating a transition from one room to the other. However, zebrafish groups exhibit heterogeneity, ranging from groups in which leadership-sharing is egalitarian to groups in which a single fish monopolises the leadership. This difference is explained by the greater mobility of these individuals [48]. Here, we emulated this behaviour by forcing the robot to perform as many transitions as possible.

We validated with experiment E2 that the robot can, in fact, lead the group with this behaviour. Here, every time the robot was with the most fish in one chamber, it exited the room to go to the *other room* (Sec 7.3).

Our results showed (figure 7.3A) that, in ten trials with different groups of fish, the robot initiated a transition of the group of fish more often than all the other fish in the group (7 / 10) or all the fish except one (3 / 10).

As in [48], a linear regression shows that the total number of initiations of each fish is linearly correlated to the total number of attempts performed (figure 7.3B). This means that the success of fish that initiate more group starts than their congeners can be explained simply by the fact that they tried to exit the room more often [48], not by their physical traits, such as size or sex. In our experiment, we applied this hypothesis to the robot behaviour, and figure 7.3B shows that the ratio of attempts and successes of the robot initiating collective departure, represented by green stars, corresponds to the fish. We also compared the distribution of the number of initiations by individuals between the reference experiment E1 and experiment E2 using a t-test and did not observe any significant difference (p-value = 0.3880). The number of initiations performed by the robot was therefore not excessive compared to that observed in fish. This experiment

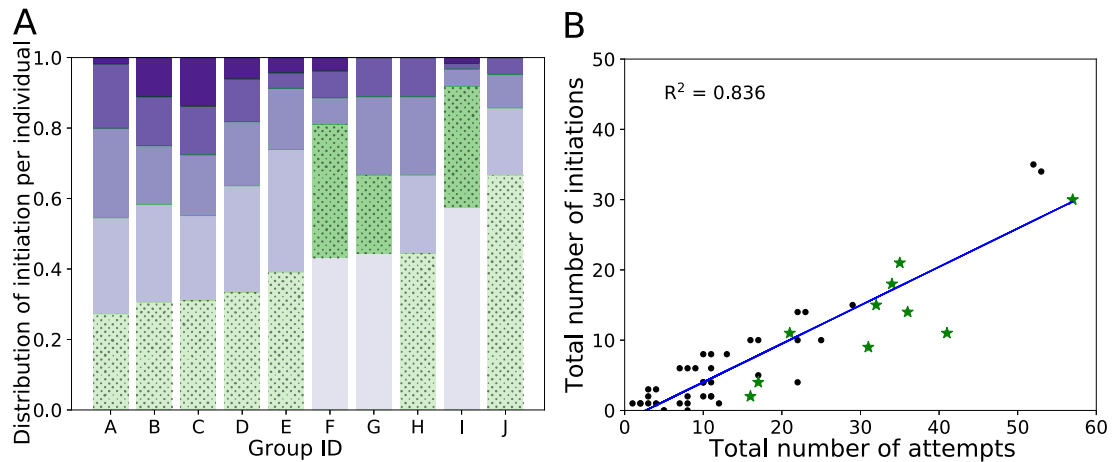


Figure 7.3: Robot initiation of transition towards the *target room*. (A) Frequency of initiation according to the intra-group ranking of the fish for initiation. For each group, we calculate the proportion of group departure that each individual initiated, as in [48]. The fish are then ranked according to this frequency. The robot is indicated in dotted green. (B) The total number of collective departures initiated in relation to the total number of attempts made by fish, in black, and by the robot, in green. The data show that the number of initiations is directly proportional to the number of attempts. Each fish has the same probability of success to initiate a collective departure when attempting. The robot, even with more initiations than most fish, has a similar probability of success.

demonstrated that we could reproduce zebrafish initiation behaviour as described in [48] with our robot during long trials. By comparing the robot trajectories in figure 7.4 during the initiation phases with a fish under similar conditions, we observed that the robot followed a trajectory with linear speeds comparable to those observed in fish. In the example figure 7.4B, we observed that fish used the same trajectories as the robot with a one seconds delay. Also, the fish exited the rooms in groups.

7.4.2 Modulation of the spatial distribution

We wanted to modulate the collective decisions of the fish with the robot and change the time spent by the fish in each room. We considered two main strategies: first, using the robot as a leader to create a collective decision of exiting one room to a *target room*, and second, attracting the fish in the *target room* using the robot as an active lure. We also combined these strategies in an experiment with two robots, one the leader and one the attractor.

Experiment E1 was our reference with five fish and no robot. We demonstrated that the fish did not have a preference for either room. We measured the time spent in each room (figure 7.5A). We looked for differences between the two distributions using a Wilcoxon test and observed no significant difference (p-value = 0.8886). Without robot interference, the fish were in each room the same amounts of time. We also observed

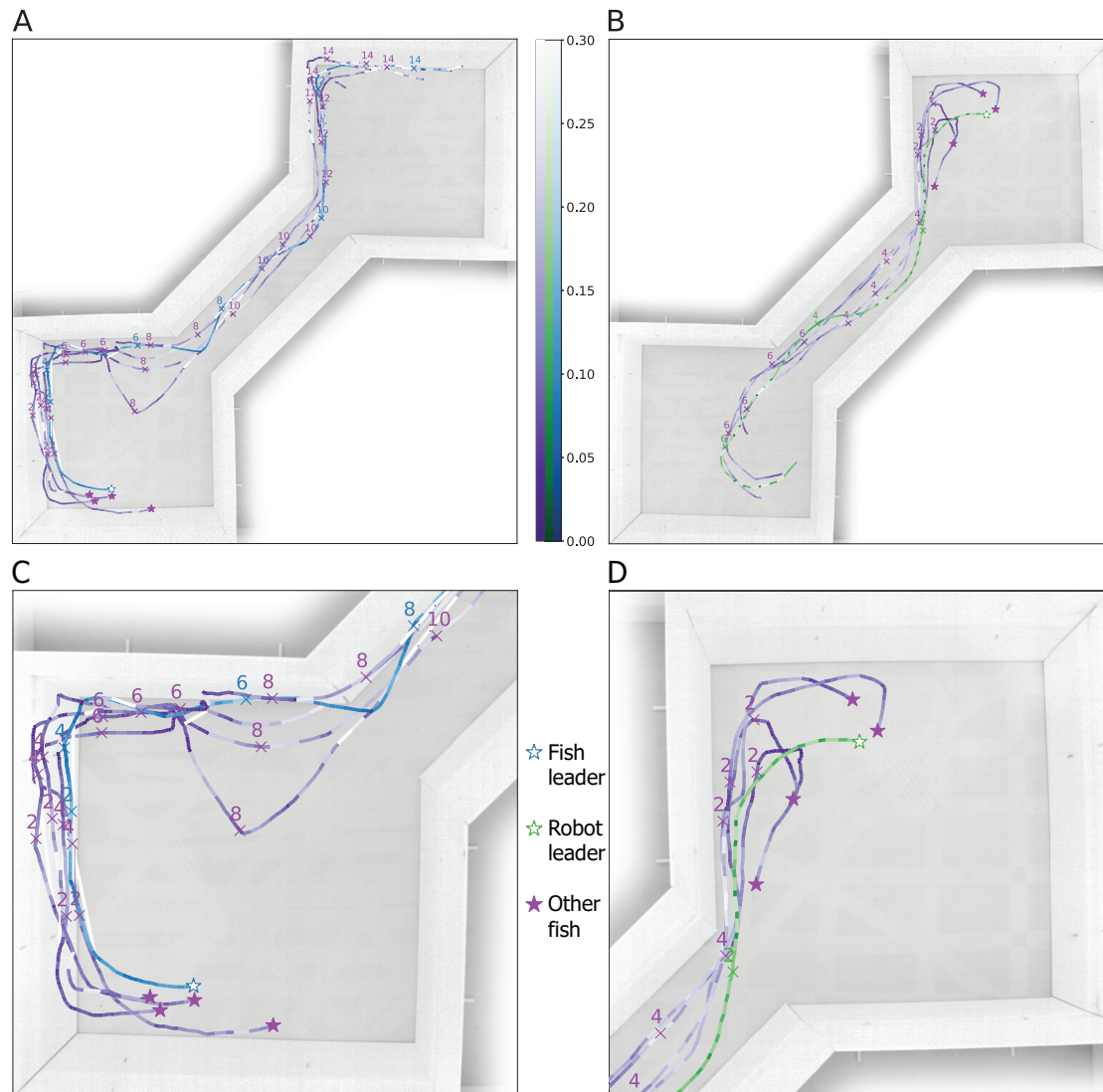


Figure 7.4: Examples of individual trajectories during a collective departure event (A) with a fish as a leader from the fish control experiment E1 and (B) with the robot as the leader, as in experiment E2. (C) and (D) are zooms of (A) and (B), respectively. The trajectory of the leader is in green for the robot, in blue for the fish and in purple for the other fish. The colour scale indicates the linear speed of the agent in m/s . A star indicates the beginning of the trajectory, with a white centre for the leaders. Crosses on the trajectory indicate the time step in seconds. By comparing the trajectories of the robot and fish, we observe that the robot succeeds in reproducing the initiating behaviour.

that they spent less time in the corridor, so this part of the set-up was considered a transition zone.

In experiment E3, we used the initiation behaviour tested in E2 to modulate the fish spatial distribution during a 15 minutes trial. Figure 7.5B corresponds to experiment E3, in which the robot acted as a leader and initiated transitions towards the *target room*. There was no significant difference between the time spent in each room (p -value = 0.3329). When using the initiation behaviour, the robot failed to modulate the spatial distribution. However, looking at the initiation successes in each of the rooms (figure 7.6), we can see that the robot succeeded in initiating collective departures to the *target room* (except for one group) (figure 7.6A) and did not lead in the other direction (figure 7.6B), as expected. The failure of the modulation of spatial distribution could therefore be explained by the fact that zebrafish have short residence times [211]. The collective departures of the robot may therefore have occurred in a timeframe close to that executed by the fish themselves.

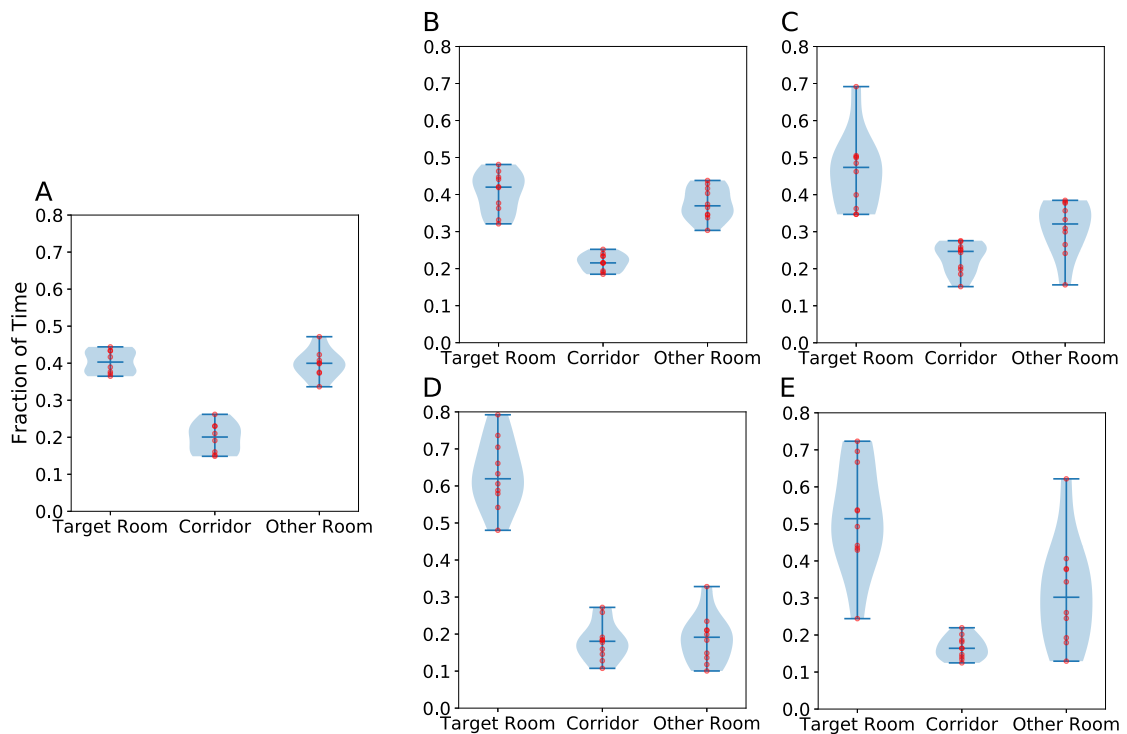


Figure 7.5: Fraction of time spent by all groups in the five experiments: **E1** Use of space for only five fish in the different parts of the two-room set-up (A), **E3** for groups comprising four fish and one robot acting as a leader and initiating transitions towards the *target room* (B), **E4** groups of four fish and one robot acting as an attracting lure in the *target room* (C), **E5** groups of four fish and two robots acting as attracting lures in the *target room* (D), **E6** groups of four fish and two robots, one acting as an initiating leader and the other an attracting lure (E). A Wilcoxon test shows no significant difference between fish presence in the rooms in (A), as fish spend the same amount of time in both rooms and transit rapidly through the corridor between the rooms (p -value = 0.8886) and (B) (p -value = 0.3329) when there is significant difference of the spatial distribution in (C) (p -value = 0.0284), (D) (p -value = 0.0051), (E) (p -value = 0.0367).

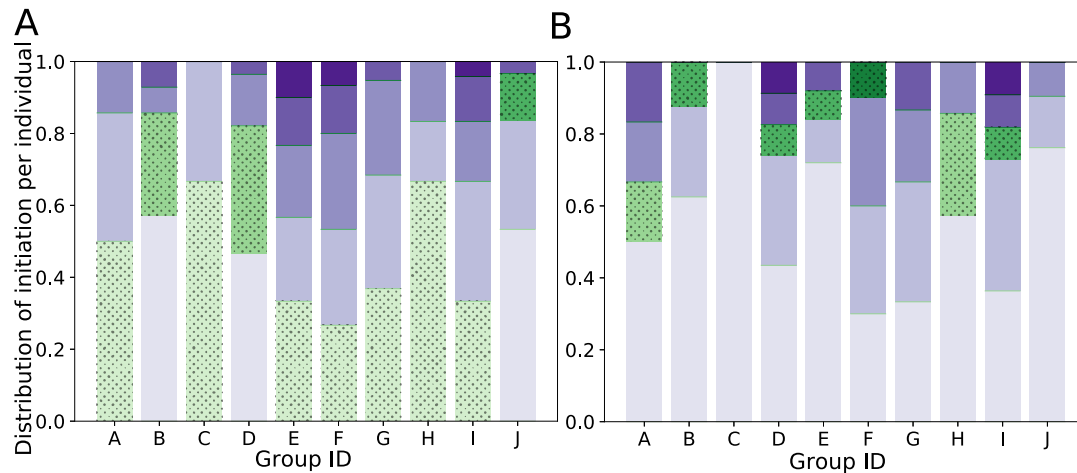


Figure 7.6: Proportion of departures initiated by each agent in experiment E3. The robot is indicated in dotted green. (A) To the target chamber. The robot is a leader. (B) From the target chamber. The robot is not the leader. The results indicate that the modulation of the transitions towards the *target room* works (A), although the residence time in the *target room* is not significantly changed (figure 7.5B).

Another strategy to modulate the spatial distribution is therefore to delay collective departures thanks to an agent that represses any exit from the *target room*.

In experiment E4 (figure 7.5C), the robot acted as a lure by swimming only in the *target room* during all the trials. We observed that fish stayed more in the *target room* (p-value = 0.0284) with a median of 0.45 against 0.31 in the *other room*. However, the variability of the time spent by the shoal in each room was quite high between trials, with values in the *target room* ranging from 0.3 to 0.7. This behaviour significantly modulated the time spent in each room by the fish, but over ten trials, three groups did not show any bias (the time spent in the *target room* being slightly less than in the *other room*).

In experiment E5 (figure 7.5D), we used two robots with the same behaviour as in E4. This experiment also biased the time spent between each room (p-value = 0.0051). The difference between the medians was higher than in E4 (0.63 against 0.18), and the variability was reduced between trials. The effect observed with one robot was amplified with two robots, and the influence of the robots along all groups was constant.

From the results above, we combined experiments E3 and E4. In experiment E6 (figure 7.5E), one robot acted as a leader to encourage the group to exit the *other room* while the other robot acted as a lure to delay the group from exiting the *target room*. There was a significant difference between the occupation of the two rooms (p-value = 0.0367). The medians were similar to the ones found in E4 (figure 7.5C), but the variability was even higher than in E4. Instead of being complementary, the initiation behaviour had a diminishing effect on the attraction behaviour.

7.5 Discussion

Previously, we have analysed the collective behaviours of small zebrafish groups in structured environments composed of two rooms connected by a corridor [48,211]. This set-up was designed to study how zebrafish groups navigate between the two rooms and how they make decisions to switch from one room to the other. In parallel, we developed an autonomous robot capable of zebrafish biomimetic behaviours that can be socially integrated into small zebrafish groups [19,43]. We further showed that a small number of those robots can modulate the collective behaviour of mixed zebrafish and robot groups in a circular set-up [21].

Here, we further explored the possibilities of modulating the behaviour of small zebrafish groups by using the same, previously integrated robot. We tested several strategies inspired by the biological results obtained by Collignon *et al.* [48] and Seguret *et al.* [211].

We used the robot to modulate collective departure over a period of about 15 minutes. The objective was to test the hypothesis that an individual with bold behaviour could exert a form of leadership by initiating transitions between the two rooms more often than other members of the fish group. We used the same behaviours as [43], but we added a behavioural rule that made the robot initiate a room transition as often as possible. Under these conditions, the robot could induce transitions more often than most of the fish. By looking at the ratio between exit attempts and initiations of movement from one room to another, we observed that the robot followed the same linear relationship as that observed in fish. These two results are consistent with what has been observed in zebrafish groups alone [48]. It also confirms the hypothesis that ‘bold’ behaviour is sufficient to explain the variations observed in the distribution of leadership of *Danio rerio*. This conclusion goes further than our previous work with autonomous robots [41–43]. Indeed, the initiations of collective departures have never been explicitly simulated by a fish robot before. In this study, we reproduced this process in a way similar to what can be observed in fish [48].

We then used the robot to bias the residence time of fish between the rooms.

Once we showed that the robot could induce group transitions between the rooms, making the robot choose one of the two rooms as the *target room* was simple. The hypothesis was that, by making more transitions towards that *target room*, the fish robot could bias the fish group presence density in favour of the *target room*. The experiments showed that this did not work, as the presence of the fish groups remained equivalent in both rooms. This may be due to the fact that zebrafish are highly dynamic fish with short residence times that keep moving in their environments, never setting long in one room [48,211]. As soon as they arrived in the *target room*, they did not stay long and made another transition towards the *other room*.

We tested another strategy to bias the residence time of the groups in favour of a *target room*. The fish robot made a choice and remained in the *target room* to become an attracting lure for the fish group. In this experiment, the results showed that, indeed, the fish groups spent more time in the *target room*. The luring strategy of using an individual (the fish robot) to attract the group is thus more efficient than inducing group transitions towards the *target room*. Moreover, this strategy was simpler in terms of behavioural program.

The recruitment effect can be enhanced by adding more robots behaving as robot-fish duets in the *target room*. In this case, the bias in favour of the target is maximal compared to the other strategies we tested in this study.

If we try to mix the two types of strategies, transition initiations and attraction, we see that the bias in favour of the target is low. In addition, the dispersion of the results showed that the group of fish was disturbed by this strategy. This disturbance was probably due to the mobility of the robots, which are not as fluid as that of fish.

This study dealt with groups comprising small numbers of zebrafish. We know that such small groups are more socially unstructured and less cohesive than larger groups. In this configuration, we chose the maximum difficulty to try to modulate the groups of fish with robots. Some of these strategies therefore allowed us to significantly bias the spatial distribution of fish groups over a long period of time by using robots moving with them. This was done previously in cockroaches [97] with robots selecting specific shelters. In fish, Bonnet *et al.* [21] succeeded in biasing the swimming direction of fish using the same robots. However, the environment used, a circular corridor, and the objective were both simpler than in our study. In the two-room set-up, the robots could not move according to a predefined path as in Bonnet *et al.* [21] but had to use a behavioural model to navigate the environment and interact with fish. This made it more complex to carry out modulation, but it allowed us to better understand decision-making within social fish groups.

Whether on collective starts or spatial distribution, we have shown that it is possible to modulate the behaviour of social fish with autonomous, biomimetic robots using strategies based on behaviours observed in animals. Our methodology allows us to test hypotheses about the functioning of specific collective behaviours. Other behaviours could therefore be tested with this same methodology such as learning behaviour or collective exploration.

Part V

Conclusion

8.1 Main contributions

Collective behaviour is a phenomenon widely studied by many disciplines. Through the spectrum of “classical” observational ethology, the use of simulation models inherited from mathematics and physics and finally robotics, there are many methods to understand and decipher the mechanisms allowing complex behaviours expressed by social animal groups. In this manuscript, I studied the collective behaviour of group of fish in zebrafish. My research has focused on the use of a constraining experimental set-up to highlight collective travel. I have studied collective movements and decision-making using tools from several disciplines, such as precise fish identification tools, behavioural model and biomimetic autonomous robots.

8.1.1 Zebrafish collective behaviour

In Chapter 1 we tested seven group sizes in a maze made of two rooms connected by a corridor. Beyond the simple observation that zebrafish move from one room to another without interruption, we highlight in our analyses that the larger a group is, the shorter the distances are between the nearest neighbours, while those separating each pair of fish increase. As for the use of space, when the zebrafish stay in one of the two rooms, they do it in subgroups of more than 80% of the group. However, the larger the group, the fewer events with all the individuals will be recorded in the rooms. We note that groups (at least 70% of the individuals) make five to six times fewer U-turns than transitions from one room to the other, for group sizes below 10 individuals. Finally, an analysis of the collective departures from one of the two rooms shows that, a few seconds before and at the time of departure, the fish adopt a very particular organisation. Indeed, the zebrafish located just after the initiator of the movement, as well as the last to leave, rarely change position between the few seconds before the exit of the group and the actual moment of the exit. Thus, we notice that there is a correlation link between the exit order and the ranking of distances in relation to the initiator of the movement as we approach the moment of departure. These results are similar to what has been observed in other social fish [205, 252] and suggest that the topology of the group is linked to a cascade of behaviour change [205] where the first fish drags another fish that will lead the next one.

In Chapter 2, we have shown that the ability to initiate a collective movement is possible for all members of the group. It is therefore a distributed leadership. In addition, we showed that the initiation process was not temporarily organised as the identity of the fish that led a departure does not provide information on the identity of the fish leading the next one. Our results also show that sharing within the group varies between groups. In some groups, leadership is distributed equally, with each individual having initiated the departures a number of times similar to the other members of the group.

In other cases, one or two individuals will lead them most of the time. If groups of two individuals mainly show the two extremes, the larger the group size, the more groups with a heterogeneous leadership distribution will appear, while limiting the most extreme cases. We also highlighted that the heterogeneous distribution of leadership was not the result of a higher success rate of some individuals that could have a higher tendency to be followed. On the contrary, the number of successful departures initiated by the fish was linearly correlated to their number of attempts. This is consistent with what has been observed in Damselfish *Dascyllus aruanus* [252], where the individuals making the most attempts are the most followed. For groups of five, seven and ten fish, the individual who has initiated the most collective departure is often the most mobile, which corresponds to theoretical prediction [261] and observation made in other species [186, 252]. This situation highlights personality differences within the zebrafish.

8.1.2 Model of the collective behaviour of zebrafish in a constrained environment

In Chapter 3, we established a multi-level, stochastic and microscopic model of zebrafish behaviour in a constrained environment, a two rooms maze. The observations in Part I show that fish exhibit different behaviours in a constrained environment depending on their spatial positions. To correctly model these specificities, we have described global behavioural rules, similar to the previous models [41, 49], and for which the parameter values vary according to three spatial zones defined by the experimental observations. The model is automatically calibrated by a multi-objective evolutionary algorithm that compares the simulations with the experimental data. This model is able to simulate different behaviours depending on the spatial context. It also reproduces the fission and fusion dynamics of the group and the transitions between the two rooms of the simulated set-up, as observed in zebrafish [48, 211].

8.1.3 Integration of robotic fish into zebrafish groups

In Chapter 4, we integrated a biomimetic and autonomous robot into a group of fish. We have sought to better understand the key points that allow a fish to recognise a conspecific. We worked on the visual aspect, via a biomimetic lure, but also on the behavioural aspect. Previous studies [138, 139, 240] had highlighted the need for a robot to be biomimetic in order to successfully integrate a group of fish with a focus on the visual aspect but without taking into account the impact of the robot behaviour on social integration. The robot movements are based on a low level control imitating the swimming of fish from scripted accelerations and decelerations, and a high level control reproducing the behaviour of the fish through a mathematical model previously developed [49] and adapted to this study. We have shown that the visual and behavioural aspects both play a role in the integration of the robot inside zebrafish groups.

To improve the integration of the robot, we used, in Chapter 5, the biomimetic models designed and calibrated in Chapter 3 to drive the behaviour of the robot. This creates a mixed-group of zebrafish and robot because we demonstrate quantitatively that: (i) the robot responds to the animals with closed-loop interactions; and (ii) the robot is effectively socially integrated into the group of zebrafish. This new approach provided a better representation of behavioural variability and allowed the robot to be socially integrated with the group of fish.

Finally, in Chapter 6, we extended this approach further. Instead of calibrating the model driving the robot using a set of neutral experiments with only fish, we have decided in this chapter to calibrate the model in real time during robot-animal experiments. Thanks to this method, which nevertheless requires substantial computer resources, we were able to improve the integration of the robot by taking into account the behavioural variations existing between groups of fish of the same species and age. This method can significantly reduce the number of experimental trials necessary to calibrate the model.

8.1.4 Modulating fish collective dynamics with robots

In Chapter 7, we succeeded in modulating the behaviour of the zebrafish by using the previously integrated robot and those autonomously and over a long period of time. We have tested several strategies in this chapter. First, by using the work developed in Chapter 2, we used the robot as a leader. The objective was to demonstrate the theory that an individual, without possessing a specific trait, but with bold behaviour, could be a leader more often than other members of the group. Using the same behavioural model as in Chapter 4, we have added a behaviour rule that forces the robot to leave the room with the group as much as possible. Under these conditions, the robot was able to lead more often than the majority of fish, but also to obtain an attempt and initiation success ratio similar to fish.

We also modulated the presence of fish between the rooms. In Chapter 1, we showed that the fish used both rooms in the same way. Here, we tried to modify the time spent in each room, in a similar way to what was done on cockroaches [97], by using two different strategies, with one or two robots. We first used an initiation strategy to drive the group in one specific room. However, if the robot has demonstrated its ability to lead the group, in this configuration it seemed unable to follow fish and it was often found out of sync with the group. As a result, this strategy has not been successful in modulating the fish group density.

The second strategy was to use the robot as an attractor. In this experiment, the robot moves autonomously following the model, but is limited in its movements to a single chamber. It imitates the behaviour of a fish that extremely prefers one room over another and does not lead. In this configuration, the robot was able to significantly

modulate the residence time between the two rooms. When using two robots with the same strategy, the space modulation was even stronger with less variability between the replicas. Finally, we applied both strategies at the same time by using two robots, one remaining in the target chamber, and the other initiating collective departures to the same chamber. We see that the bias in favour of the target is lower than in the attraction strategy with two robots.

8.2 Perspectives and concluding remarks

The studies discussed in this manuscript have explored group dynamics in structured environment. They provided a better understanding of the influence of group sizes on collective movement, transition phenomena and decision-making. They have also expanded our understanding of leadership in cohesive groups such as the zebrafish. Part of my work has focused on the modelling of zebrafish collective movements in a constrained and structured environment, a notion rarely developed in similar modelling works, with a few exceptions with explicit formalisation of fish interactions with tank walls [38, 49]. This aspect of my work has been used for the integration of an autonomous biomimetic robot into a zebrafish group. It has allowed us to better understand the different aspects necessary for a fish to recognise and interact with one of their congeners. This research therefore focused on both the visual and behavioural aspects translated in this context by biomimetic lure and individual-agent model. Finally, thanks to robotics, we were able to explore in greater depth specific aspects of zebrafish behaviour, particularly leadership and decision-making.

While this work has relied on a methodology combining a variety of approaches - biological observation, modelling and robotics - the objective of this manuscript has remained to provide a better understanding of the collective behaviour of the zebrafish, and on a broader scale of social fish.

However, my research leaves much room for improvement. The results of this research focus on a social fish species, the zebrafish, and it would be interesting to obtain a greater transversality on the issues addressed by studying in a similar way other social species, including more cohesive species such as *Hemigrammus rhodostomus*. Studies in more complex environments could highlight more accurate decision-making and exploratory behaviours like Y-maze [253], T-maze [124] or Plus-maze [155, 220]. Finally, it would be interesting to study in more detail the personality of individuals within the group and their impacts on the dynamics of collective movement.

The model developed here makes it possible to solve in a simple way the problem of simulating animal behaviour in complex environments often used in ethology. Maintaining identical behavioural rules, but modulating the parameters according to zones, allows to quickly produce models adapted to a variety of situations. Nevertheless, a number

of questions could be explored to obtain a better understanding of the behaviour of the zebrafish. For example, linear velocity has been simplified in this study by randomly picking the instantaneous speed in a distribution from the biological data, without taking into account the previous velocities (if the fish accelerates) or the angular speed (if the fish is rotating). Being able to simulate burst-and-coast swimming movements, as it has been done in Calovi *et al.* 2018 [38], could make our model more accurate while providing information on the influence of swimming on individual decision making [86]. Similarly, it would be interesting to observe whether adding heterogeneity in the model parameters between the simulated agents provides information on the fish personality.

My work is therefore part of the current methodological approach [39, 86, 237, 256] with a first step of biological experimentation with a precise recording of observations and an analysis of data to identify behavioural rules. This is followed by a formulation of these rules in the form of a mathematical model. A comparison of the model simulations against the biological data makes it possible to discuss the relevance of the behavioural rules. However, the use of robots makes it possible to add a new step to this approach. There is no doubt that they are an innovative tool in ethology. The development of robotics has enabled the scientists to use increasingly sophisticated tools to study animal behaviour. My work is the result of this trend and proposes significant advances in the field. By comparing the mathematical model directly with biological agents, the step of integrating the robot into the group of fish makes it possible to go further than the simulations to validate or not the behavioural rule hypotheses. But the last part of my work allows me to go even further as the modulation of social animals involves the replication of specific decision-making mechanisms with the robot. The success of the modulation therefore validates our understanding of these mechanisms *a posteriori*. In my work, I automatically modulate the behaviours of a group of zebrafish over long periods of time, validating our knowledge of the social behaviour of this fish. My work is therefore a new step in the use of robots in ethology and paves the way for more studies of this kind that could provide a better understanding of other mechanisms, such as collective exploration.

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