Robots mediating interactions between animals for interspecies collective behaviors

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Self-organized collective behavior has been analyzed in diverse types of gregarious animals. Such collective intelligence emerges from the synergy between individuals, which behave at their own time and spatial scales and without global rules. Recently robots have been developed to collaborate with animal groups in the pursuit of better understanding their decision-making processes. These bio-hybrid systems make cooperative relationships between artificial systems and animals possible, which can yield new capabilities in the resulting mixed group. However, robots are currently tailor-made to successfully engage with one animal species at a time. This limits the possibilities to introduce dis-

tinct species-dependent perceptual capabilities and types of behaviors in the same system. Here we show that robots socially integrated into animal groups of honeybees and zebrafish, each one located in a different city, allow these two species to interact. This interspecific information transfer is demonstrated by collective decisions that emerge between the two autonomous robotic systems and the two animal groups. This novel bio-hybrid system works at any distance thanks to the robots and operates in water and air with multiple sensorimotor properties across species barriers and ecosystems. These results demonstrate the feasibility of generating and controlling behavioral patterns in bio-hybrid groups of multiple species. Such interspecies connections between diverse robotic systems and animal species open the door for new forms of artificial collective intelligence, where the unrivalled perceptual capabilities of the animals and their brains can be used to enhance autonomous decision-making, which could find applications in selective "rewiring" of ecosystems.

Introduction

Robotics has become a valuable tool in the study of animal behavior. For instance, robots have been introduced into groups of animals by building them to emit certain cues that are used by the animals within individual-level interactions. These robots can be simple observers (*1*), or sample the biodiversity (*2*). Moreover, they can act on certain animal behaviors, to modulate, for instance, the movements of cattle (*3*), factors involved in mate choice (*4*) or simulate predator-prey interactions (*5*). Several studies have involved bio-hybrid systems composed of groups of robots and animals, where the artificial devices were working in a closed-loop according to the change of behavior of the animals and thus autonomously interacted with them (*6–16*). This approach is possible when the designed robots are capable of socially integrating into groups of animals by mimicking some of the signals used during social interactions (*6*). These closed robot–animal interaction loops can provide access to richer dynamics as compared to pre-programmed or human-operated robot approaches, and can be used to test hypotheses about the way animals interact and how self-organized collective behavior can emerge from these local interactions (*17–19*) such as identifying behaviors in which a small fraction of agents can change group-level behaviors (*6*). These systems also represent a novel kind of bio-hybrid information and communication technologies (ICT) system, as the animals can enrich the capabilities of the machines, and vice versa (*20*). However, until now, studies involving bio-hybrid systems only focused on the interaction between one particular group of animal species and one particular group of specifically-designed robotic systems. The coupling of several bio-hybrid systems that would allow the study of how collective decision-making can arise at a larger scale, among multiple individuals of different species, with their own sensing and acting properties, has not yet been explored to the best of our knowledge.

Honeybees (*Apis mellifera* L.) and zebrafish (*Danio rerio* L.) are two animals of great interest for the scientific community. Honeybee colonies as superorganisms show various self-organized collective behaviors driven by feedbacks that amplify and stabilize the actions leading to optimal collective decisions at the colony level (e.g., nest site selection or forager recruitment) that are crucial for the survival of the colony (*21, 22*).

The zebrafish is a model organism in genetics and neurophysiology (*23, 24*), thus its behavior is well studied. In addition, the zebrafish is a social species forming shoals both in nature and in laboratory conditions (*25*). It is a common animal model for studying the link between individual variability and collective behaviors (*26*). Even though the dynamics of interactions differ between honeybees and zebrafish, as well as many other factors such as their environments and the mechanisms used in local interactions, both honeybees and zebrafish exhibit decision-making at the collective level, opening the possibility to facilitate some form of indirect exchange of information between groups of the two animals.

In this article, we introduce an innovative methodology to create interaction links at a collective level between animal species. In particular, we showcase this by selecting two animal species that would not directly interact in nature, i.e. zebrafish and honeybees. By exploiting selected mechanisms in gregarious animals, robotic agents were designed to integrate themselves into these two animal groups, and to participate in the self-organized collective choices, in binary choice systems (*6*). Using these two bio-hybrid systems, we established a long-distance communication channel between two types of robotic agents in order to create an interaction link between two animal groups of different species and translate the information between the two bio-hybrid systems. These robotic agents allowed these two bio-hybrid systems to share their collective decision-making, which enabled the emergence of a global consensus. We observed how the collective decision from one species can be transferred to another via information exchanged between the robots. Here, we make the first steps to producing new bio-hybrid systems that can make use of multiple sensorimotor properties across species barriers and ecosystems enhanced by different type or robotic systems.

Results

The robotic design approach we followed for animal-robot interaction was based on the methodology to create mixed groups of robots and animals in order to study self-organized collective behaviors (*20*). First, the robot presence is accepted by the animal group in a way that the usual animal behavior is not modified; second, the active behavior of the robots can modulate the animal behavior in a parsimonious way. This means that the robotic agents have to be designed to be socially accepted and capable of reaching consensus with the animal groups. Such bio-hybrid groups have similar properties to the animal ones but can also enhance collective behavior by sharing properties between the animals and the robots (*6*). Due to the fact that self-organized collective behaviours are frequently amplification phenomena, a small number of robotic agents can be enough to influence the whole group (*27–29*).

The system we developed is shown in Fig. 1. For each species, we created the simplest and smallest set of robotic agents that can either autonomously reproduce some of the signals used by animals during their social interactions or emit physical cues that are present in the animals' natural environment and to which the animals will react in a predictable way. On the side of honeybees, these robots produce heat (*12*), which is an attractive cue for young honeybees as they rely on a specific thermal environment in the brood nest area (*30*). On the fish side, a lure with the same size and shape ratio of zebrafish moves in the water by reproducing the same motion patterns (i.e., trajectories, speed and accelerations) as the fish (*31*). It has also already been demonstrated in (*12*) and (*15*) that the animals respond to the stimuli created by these two robotic devices the same way that they are responding to their conspecifics, and that the robotic agents and the animals can reach consensus (see Text S2). For sensing purposes, the bee-robots are equipped with proximity sensors to estimate the density of bees close to each robot. In the fish-robot system, a camera continuously films the aquarium from above, and a blob detector is used to determine the position of each agent (fish and robots). This sensory information can be used either to close the interaction loop between robots and animals (*12, 14, 15, 32*), or also to transmit information from one species to another. On both sides, there is a binary collective choice for the animal groups, which is a common setup for studying self-organization in animal groups (*33*). The honeybees have to choose around which of the two bee-robots they will aggregate. The fish are constrained inside a ring, and they have the choice to move in the clock-wise (CW) direction, or counter clock-wise (CCW) direction.

The experimental setups for fish and bees were located in two different cities. The fish setup was located at Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, and the bee setup was located at the University of Graz, Graz, Austria. Therefore, these experiments not only tested the possibility to connect two different animal species, but also to perform these complex tasks through a long-distance link, as the two universities are separated by a distance of approximately 680 km as the crow flies.

We performed three sets of experiments, each with different connectivity between the bio-hybrid systems (Fig. 2). Our main goal was to connect the two bio-hybrid systems in a closed loop, such that each animal group contributes to the overall system dynamics and collaborates with the other group, so that a consensus decision emerges from the system (Fig. 2 d). To examine the interactions in more detail we also tested two further settings, where only one bio-hybrid system has a closed-loop interaction and shares information with the other system (Fig. 2 b and c). As noted above, each bio-hybrid system is faced with a collective binary choice: left or right side of the arena for bees, and CW or CCW swimming direction for fish. In each bio-hybrid system the robots stimulate the animals and receive sensory information about the animals (see Text S1).

In experiment 1 (B $\rightarrow$ F) the bee bio-hybrid system exhibits a self-contained decision-making dynamics and also transmits signals to the fish bio-hybrid system, which operates in an 'open-loop' mode.

In experiment 2 (B $\leftarrow$ F) the fish bio-hybrid system governs the overall dynamics through coupling of the fish swimming direction to the fish-robot swimming direction, and transmitting this direction signal to the bee-robots that operate in open-loop mode.

In experiment 3 (B $\leftrightharpoons$ F) both bio-hybrid systems are connected in a single closed-loop: the sensed fish direction is used to determine the bee-robot temperatures, and the sensed bee densities are used to determine the fish-robot swimming direction. In this experiment, each animal species has to select from two identical choices with their respective robots, and the robots are sharing this information to allow the emergence of a global consensus in the system.

To provide a control condition, we produced a surrogate dataset that uses bee-side results and fish-side results where these sub-systems act without influence from the other ($\text{B} \not\leftrightarrows \text{F}$).

Fig. 3 a shows time-series for selected runs of each condition. For the case of $\text{B} \rightarrow \text{F}$, where the bees influence the collective decisions of fish, we can observe that the bees took $\sim$6 min to make a collective decision by aggregating more near the right bee-robot. When the right bee-robot started to detect that the majority of bees was located around it, this information was transmitted to the fish-robot which started to rotate more in the CW direction. After some time, we can observe a bias of the fish collective decisions that were influenced by the fish-robot rotating more in the CW direction (see Movie S1).

In the presented run of $\text{B} \leftarrow \text{F}$, it is possible to observe that the fish were continuously changing swimming direction, which results in the measure fluctuating around 50%. This behavior influenced the fish-robot to also switch swimming direction, and this information was transmitted to the bee system. For example, when the fish were mostly swimming in the CCW direction, the left bee-robot heated more than the right bee-robot which influenced the bees to aggregate more around the left bee-robot. Because of the way fish were continuously switching swimming direction, the side with bee majority also oscillated (see Movie S2).

Finally, we also show a run of $\text{B} \leftrightarrows \text{F}$. We can observe that in the first half of the experiment, the two species did not succeed in deciding. This is probably mainly due to the fish which were swimming in each direction around half of the time during this period. However, after 20 min, the two systems stabilized and the two animal groups made a collective decision together (see Movie S3). More broadly across the experiments, bees made strong decisions frequently in $\text{B} \rightarrow \text{F}$ as we have observed in prior work (*34*), the ability to reach and maintain aggregations was disrupted by the influence of the fish in $\text{B} \leftrightarrows \text{F}$ and even more so in $\text{B} \leftarrow \text{F}$ (see Text S5).

To evaluate the coordination between the animal species, we sampled the measurements of collective behavior at each second, and then averaged the results over each minute.

For each run we computed a collective decision coefficient Q_{BF} that is given by

$$Q_{BF} = 100 \times \sum_{t}^{T} \frac{|C_t^B - C_t^F|}{T}, \tag{1}$$

where C_t^B and C_t^F are the collective decisions of bees (bee density in one of the two halves of the arena) and fish (swimming direction, CW or CCW) respectively, at a given time t, and T the total duration of the experiment.

Fig. 3 b shows the distribution of collective decision coefficient Q_{BF} for all experiments. We find that the four distributions differ statistically (Kruskal-Wallis $p < 0.05$), and a post-hoc analysis using the Tukey's honest significant difference criterion shows that the mean ranks of the distribution of conditions B $\rightarrow$ F, B $\leftarrow$ F and B $\leftrightharpoons$ F significantly differ from condition B $\not\leftrightharpoons$ F, while conditions B $\rightarrow$ F, B $\leftarrow$ F and B $\leftrightharpoons$ F have no significantly different distributions. It seems that B $\leftarrow$ F and B $\leftrightharpoons$ F obtained a higher score than B $\rightarrow$ F, due to the fact that the fish are less influenced by the fish-robot in B $\rightarrow$ F than the bees by the bee-robot in B $\leftarrow$ F and B $\leftrightharpoons$ F, as observed in (*12*) and (*14*). Overall, this shows that we succeeded in designing a system where two animals species are capable of collectively interacting through the robotic devices.

To investigate information exchange between the two animal groups, we measured the transfer entropy (*35*) in each direction (Fig. 4)(see Text S4). The transfer entropy measured between bees and fish indicates that the driving species in B $\rightarrow$ F and B $\leftarrow$ F transfer information to the other species; and there is no significant transfer in the reverse direction, as expected. Furthermore, in B $\leftrightharpoons$ F information is exchanged in both directions, although the exchange from bees to fish appears to be stronger. Conversely, no significant transfer was measured in the control condition B $\not\leftrightharpoons$ F where the two bio-hybrid systems were not connected electronically. A comparison of the distribution of local transfer entropy values between experimental conditions reveals a significant difference between the $TE_{B \Rightarrow F}$ for the cases where the bee–fish link is present (B $\rightarrow$ F and B $\leftrightharpoons$ F) and where it is not (B $\leftarrow$ F); but that there is no significant difference between the distributions for B $\rightarrow$ F and B $\leftrightarrows$ F (Mann-Whitney U test, p < 0.05 plus Bonferroni correction). Similarly for the $TE_{F \Rightarrow B}$ distributions, B $\rightarrow$ F differs from B $\leftarrow$ F and B $\leftrightarrows$ F, but B $\leftarrow$ F and B $\leftrightarrows$ F do not differ significantly (see Text S5 and Fig. S3).

The evidence from this analysis of transfer entropy shows that the actions of one species are informative in predicting the future states of the other species, when the robot–robot link is active. Note that this technique infers relationships from observational data, and thus can report significant relationships between pairs of variables that "appear to be causally linked" (*36*) where there is not a direct causal relationship, such as when a third, unobserved variable causes changes in both the observed variables. However, comparison between the various experimental conditions that utilize different robot–robot links supports the conclusion that the collective behavior of fish is the underlying cause of the transfer entropy between fish and bees.

Discussion

We developed an autonomous robotic system capable of coordinating the collective behavior of two animal species using socially integrated robots. The designed system involves a group of zebrafish and a group of honeybees located in two different cities connected through the Internet. This system is thus scalable, as any bio-hybrid system in any location at any distance with a data connection can remotely connect to such an interspecies network, thus enabling the behaviors of distant animals to be coordinated. Moreover, the systems that we have designed support a larger number of robotic agents to interact with either group of animals (*12, 14*). However, in this study we took a parsimonious approach (*20*) that strives to use the smallest number of robots and animals necessary to demonstrate the novel interspecies interactions.

Each experiment lasted 32 minutes, and showed that the two species could reach consensus for several minutes or longer periods, and further studies could be designed to test these effects over longer time scales.

We demonstrated the interaction between the two animal groups by analyzing the statistical coherence of the collective decisions of the two species. This coherence is quantified by the transfer entropy between them for three different scenarios: the bees influence the collective decisions of the fish; the fish influence the collective decisions of the bees; and mutual influence between the two species. We also showed that when the two bio-hybrid systems were not connected, no information could flow and the distribution of the collective decision difference was significantly different than for the three other conditions where the two systems were connected. It shows that, using the interspecies link that we have designed, animal groups that do not collectively interact in nature can reach a consensus.

The presented system shows that it is possible to mediate the interactions between animal species. Here, we performed these experiments in laboratories, but one can envisage being able to insert such artificial agents within the groups of animals in the wild. This approach could also be generalized to other living species, such as plants (*37*), fungi or even microorganisms, to allow systems to interact even at different scale. It would then be possible to, on the one hand, exploit the unrivaled sensory properties of the living systems, their behaviors and their ease to live in the wild, and, on the other hand, to influence their choices and to add physical properties like telecommunication and other capacities. This approach could also enable the study of information flow in ecosystems and natural phenomena such as cascade effects, or other effects that might responsible for the collapsing of ecosystems (*38*), and identify solutions to repair broken links in these ecosystems.

Various studies have been done on how information is transmitted within a group of animals of the same species (*36, 39, 40*). The system we developed allows the study of the information transfer between agents whose properties fundamentally differ (communication channel, movement dynamics), aiming towards a better understanding of how bio-hybrid and multi-species systems are capable of exchanging information. One can also envision future applications in which the robotic systems would be able to learn and to adapt their behavior to animal species. Indeed, elsewhere we have already begun to explore these possibilities in the two individual bio-hybrid systems (*41, 42*) based on methodology for continuous real-time adaptation of multi-level behavioural models by evolutionary algorithms. We envision robotic systems that can discover by themselves new properties of bio-hybrid artificial intelligence towards synthetic transitions (*43*) and organic computing devices (*44*), where robots could passively evolve among animals.

Materials and Methods

Animals

The experiments performed with honeybees (*Apis mellifera*) were conducted with freshly emerged bees, aged from 1 h to 24 h. Bees this age are not yet able to actively produce heat (*45*) nor able to fly. To provide bees of this specific age for the experiments brood combs with sealed pupae were removed from colonies and hatched in incubators at a relative humidity of 60% and a temperature of 35 °C. Every morning all bees that had hatched over night were brushed off the combs and kept in ventilated plastic boxes on heating plates set to 35 °C. The bees were provided with honey *ad libitum*. All bees were reintroduced into the hives at the end of the day. Each individual was only tested once in an experiment and individuals with visible damage, i.e., missing or mutilated extremities (legs, antennae, wings), or attached varroa mites were not used in experiments. The experiment with honeybees were performed in Graz, Austria, and, for such experimentation with non-vertebrates, no restrictions to experimentation apply and no specific ethical board approval of experiments is required.

On the zebrafish side, the experiments performed in this study were conducted under the authorization N°2778 delivered by the Department of Consumer and Veterinary Affairs of the Canton de Vaud (Switzerland) after approval by the state ethical board for animal experiments.

The fish were bred following the guidance of (*46*), and that was imposed by our state and institution.

For the experiments, we used 100 wild-type zebrafish *Danio rerio*, with short fins. These zebrafish were acquired from a pet shop (Qualipet, Crissier, Switzerland), and were stored in two 60 litre housing aquariums. The average total length of our zebrafish was ∼4 cm. The water temperature of the housing aquarium was 26 °C. The fish were fed twice a day with commercial food using a food distributor. The housing aquarium environment was enriched with plastic plants, cladophoras, gravel, rocks, and aquatic snails. Each individual was only tested once per day, but the same fish could have been tested for several runs that are presented in this study.

Robotic bee arena

The bee experimental setup is shown in Fig. 1, a2–e2. The bees were placed on a horizontal surface, covered by a sheet of pressed beeswax, which was placed above an acrylic glass (PMMA) floor. The floor had 6.0 cm holes cut for the thermal control surface of the robotic devices. 2.0 cm holes were cut in the wax for the robot heads to protrude, where the infra-red (IR) sensors to detect the bees are sited (Fig. 1 j) (*12*). A plexiglass stadium-shaped wall of length 16.5 cm, width 6.0 cm and height 5 cm was placed over two robots forming an 'arena' with 91 cm^2 for the bees to move within (Fig. 1 h). The robots were separated by 9.0 cm from centre to centre. The entire setup was in a darkened room without windows, at Karl-Franzens-University in Graz, Austria.

The robots are specifically designed to interact with juvenile honeybees, and can generate stimuli with several modalities that the bees are sensitive to, including heat and vibrations. Because the bee-robot is programmable by a computer and can carry out a complex series of actions automatically using sensors and actuators, we consider it as a robot, even though it is a stationary device. The robots are also equipped with IR and temperature sensors (*12*).

Beaglebone single-board computers manage the robots, including executing the user-level controller, which in turn depends on the low-level actuator regulation and sensor readings through an application programming interface (API). These simple computers provide data logging and communication with other robot controllers and the host PC, which was connected by internet to the fish bio-hybrid system PC host.

The arena was filmed using a Basler ac2040–25gmNIR camera that is sensitive to IR light. The camera was mounted at 123 cm above the arena, and at full resolution of 2040×2040 px captures a 100 cm×100 cm region. The relevant part of the arena was cropped from the entire frame for each experiment (Fig 1 h). The arena was surrounded by white sheets to provide a cleanly lit environment, and three IR LED devices (each device comprises 48 LEDs with peak wavelength at 940 nm and consumes 7.2 W) were placed above the setup to provide IR light for the camera.

Robotic fish arena

The fish experimental setup is presented in Fig. 1, a1–e1 (*47*). It consists of an aluminum structure that supports an aquarium of 100 cm×100 cm×25 cm made of glass. The bottom surface of the aquarium was covered with white teflon sheets to avoid the reflection of light on the glass and to have a smooth surface for the motion of the module moving inside the aquarium (Fig. 1 i). The tank was filled with water up to a level of 6 cm, and the temperature was set to 26 °C. The whole setup was confined behind white sheets to isolate the experiments from the rest of the room and to ensure consistent luminosity. Three 110 W fluorescent lamps were placed around the setup and oriented towards the white sheets to provide indirect daylight-level lighting of the tank.

Underneath the tank, a wheeled mobile robot moved on a fixed support. The robot transmitted 2D motion to a fish-lure module that was moving inside the tank using magnets, and was able to achieve the required speeds and accelerations to reproduce the fish displacements underwater (*31*). The wheeled mobile robot was continuously powered using brushes that acquire power from two conductive plates, and was controlled via a wireless Bluetooth link. The fish-lure is the part that is visible to the fish (Fig 1i), and with which the fish interact. The lure had dimensions close to those of the zebrafish and its tail beats when it is moving at a constant speed. We have shown in previous studies that this robot can be socially accepted and participate in the zebrafish collective behaviors (*14, 15*).

To constrain the zebrafish movements, an arena composed of an outer circular wall and an inner circular wall forming a circular corridor was placed inside the tank (Fig. 1 g). This is a common setup to study the collective behavior of fish (*14, 40, 48–50*), and offers a binary choice for the fish, as they can either move in a CW or a counter-clockwise CCW direction, which will be used to evaluate the effect of the robots on the collective decisions of the fish (*14*). The dimensions of the corridor were as follows: an external diameter of 58 cm, an internal diameter of 38 cm, and a corridor width of 10 cm.

Long-distance communication

To demonstrate the applicability and potential impact of the interspecies link, we purposefully chose two species that do not interact in nature (zebrafish in the water, honeybees on land). Thus the two species will never get in direct physical contact in their natural habitats. To stress this point even further, we separated them by a significant physical distance. This raised additional challenges, like time-delays and network trafic. Solving those challenges demonstrates the general applicability of our system. This also shows that the system is scalable, as we can connect two bio-hybrid systems that are located at “any distance”, as soon as they can be connected to the internet.

To enable the coordination and collective choice experiments across the two spatially separated species, an inter-species communication protocol was designed. The protocol is built on ZeroMQ (*51*) and Google's 'Protocol Buffers'[1] package, and supports sending aggregation information from the bee-robot to the fish-robot, as well as sending swimming direction information in the other direction, from the fish setup to the bee-robot controller. The message format is listed in Table 1.

Table 1: The messages exchanged in the interspecies communication protocol. The $<$ and $>$ symbols imply a variable value in the protocol.

Direction	Target	Sender name	Data format
Fish $\rightarrow$ Bees	Bee-robot ID	Fish-robot	fish-$<$direction$>$
Bees $\rightarrow$ Fish	Fish-robot	Bee-robot ID	bee-$<$ density$>$

Experimental procedure

The experiments with zebrafish took place at the École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, and the experiments with honeybees at the University of Graz, Graz, Austria, between July 21 and August 16, 2017.

Each interaction experiment with the recording of data lasted 32 minutes. We predetermined a fixed starting time for each experiment to be synchronized between Graz and Lausanne. For both bio-hybrid setups, we established an experiment procedure that was used for each experiment.

In total, we performed 25 runs of $\text{B} \rightarrow \text{F}$, 21 runs of $\text{B} \leftarrow \text{F}$, 26 runs of $\text{B} \leftrightharpoons \text{F}$, and 21 runs of $\text{B} \not\leftrightharpoons \text{F}$. However, several runs had to be removed from the analysis, either due to a problem during the experiment, such as a honeybee escaping from the arena, or a technical problem during the data acquisition, such as failure of the video recording or logging of robot data. Therefore, the analysis in this study took into account 22 runs of $\text{B} \rightarrow \text{F}$, 19 runs of $\text{B} \leftarrow \text{F}$, 23 runs of $\text{B} \leftrightharpoons \text{F}$, and 19 runs of $\text{B} \not\leftrightharpoons \text{F}$. The details concerning the replicates that had to be

[1] `https://developers.google.com/protocol-buffers/`

excluded can be found in the supplementary material, in the folder containing the experimental data.

On the bee side, we performed each experiment with groups of 12 honeybees in the stadium-shaped arena around two bee-robots as described above.

This group size was determined in preliminary experiments to best facilitate the density-dependent collective thermotaxis of the honeybees without leading to crowding effects (for density dependency also see (*30*)).

The ambient temperature of the experimentation room was 27°C during experiments. Air-conditioning was only used between experiments to regulate the room temperature, and to minimize disturbances during experiments. Before each experimental run the wax floor in the arena was replaced to remove any chemical cues for the bees. Both robots were initially set to a temperature of 28°C before starting the experiment. After starting the recording and initializing the robot controller, the IR-sensors were calibrated. As soon as the calibration phase was over, 12 randomly chosen bees were transferred into the arena. In contrast to the fish, no acclimatization time is needed and the experiment started synchronized with the fish side. After the experiment, the data was stored for analysis and the bees were removed from the arena.

On the fish side, we performed the experiments using groups composed of six agents: one fish-robot and five zebrafish. This was determined in preliminary experiments to obtain a strong observed effect of the robotic agent on the animals, as well as homogeneity of the overall mixed group (*14*).

The water in the experimental tank was maintained at the same temperature (26°C) and water quality as the water of the housing aquarium to minimize the effect of the water transition on the zebrafish. In the morning of an experiment session, zebrafish were selected at random from their housing aquarium and were maintained inside a transfer tank next to the experimental tank during the experiment. Then, five zebrafish were selected among the entire group with a hand net from the transfer tank and transferred into the experimental tank. We let them acclimatize for 10 minutes inside the experimental tank before starting an experiment, as we have observed that for the first five to ten minutes, the behavior of the zebrafish was not the same compared to the behavior during the rest of the experiment, probably due to the stress during transfer and acclimatization to the new environment (*14*). After each experiment, the fish were placed in a second transfer tank near the experimental setup, so that they could not be reused for further experiments during the same day. After the experiment session, all the fish were put back into their housing aquarium.

Data analysis

We quantified the bees over the course of each experiment in two primary ways: the bees' locations, and the rate of motion. The first is particularly challenging due to the level of occlusion and interference from robot IR sensors in the films recorded with an IR-sensitive camera. Accordingly, this was performed by hand, using a tool that was developed to provide guidance as to where the bees are located – its suggestions are then manually corrected where necessary. We annotated an average of 100 frames per experiment (112 in B $\rightarrow$ F, 107 in B $\leftarrow$ F, 96 in B $\leftrightharpoons$ F). Given how labour-intensive this task is, it is not feasible to label at a rate that makes motion estimates derivable from the same data. However, optical flow can be computed automatically and provides us an approximation of motion in the bee group, without the need for individual identities.

On the fish side, the high–resolution (1024$\times$1024 pixels) videos created were processed using the open-source software idTracker (*52*) to retrieve the individual trajectory of each agent separately. idTracker allows the reliable identification of six agents in a 32–min high–definition video. There were no false positives and no propagation of identification errors, and fish were identified correctly in 95% of the time-steps on average.

The agents' positions estimated using idTracker were used to compute multiple values such as individual direction of swimming, linear speed, inter-individual distances, and number of switches of direction.

The analysis described above produced a time series of group behavior for each species in each replicate (Fig. 3 a). We used these time series in further steps of analysis to better characterize the whole system. This includes transfer entropy measures from information theory, differences in collective choices, and correlations between the animals and robots, and between the animal species (see Texts S2, S3, S4 and S5).

Supplementary references: (*53*) (*54*) (*55*) (*56*) (*57*)

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materials availability: All data needed to evaluate the conclusions in the article are present in the paper or the Supplementary material.

Supplementary materials

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Text S2: Correlation between robotic agents and animals

Text S3: Optical flow as a measure of motion in the bee arena

Text S4: Application of transfer entropy analysis

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Movie S2: Example of an experiment of condition $\text{B} \leftarrow \text{F}$.

Movie S3: Example of an experiment of condition $\text{B} \leftrightharpoons \text{F}$.

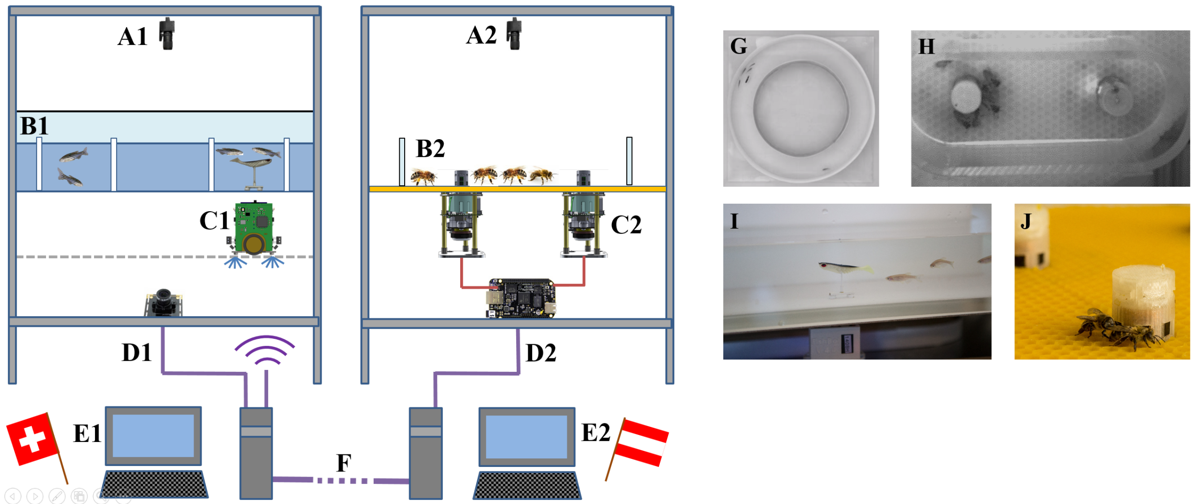

Figure 1: Automated setup for interspecies experiments composed of two animal species (zebrafish and honeybees) and two artificial devices (fish- and bee-robots). The two setups are composed of a metallic frame, with a camera (A1–2) to capture the arenas in high-definition. The fish arena (B1) includes a tank filled with water and a circular corridor (G). This space constrains the zebrafish and lure, presenting a binary choice: the mixed group can move either CW or CCW (G, I). Underneath the tank (C1), a wheeled mobile robot moves, which also moves its lure via magnetic coupling. The honeybees are contained within a silicon oil coated plexiglass arena (B2) with two bee-robots, forming a binary choice: the honeybees will decide to aggregate around one of these bee-robots (H). The "head" of each immobile robot incorporates 6 IR sensors (J). The main bodies are mounted below the arena floor (C2), and include a Peltier element to modulate the local temperature inside the arena. The two setups are interfaced (D1–2) with computers (E1–2) on which programs controlled the robots in a closed-loop. The fish setup (in Lausanne, Switzerland) was connected virtually (F) to the bee setup (in Graz, Austria).

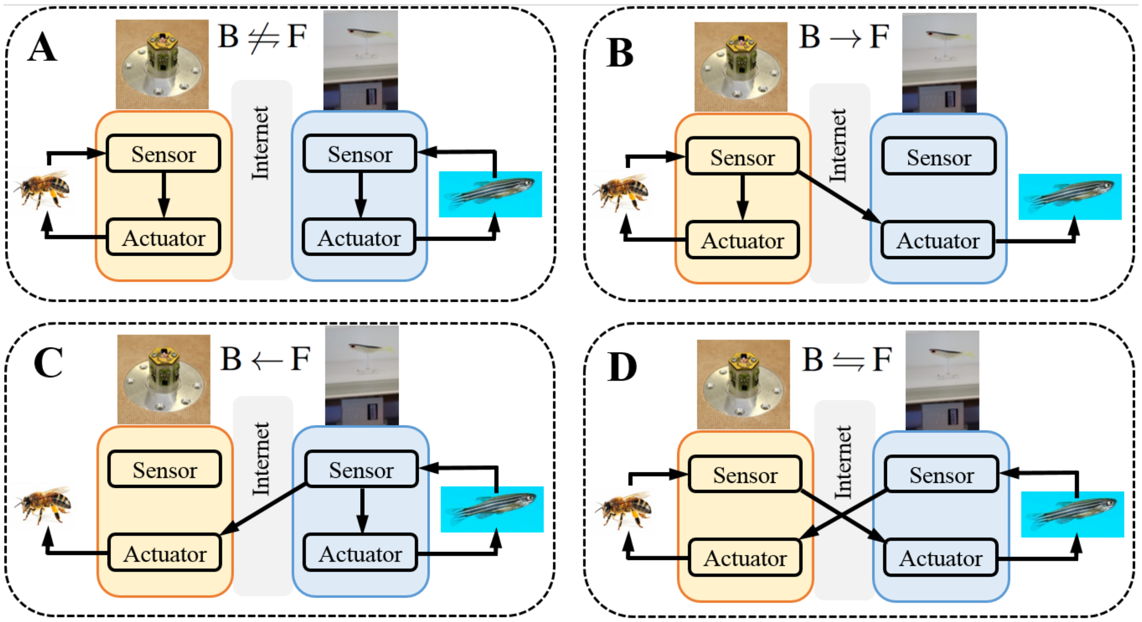

Figure 2: The four conditions implemented to test connectivity between bee and fish experimental setups. A) Control condition, where the robots interact with the animals in a closed-loop, but do not exchange information between the setups. B) Condition B $\rightarrow$ F where the fish-robot behavior is modulated according to what was sensed by the bee-robot, which is interacting in a closed-loop with the honeybees in a self-contained decision-making dynamics. C) Condition B $\leftarrow$ F where the bee-robot behavior is modulated according to what was sensed by the fish-robot, which is interacting in a closed-loop with the zebrafish in a self-contained decision-making dynamics. D) Condition B $\leftrightarrows$ F where the bee-robot behavior is modulated according to what was sensed by the fish-robot and the fish-robot behavior is modulated according to what was sensed by the bee-robots. It establishes a long distance closed-loop interaction between the two bio-hybrid systems which can share their collective decision-making, to allow the emergence of a global consensus

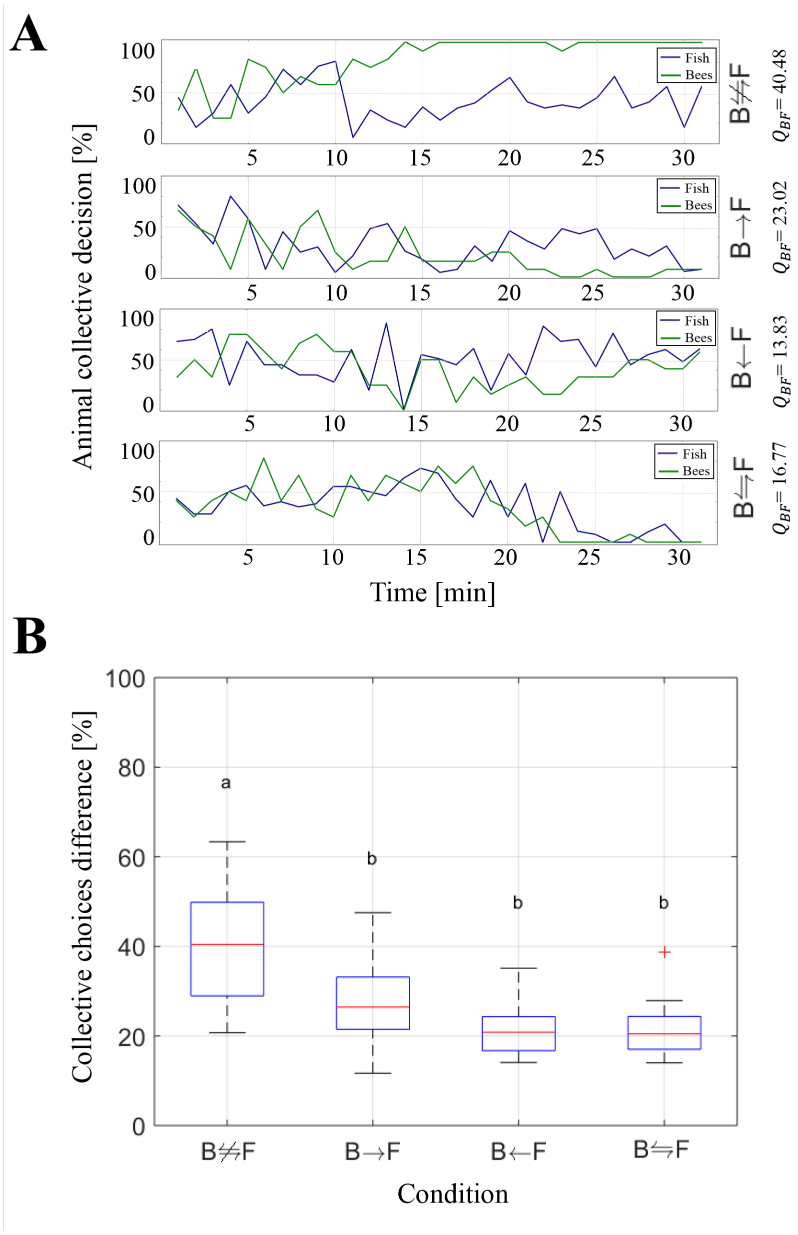

Figure 3: A) Time-series of collective decisions in each species (bees and fish) for selected runs of each condition. The fish choose their rotation direction (% CW), the bees choose their resting place (% right side). B) Collective decision difference coefficient Q_{BF} for all runs of each condition, reflecting how closely correlated the behavior is across the two animal species. With bi-directional link, B $\leftrightarrows$ F, the low Q_{BF} values indicate a highly coordinated system. The four distributions differ statistically (Kruskal-Wallis $p < 0.05$), and a post-hoc analysis using the Tukey's honest significant difference criterion shows that the mean ranks of the distribution of conditions B $\rightarrow$ F, B $\leftarrow$ F and B $\leftrightarrows$ F significantly differ from condition B $\not\leftrightarrows$ F, while conditions B $\rightarrow$ F, B $\leftarrow$ F and B $\leftrightarrows$ F have no significantly different distributions.

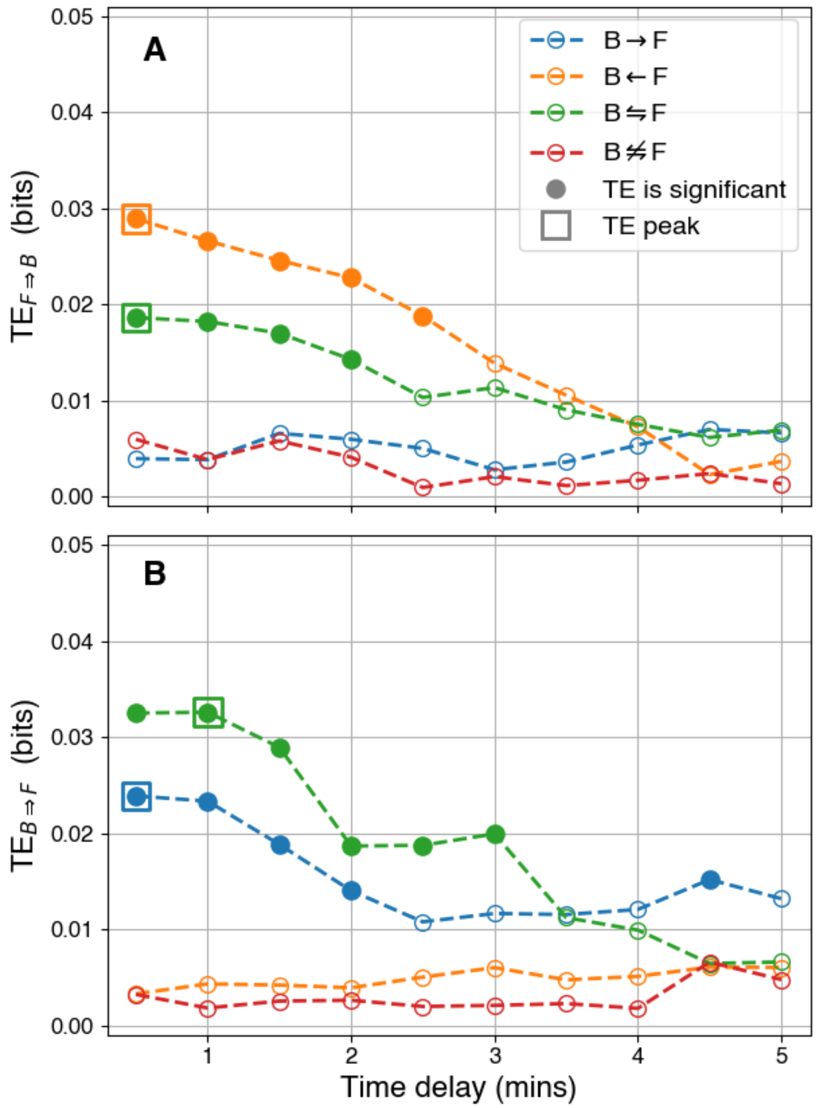

Figure 4: Transfer entropy (TE) is a directional information-theoretic measure that can indicate dependencies between actors in a complex system. We measured (A) $TE_{F\Rightarrow B}$, from fish to bees, and (B) $TE_{B\Rightarrow F}$, from bees to fish. When the two bio-hybrid systems are connected, we found substantial TE in at least one direction. The solid markers indicate a statistical significance (Mann-Whitney U test, p$<$ 0.05 plus Bonferroni correction). Specifically, in B $\rightarrow$ F we have only information flowing from bee-robots to fish-robots, and this is clearly reflected in high $TE_{B\Rightarrow F}$ only. Conversely in B $\leftarrow$ F we only have high $TE_{F\Rightarrow B}$. The condition B $\leftrightharpoons$ F shows significant bi-directional information exchange between the animals.

Supplementary Material

Robots mediating interactions between animals for interspecies collective behaviors

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This PDF file contains the following:

Other Supplementary Material for this manuscript includes the following:

Text S1: Robot controllers

Bee robot controller

The robot controller is designed to operate the same procedure for each of the experimental settings, and on both robots in each experiment, differing only in input and output parameteri-

sation (Tab. S1) to switch between experimental conditions (Fig. 2). The robots modulate the local environmental temperature T, in the range 28°C –36°C, by means of their Peltier devices. **The sensory information** is sampled and read into buffers and a moving average is computed on every update. The bee-robots have 6 IR proximity sensors that can detect the presence of nearby bees. At the start of each experiment the IR sensors are re-calibrated to the specific local conditions, by taking 50 measurements without any bees present. The threshold used to deem that a sensor was triggered was 1.1× the highest value observed, for each sensor on each robot independently. During runtime the sensors are read every 0.5 s and their values are recorded in a FIFO memory with 60 slots, so to facilitate computation of a moving average.
The actuator on each robot used in these experiments is the Peltier device, which modulates the temperature on the wax in the vicinity of the robot. The temperature is managed at two levels: an 'executive' target T_{tgt}, where the robot currently aims to reach, and a 'fine-level' temperature, which ensures that the Peltier is used within operational bounds. Specifically, the reference temperature T_{ref} of the Peltier is allowed to change by small amounts (maximum of of 0.15°C from the current temperature), and a new T_{ref} value can only be set when the previous T_{ref} has almost been reached, and at a shortest interval of 5 s.

Table S1: Parameters of interaction network

		B $\rightarrow$ F	B $\leftarrow$ F	B $\leftrightharpoons$ F
$w_{1,2}$	cross-link	-1.5	0	0
$w_{i,i}$	self-weight	$+1.5$	0	0
$w_{f \to b}$	fish input	0	1	1
$w_{b \to f}$	output to fish	(on)	(off)	(on)

The controller procedure executed by the bee-robots is as follows.
Initialisation:

1. Initialise memories M_{self} and $M_{j \neq self}$ each with m_b elements
2. Initialise memory M_{fish} with m_f elements
3. Define trigger thresholds for each sensor on each robot:
   (a) $s \leftarrow$ record 50 sensor readings for each sensor
   (b) $t_i = 1.1 \times \max \mathbf{s_i}$ for each sensor i

Every 0.5 sec:

1. Update own information:
   (a) read own sensors

i. $x \leftarrow$ read own sensors above trigger threshold ($x \in [0, 6], x \in \mathbb{Z}^+;$)
ii. $s_{self} \leftarrow$ compute instantaneous fraction of active sensors ($s \in [0, 1], s \in \mathbb{R}$)
iii. push s_{self} into FIFO memory M_{self}.

(b) process received messages from neighbouring bee-robots

i. save $\hat{s}_j$, the time-averaged signal, for each neighbour j.

(c) process received messages on neighbouring fish-robots

i. push s_j, an instantaneous signal, into FIFO M_j for each neighbour j.

(d) update moving averages, $\hat{s}$.

i. $\hat{s}_{self} \leftarrow \text{avg}(M_{self})$
ii. $\hat{s}_{fish} \leftarrow \text{avg}(M_{fish})$

2. Share time-averaged signal to information to others:

(a) emit $\hat{s}_{self}$ to all neighbouring robots (bee-robot and fish-robot)

3. Compute output temperature:

(a) compute temperature bonus b, as a weighted sum of the time-averaged inputs: $b_i = \sum_{j \in \mathcal{N}} w_{j,i} \hat{s}_j$, where i refers to the robot doing the computation, i.e., self.

(b) update target temperature $T_{tgt} = T_{min} + r \cdot b$, for the temperature range $r = 8$.

(c) fine temperature regulation limits the maximum rate of change and only updates the reference target T_{ref} once the previous one is nearly reached.

We set $m_f = 120$ and $m_b = 60$. The interaction parameters that differ between experimental conditions are defined in Tab. S1.

Fish-robot controller

The fish-robot controller is designed with the same principle as the one presented in (*14*).
The actuator The wheeled mobile robot was controlled using a Proportional-Integral-Derivative (PID) control for the rotational speed, while maintaining a constant linear speed of 8 cms^{-1}. The target positions that the robot had to reach were generated 10 degrees from the robot location in the direction of the movement (CW or CCW) by the control software. The speed commands of the wheels were sent to the robot via Bluetooth. This resulted in the robot rotating either CW or CCW. A Braitenberg-based obstacle avoidance mechanism (*53*) that retrieved the signals from the IR proximity sensors of the robot ensured that the robot avoided the walls. The robot thus mimicked the fish swimming behavior in the narrow corridor, with the robots swimming along the corridor walls of the circular corridor and with oscillations between the two walls.

Text S2: Correlation between robotic agents and animals

To assess whether the robotic agents were able to join the animal groups in their collective decision-making, we tested for each closed-loop system (bee vs bee-robot, fish vs fish-robot) whether the robots and living agents were capable of making the same decisions over the time and each experiment.

Results of linear regressions performed on these data are shown in Fig. S1. We observed that there is a significant correlation for both bio-hybrid systems, with coefficient of correlation of 0.81 for the fish and fish-robot, and 0.83 for the bees and bee-robots (p-values < 0.05 for both cases). It shows that the robotic agents are able to make the same collective decisions as the animal, when configured to follow it.

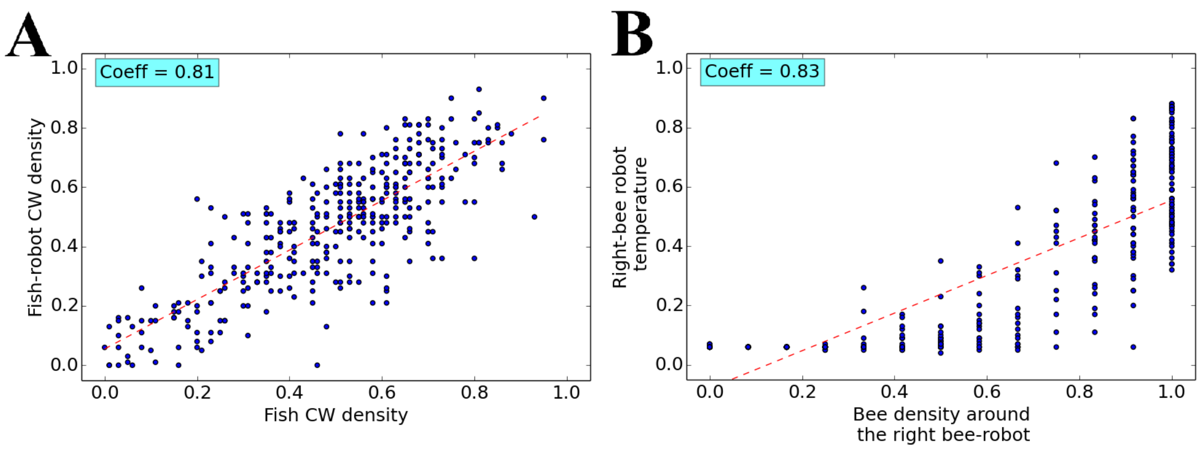

Figure S1: A) Correlation between the zebrafish and the fish-robot for condition B $\leftarrow$ F where the robot was controlled in a closed-loop according to the fish collective decision to move either CW or CCW. B) Correlation between the honeybees and the bee-robot for the condition B $\rightarrow$ F where the bee-robots were controlled in a closed-loop according to the bees decisions to aggregate around one of the two robot. Each data point represent a collective decision during a minute of an experiment. A linear regression was performed on the data, and we obtained for the fish and fish robot a correlation coefficient of 0.81 (p-value < 0.05) and for the bees and bees-robots a correlation coefficient of 0.83 (p-value < 0.05).

Text S3: Optical flow as a measure of motion in the bee arena

To obtain information on the movement of the bees over time, we computed optical flow in the videos of each experiment. Optical flow provides an approximation for the velocity that is perceptible in an image sequence, which can be used to describe the motion of groups of animals without the need for individual tracking. We based our approach on that described by

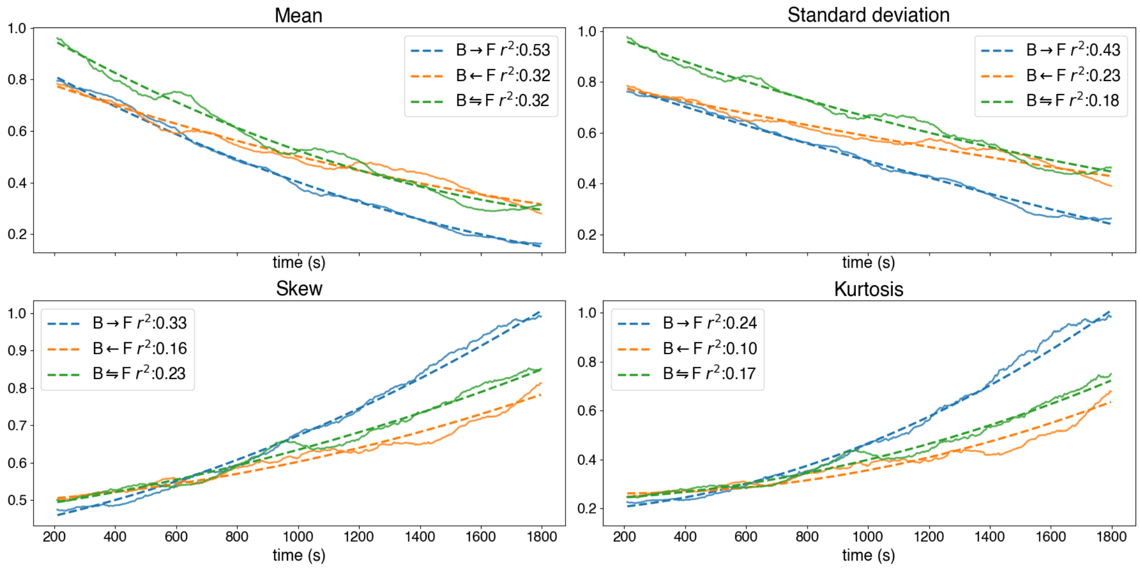

Figure S2: Time series of the moments of the optical flow distribution, extracted from films of the bee arena. Each graph is normalized to show the maximum value at 1. The solid line shows the mean of the distribution over all replicates in each condition, while the dashed line shows a quadratic OLS fit (with residual r^2 reported in the legends). For each moment, $\mathrm{B} \to \mathrm{F}$ and $\mathrm{B} \leftarrow \mathrm{F}$ show the most different dynamics, with $\mathrm{B} \leftrightharpoons \mathrm{F}$ appearing to be a mixture of these two extremes.

(*54*), in which flow vectors are calculated for each pixel using the Lucas-Kande method, and then the average magnitudes are computed for 8 x 8-pixel blocks. For each pair of video frames, we arrive at a motion distribution for the bee population as a whole. We then compute the first four moments of this distribution (mean, standard deviation, skewness, and kurtosis) over time. We followed this approach, pre-processing the frames to classify each pixel as a bee or not a bee, using a method similar to (*55*).

Figure S2 shows the time-series of optical flow distribution moments, averaged across all replicates in each condition. We used an interval of 25 frames (similar results were obtained with intervals in the range 10–100 frames). These data show a general trend of bee activity decreasing over time (decreasing mean and standard deviation), but also an increase in skewness and kurtosis. The latter changes indicate an increase in the deviation of distributions from a unimodal Gaussian distribution, and can be understood as follows: if bees tend to move at approximately the same speed while walking and approximately zero while sitting, we will likely observe two modes. When most bees are walking the distribution will be dominated by the faster mode; while when the majority of bees are sitting the distribution will be dominated by the slower mode. However, one or two exploratory bees will boost the second mode, causing high skewness and kurtosis.

Considering the differences between the conditions, we see that the average motion of bees decreases the most in $\mathrm{B} \to \mathrm{F}$; the rate of decrease is the smallest in $\mathrm{B} \leftarrow \mathrm{F}$. The distribution becomes most skewed in B $\rightarrow$ F, and least in B $\leftarrow$ F. The skewness increases when most bees have settled but there are a small number still exploring. Overall, we see that the bee population movement is influenced significantly by the information from the fish population, since the B $\leftarrow$ F time-series differ the most from the other conditions B $\rightarrow$ F and B $\leftrightharpoons$ F.

Text S4: Application of transfer entropy analysis

Transfer entropy (TE) from Y to X is defined by (*35*) as

$$TE_{Y \Rightarrow X} = \sum p(x_{n+1}, x_n^k, y_n^l) \cdot \log \frac{p(x_{n+1}|x_n^k, y_n^l)}{p(x_{n+1}|x_n^k)}, \tag{S1}$$

where $x_n^{(k)}$ is an embedding with k elements, $\{x_{n-k+1}, x_{n-k+2}, \ldots, x_n\}$. It was originally described for discrete variables, but via estimators we can also apply the same measures to continuous data (*56*).

Following the commonly used method for detection of lags (see e.g. (*40*,*56*)), we computed the transfer entropy for a variety of lags ($\in \{1, 11\}$ samples). We applied these measures to the ensemble of all data for each condition, sampled at 30 s, and a Gaussian estimator with $k = l = 5, \tau_k = \tau_l = 1$, using the JIDT package (*57*). While there is not a sharp peak it is clear that the shorter lags maximize the transfer entropy (see Fig. 4). Beyond testing each transfer entropy measure for significant difference to zero (as shown with the filled markers in Fig. 4), we also compared the distributions of pointwise TE values for each of the conditions B $\rightarrow$ F, B $\leftarrow$ F and B $\leftrightharpoons$ F. Additional statistical tests use the Mann-Whitney U test, comparing the distribution of pointwise TE measurements for the different conditions B $\rightarrow$ F, B $\leftarrow$ F and B $\leftrightharpoons$ F (Fig. S3).

Text S5: Modulation of animal collective behavior

On the fish side, the zebrafish dynamics are rather volatile (Fig. 3 a), and in experiment B $\leftarrow$ F in particular, where the fish dynamics govern the robots in both subsystems, this has a significant effect on the rate of decision-making in the bee population (see below). In the reverse direction (B $\rightarrow$ F), the fish dynamics are not significantly stabilized by the influence of the bees over 32 minutes of experiments; this is consistent with our previous results showing that the fish-robot is able to modulate the fish behavior significantly, but this effect is not constant over the entire experiments due to the volatility of the fish (*14*).

The three different configurations led to different rates of group-level behavior in the bee population, in particular in the rate of collective decision-making. We computed the Collective Decision Index (CDI) as a measure of how much time a bee group spent in a strong aggregation on one side of the arena or the other (Eqn. S3).

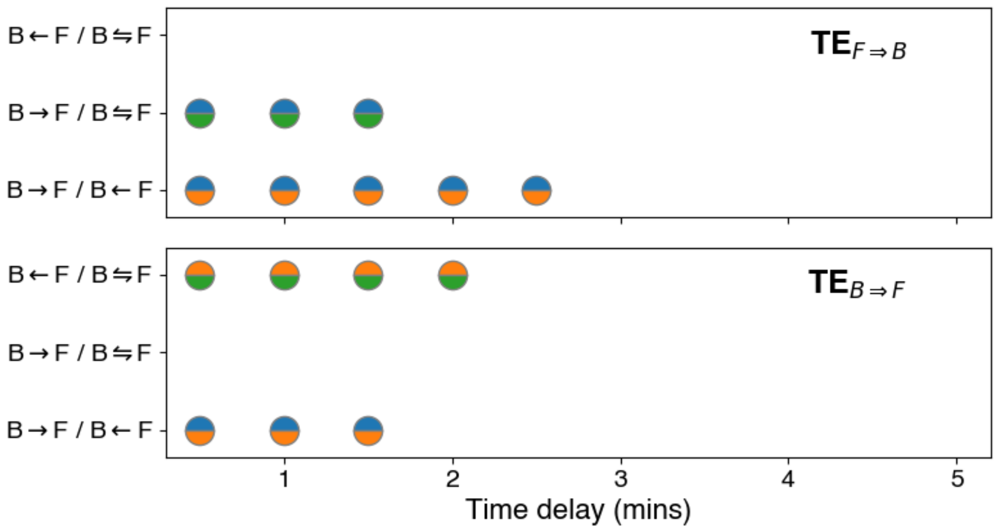

Figure S3: Statistical comparison of the transfer entropy distributions, using a Mann-Whitney U test. When the measured distributions are significantly different, a marker is placed (with $\alpha < 0.05$ plus a continuity correction). The markers use the same pair of colors as for the different experiments in Fig. 4. We can observe that where the robot–robot links are present, the distributions significantly differ from conditions where the links are absent.

We find the minimum number of bees d that must be together to be considered as a collective decision from the binomial distribution, following the methodology of (*6*), given n bees and confidence value p. For $n = 12, p < 0.05$ at least $d = 10$ bees must be together. Given $R(t)$ of the n bees are in the right half of the arena at timestep t, and a threshold of d bees, the binary value $c(t)$ indicates whether the population has significantly decided or not in that timestep,

$$c(t) = \begin{cases} 1 & \text{if } \max\left(R(t), 1 - R(t)\right) \geq d, \\ 0 & \text{otherwise.} \end{cases} \tag{S2}$$

The mean value of $c(t)$ over some interval $[t_1, t_2]$ gives a fractional time budget,

$$CDI = \frac{1}{t_2 - t_1 + 1} \sum_{t_1}^{t_2} c(t), \tag{S3}$$

where CDI lies in the range $[0, 1]$. For comparison here we consider a group of bees to have made a strong collective decision when CDI$\,>0.5$, measured over last 10 mins.

Over all runs of B $\rightarrow$ F, bees make a strong decision in 15/22 (68%) of the runs. Of these, 10 are to the right and 5 to the left (unbiased, two-sided Binomial test, $p = 0.30$). This dynamic is typical of our bee bio-hybrid system (*34*). Conversely, the volatility in fish swimming behavior caused the bees difficulties in reaching a collective decision, with only 1/19 runs of B $\leftarrow$ F reaching a strong decision (CDI > 0.5). The fully closed-loop system of $\text{B} \leftrightarrows \text{F}$ led to strong decisions in the bee group for 8/23 (35%) runs (6 R and 2 L, unbiased, two-sided Binomial test, $p = 0.34$).

These results are consistent with those shown in Text S3, but note that there is a difference in what is revealed: if the majority of bees had stopped together, and thus made a collective decision, the mean optical flow observed would be reduced. However, if the bees had split into two groups, also stopped, the optical flow would have reduced but the CDI would not be increased. Accordingly, both views confer valuable information regarding the bee group-level behavior.

Text S6: Links to the software

All the code that runs in the system is available online.

The high-level control software, which includes the multi-agent tracking and behavioral control of the fish-robot can be found in `https://github.com/assisi/CATS2`.

The firmware of the Fish-robot can be found in `https://zenodo.org/record/1467084`.

On the Bee side, the python API that is used to conduct experiments with the bio-hybrid system composed of bees and bee robots can be found in `https://zenodo.org/record/1320079`.

The controllers executed on the bee-robots, as described in Text S1, are written using this general API, and can be found in `https://github.com/assisi/coord-interspecies`.