UNIVERSIDADE DE LISBOA
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Behavioural dynamics and decision-making of cognitively-developed invertebrates (cephalopods) in social contexts

" Documento Definitivo"

Doutoramento em Biologia
Especialidade de Etologia

Eduardo André Silva Sampaio

Tese orientada por:
Professor Doutor Rui Afonso Bairrão da Rosa
Doutor Simon Gingins

Documento especialmente elaborado para a obtenção do grau de doutor

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“The answers you get depend upon the questions you ask”

Thomas Kuhn

Contents

Part I

Part II

List of Abbreviations

T1-O	Treatment 1 Observer
T1-D	Treatment 1 Demonstrator
T2-O	Treatment 2 Observer
T2-D	Treatment 2 Demonstrator
T3-O	Treatment 3 Observer
T3-D	Treatment 3 Demonstrator
T4-O	Treatment 4 Observer
T4-D	Treatment 4 Demonstrator
T5-O	Treatment 5 Observer
T5-D	Treatment 5 Demonstrator
BTG	Black tip grouper
GG	Green Goatfish
LGT	Lyretail grouper
OCT	Octopus
YG	Yellowsaddle goatfish
YGJ	Yellowsaddle goatfish juvenile
HSD	Honest significant differences

List of Figures

also characterized by a mostly white body but with a blue line across the mantle intersected by smaller blue lines, and darker tones of brown in the arms as well as in the tip and across the mantle.

Figure 3.1. Time-to-event analyses on the probability of attacking the stimulus (i.e. not reaching learning criterion) across trials, for **A.** inhibition by social learning test (T2); and for **B.** all different treatments (excluding T5). T2 demonstrators (T2-D) and observers (T2-O), T3 pre-exposed to stimulus (T3-P), and both subject conditions (observers and demonstrators) of T1 and T4, which are pooled per treatment since probability of attacking was the same (i.e. zero). Table of numbers depicts sample progression across number of trials.

Figure 3.2. Mean number of attacks per trial (model and data points) by different treatment subjects: T2 demonstrators (T2-D) and observers (T2-O), and T3 pre-exposed to stimulus (T3-P).

Figure 3.3. Mean latency time (seconds) to attack the stimulus by demonstrators (D) and observers (O), in **A.** the inhibition by social learning test (T2-D and T2-O), and **B.** in the positive control test (T5-D and T5-O).

Figure 4.1. Zoomed in snapshots taken from Video S1 (Event 1). **A.** *O. cyanea* (red/brown) lunges towards an *H. atra* individual, **B.** sitting on top and grabbing it with the arms and suckers. Afterwards, **C.** the octopus rolls/pulls the sea cucumber towards itself, **D.** ultimately placing it as a den cover.

Figure 4.2. Zoomed in snapshots taken from Video S1 (Event 2). **A.** *O. cyanea* lifts up the *H. atra* individual, **B.** proceeding to use it both as den cover and vantage point to observe the environment.

Figure 5.1. Example of a multi-specific hunting group composed by a day octopus *Octopus cyanea*, a yellow-saddle goatfish *Parupeneus cyclostomus*, a smooth cornetfish *Fistularia commersonii*, and a blacktip grouper *Epinephelus fasciatus* (Video S1).

Figure 5.2. Image sequence depicting the behavioural action of *O. cyanea* “punching” (white arrows) a yellow saddle goatfish (*P. cyclostomus*) partner during interspecific multi-collaborative hunting (see Video S3).

difference between the two scores is (i.e. wider arrows indicate larger differences). Lastly, brighter colors indicate a higher weighted degree centrality (i.e. the sum of all arrows' wideness around a specific node, irrespective of direction) and darker colors indicate lower weighted degree centrality.

Figure 6.7. Directed social networks based on pairwise anchor interactions per minute, posteriorly ordered in the y-axis by the continuous value dictated by the score index in Supplemental Figure 9. Below, x-axis is ordered as the rank-transformed value of the same index. Direction of arrows indicates which species has the highest positive score in the sum of pairwise interactions, while arrow (i.e. edge) wideness indicates how large the difference between the two scores is (i.e. wider arrows indicate larger differences). Lastly, brighter colors indicate a higher weighted degree centrality (i.e. the sum of all arrows' wideness around a specific node, irrespective of direction) and darker colors indicate lower weighted degree centrality.

Figure 6.8. Directed social networks based on pairwise pull interactions per minute, posteriorly ordered in the y-axis by the continuous value dictated by the score index in Supplemental Figure 10. Below, the x-axis is ordered as the rank-transformed value of the same index. Direction of arrows indicate which species has the highest positive score in the sum of pairwise interactions, while arrow (i.e. edge) wideness indicates how large the difference between the two scores is (i.e. wider arrows indicate larger differences). Lastly, brighter colors indicate a higher weighted degree centrality (i.e. the sum of all arrows' wideness around a specific node, irrespective of direction) and darker colors indicate lower weighted degree centrality.

Figure 7.1. Graphic design representing the unveiled social aspects of the lives of cephalopods in the framework of this dissertation: squid location probing and complex sexual dynamics (Chapter 2), cuttlefish social learning of predatory behavior modulation (Chapter 3), and octopus social tool use (Chapter 4) and collective hunting with fish (Chapter 5 and 6). Credit: Maria Rita Pegado.

Figure 7.2. A simplified schematic of the phylogenetic tree of life. The most common ancestor between cephalopods (as well as other invertebrates) and vertebrates dates to ~500 million years ago (red diamond). Adapted from Schnell et al. 2021 and Vitti 2013.

List of Tables

List of Papers

As author of the present dissertation, I hereby declare that I contributed for the conception of each chapter. I was responsible for rearing animals, designing and executing experimental setups, collecting and analyzing the data, and writing each manuscript. The present dissertation is composed of one book chapter in preparation (from which Chapter 1 is partly based), and five scientific papers from Chapter 2 to 6, of which three are published and one is submitted.

Chapter 1

Rosa R, Roura A, Amor M, Gleadall I, Guerra A, González A, Lopes VM, Pereira J, Pierce G, **Sampaio E**, Villanueva R. 2022. Common octopus, *Octopus vulgaris*. in Octopus Biology and Ecology. eds: Rosa R, Pierce G, Gleadall I, Villanueva R. Elsevier, Netherlands. (*in prep*)

Chapter 2

Sampaio E, Cheng SH, Rosa R. 2021. Location probing by males complicates sexual dynamics and successful mate-guarding in squid groups. *Ecology*. e03529. doi:10.1002/ecy.3529.

Chapter 3

Sampaio E, Ramos CS, Bernardino BLM, Bleuven M, Augustin ML, Moura E, Lopes VM, Rosa R. 2020. Neurally underdeveloped cuttlefish newborns exhibit social learning. *Animal Cognition*. 618, 388–398. doi: 10.1007/s10071-020-01411-1.

Chapter 4

Sampaio E, Rosa R. 2021. Evidence of social tool use by octopuses. *Biology Letters.* (*submitted*)

Chapter 5

Sampaio E, Seco M, Rosa R, Simon G. 2020. Octopuses punch fishes during interspecific collaborative hunting. *Ecology*. 102(3), e03266. doi: 10.1002/ecy.3266.

Chapter 6

Sampaio E, Sridhar V, Fritz F, Strandburg-Peshkin A, Nagy M, Nührenberg P, Rosa R, Couzin ID, Gingins S. 2022. Shared decision-making and hierarchical species dynamics in multispecific collective hunts between octopus and fishes. (*in prep*)

Abstract

The entirety of social interactions and contexts, both with related and unrelated individuals (including heterospecifics), functions as critical sources of selective pressure on an organism and are considered to be key in explaining the evolution of extant fauna. Nonetheless, there is a large gap of knowledge on how social information in the natural environment can shape decision-making processes and behavioral dynamics in cephalopods. This dissertation aimed to comprehensively fill this gap by studying what are classically defined as (semi-)social (squid and cuttlefish, *Part 1*) and asocial (octopuses, *Part II*) cephalopod groups. Within *Part I*, Chapter 2 reports an important, new courtship behavior that increases the options of sexual tactics during squid mating seasons and may represent paternal investment; and Chapter 3 unveils the existence of complex forms of social learning by observation in cuttlefish, through a multi-treatment experiment. In *Part II,* Chapter 4 details the first direct observations of social tool use in non-social animals, reporting octopuses using sea cucumbers as den covers. Moreover, by analyzing interspecific hunting groups of octopus and fish, in Chapter 5, the occurrence of partner-control mechanisms by the octopus are reported, and, in Chapter 6, it is shown that decision-making in these groups is a shared process, with fish deciding *where* the group goes and the octopus deciding *if* the group goes. This thesis ultimately demonstrates that all coleoid cephalopod groups (squid, cuttlefish, and octopuses) have complex conspecific/heterospecific social interactions, with the availability of social information markedly shaping decision-making towards maximizing individual fitness. Thus, I propose that sociality potentially had a key role in the evolution of cephalopod behavior and cognition.

Keywords: decision-making, sociality, evolution, cephalopods.

Resumo

A totalidade das interações sociais e contextos, tanto com indivíduos relacionados como não-relacionados (incluindo heteroespecíficos), funcionam como fontes críticas de pressão selectiva sobre um organismo e são considerados factor-chave para explicar a evolução da fauna existente. No entanto, há uma grande lacuna de conhecimento sobre como a informação social pode moldar os processos de tomada de decisão e a dinâmica comportamental dos cefalópodes. Esta dissertação teve como objetivo preencher esta lacuna de forma compreensiva, ao estudar o que são definidos como grupos de cefalópodes sociais (lulas e chocos, *Parte 1*) e asociais (polvos, *Parte II*). Assim, na *Parte I*, o Capítulo 2 relata um novo e importante comportamento de corte que aumenta as opções de táticas sexuais durante a época de acasalamento das lulas, podendo representar investimento paterno; e o Capítulo 3 revela a ocorrência de formas complexas de aprendizagem por observação em chocos, através de uma experiência multi-factorial. Na *Parte II*, o Capítulo 4 detalha as primeiras observações diretas de uso de ferramentas sociais em animais não-sociais, ao mostrar polvos a usar pepinos do mar como cobertura. Adicionalmente, ao analisar grupos de caça heterospecíficos de polvo e peixes, o Capítulo 5 descreve mecanismos de controlo de parceiros empregues pelo polvo, e o Capítulo 6 revela que a tomada de decisão nestes grupos é um processo partilhado, onde os peixes a decidim *onde* o grupo vai e o polvo decide *se* o grupo vai. Esta tese demonstra que todos os grupos de cefalópodes coleóides (lulas, chocos e polvos) têm interacções sociais coespecíficas/heteroespecíficas complexas, com as informações sociais a modelar tomadas de decisão para maximizar o *fitness* individual. Assim, propomos que a socialidade teve um papel-chave na evolução do comportamento e cognição dos cefalópodes.

Palavras-chave: tomada de decisões, socialidade, evolução, cefalópodes.

Resumo alargado

A teoria da seleção natural providencia uma estrutura téorica bem-sucedida para a seleção de várias características de um organismo, com o fim de construir um organismo inteiro bem-adaptado. Além das pressões exercidas pelo ambiente abiótico, a totalidade das interações e contextos sociais, tanto com indivíduos relacionados como não-relacionados, funcionam como fontes críticas de pressão selectiva sobre um organismo, sendo fundamentais para explicar a evolução da fauna extante. Assim, os organismos interagem com coespecíficos, mas também podem interagir com heteroespecíficos em contextos ecológicos mais complexos do que dinâmicas de predador-presa (por vezes referidas como interacções socio-ecológicas).

Partilhando o último ancestral comum com os vertebrados há cerca de 500 M atrás, os membros dos cefalópodes são modelos únicos para estudar as pressões selectivas subjacentes à tomada de decisões dos animais. Os dois principais caminhos propostos para a evolução da cognição são a Hipótese da Inteligência Social (geralmente baseada em interações sociais com coespecíficos relacionados) e a Hipótese da Inteligência Ecológica (geralmente baseada em desafios de predação ou dinâmicas predador-presa). No entanto, muito pouco é sabido sobre a vida social dos cefalópodes. Assim, o objetivo da presente tese foi compreender como a disponibilidade de informação social e interacções com outros indivíduos (coespecíficos e heteroespecíficos) pode moldar a tomada de decisão e a dinâmica comportamental dos cefalópodes. Aproveitando a vasta gama de fenótipos sociais dentro deste grupo animal, estudou-se ordens que geralmente são sociais (lulas e chocos, parte I) e asociais (polvos, parte II) de cefalópodes.

Na parte I, pretendeu-se investigar como é que a presença de conspecíficos pode moldar o comportamento de lulas e chocos. No Capítulo 2, demonstrou-se a existência de novas dinâmicas de corte e estratégias sexuais em grupos de lulas durante a época de acasalamento. Estas

descobertas mostraram que a competência no comportamento e na escolha de estratégia adequada durante o acasalamento podem ser ainda mais complexos do que se pensava anteriormente. O trabalho aumenta as hipotéticas estratégias sexuais em, pelo menos, um ramo de opções adicionais para cada um dos indivíduos envolvidos. Concomitantemente, pode ter sido fornecido a primeira evidência de cuidados paternais em cefalópodes.

De seguida, no Capítulo 3, revelou-se que chocos recém-nascidos aprendem a adaptar o seu comportamento de predação baseados na observação de coespecíficos. Esta aprendizagem ocorre através de processos complexos, baseados no entendimento das acções dos coespecíficos mas também no reconhecimento das propriedades do objecto. Nesta fase crítica da vida, esta capacidade de contornar o processo de tentativa e o erro para adquirir conhecimentos do meio ambiente pode significar evitar ações extremamente dispendiosas, conferindo uma vantagem evolutiva aos recém-nascidos que incorporam informações fornecidas por coespecíficos.

Na Parte II, foram avaliadas as interacções sociais heteroespecíficas entre polvos e outros indivíduos que normalmente co-ocorrem no mesmo habitat e domínios funcionais. Especificamente, no Capítulo 4, revelou-se que os polvos são capazes de manipular fisicamente heteroespecíficos, utilizando pepinos do mar como defesa e ponto de vista privilegiado. Este capítulo constitui a primeira evidência da utilização de ferramentas sociais numa espécie não-social, sugerindo que a complexidade do ambiente heteroespecífico pode ser suficiente para que os indivíduos aprendam a explorar as propriedades de outros indivíduos (ou 'objectos vivos').

Finalmente, nos Capítulos 5 e 6, explorou-se as dinâmicas e mecanismos subjacentes aos grupos de caça formados entre o polvo e várias espécies de peixes. Ficou patente que que os polvos seguem ativamente os peixes, mantêm grupos com os mesmos parceiros durante mais de uma hora, e especificamente discriminam entre fontes de informação social quando escolhem quem seguir.

Também desenvolveram mecanismos de controlo de parceiros sob a forma de socos para regular o comportamento dos parceiros. De realçar que o sistema de peixe-polvo não se enquadra num modelo de predador nuclear-seguidor, com o movimento de grupo a basear-se em tomadas de decisão partilhada, indicando uma natureza colaborativa. Como tal, este sistema cria uma intrincada rede de interações sociais onde cada indivíduo tem em conta a posição específica de múltiplos parceiros, características cinéticas e comportamento, para decidir sobre as suas próprias ações.

Desta tese podemos concluir que os cefalópodes são, de facto, animais sociais. Demonstrámos que as agregações de indivíduos podem motivar a flexibilidade comportamental e o desenvolvimento cognitivo, expandindo: a) a gama de estratégias comportamentais específicas individuais e táticas sexuais existentes, e b) potencialmente a natureza subjacente dos processos de seleção sexual em cefalópodes. Além disso, os cefalópodes que se agregam enquanto sub-adultos, são capazes de formas complexas de aprendizagem social que podem naturalmente ser maximizadas quando em grupos. Por último, mostramos que mesmo cefalópodes solitários em termos coespecíficos, podem ter interações com heteroespecíficos mais complexas do que simples dinâmicas predador-presa, implicando flexibilidade comportamental e discriminação/compreensão da informação social, esbatendo assim as linhas entre as Hipóteses de Inteligência Social e Ecológica. Conjuntamente com as interacções predador-presas englobadas pela Hipótese da Inteligência Ecológica, proponho que interações sociais complexas com outros indivíduos (conspecíficos e/ou heteroespecíficos) poderão ter desempenhado um papel fundamental na formação da evolução da tomada de decisões, comportamentos e cognição em cefalópodes. Tentativamente podemos denominar a união destas duas visões como a "Hipótese de Inteligência Socio-Ecológica".

1 General Introduction

1.1. Life and Evolution

"… from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved" (Darwin 1859). It was with these words that Charles Darwin (together with Alfred Russel Wallace) illuminated our understanding on the relatedness and diversity existing in the natural world. The theory of natural selection provides a framework for how various features or traits of an organism are selected to construct a well-adapted whole organism. After the discovery of Mendelian genetics and gene heritability, Ronald A. Fisher united these two principles into what is known as the "fundamental theorem of natural selection" (Fisher 1930), i.e. genetic variation (or drift) and trait inheritability under selective pressures drive natural selection at the organism level. Organisms are therefore highly adaptable, changing and evolving characteristics according to environmental challenges and selective pressures.

Darwinian fitness is measured as reproductive success, or the individual's ability to pass its genes to the next generation while interacting with its environment. Over time, genes of individuals with higher fitness increase in frequency within a population, increasing the mean fitness of the population (West and Gardner 2010). Importantly, natural selection is not necessarily a directional process aiming to increase organismal complexity every new generation. If a given population is already well-adapted to its surrounding environment, there is no effective pressure for change, be it increasing or decreasing in complexity. As such, in nature, we find multiple examples of well-adapted species that remain largely unchanged over the course of millions of years (Vitti 2013). We usually call them "living fossils" (e.g. the nautilus).

This way of viewing and understanding evolution revolutionized the thought pattern in several related fields of science, among which are animal behavior and cognition. Researchers studying animal behavior have long realized that an "evolutionary hierarchical ladder" of increasing complexity bound by phylogeny - from bacteria which base their decision-making on stimulus-response to human abstract thinking -, is a flawed concept (Dugatkin 2014). There is now ample evidence that complex behaviors emerged independently several times across the tree of life, underpinned by markedly different life histories and brain architectures (Jozet-Alves et al. 2013, Roth 2015, Loukola et al. 2017, Fayet et al. 2020). Thus, understanding the ultimate causes of animal behavior has shifted from solely examining genetic relatedness across phylogenies, to a more comprehensive framework also encompassing the surrounding environment (Jakob von Uexküll's *umwelt*, Figure 1.1) and its selective pressures experienced by individuals, in an effort to explain the independent, but often convergent, evolution of complex behavior and cognition.

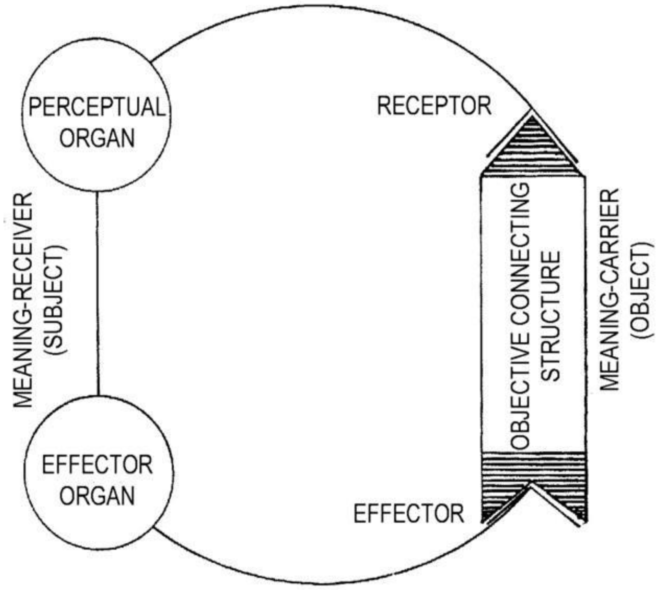

Figure 1.1. Schematic description of Jakob von Uexküll's *umwelt* model, where 'object' represents any environmental feature that interacts with an organism's sensorial or perceptual system (in *Ecosemiotic Basis of Locality*).

1.2. Social interactions

The poet John Donne famously authored the expression *“No man is an island”*, alluding to how human beings are connected and dependent on each other. Given context, the same could be said for any animal that exists on planet Earth. William Donald Hamilton notably demonstrated that gene spread, and consequently fitness, can increase through the individual’s direct and indirect impacts from interactions with other related individuals that maximize the transmission of copies of the same gene - defined as “inclusive fitness” (Hamilton 1964). Moreover, beyond what is also known as Hamilton’s “kin selection”, the Darwinian fitness of an individual is dependent on the result of interactions with other individuals throughout their life history regarding several ecological aspects, which for animals can include predation, avoiding predation, defending resources, mating, and parenting (Ashton et al. 2020, Oliveira and Bshary 2021). In fact, even if we exclude heterospecific predator-prey contexts, it is easily understandable that the impact on Darwinian fitness can be far higher for an individual that is attacked by a heterospecific due to resource competition, compared to e.g. cooperative hunting between conspecifics not resulting in prey acquisition for one of the partners (Grether et al. 2009). Thus, the entirety of social interactions and contexts, both with related and unrelated individuals, and between different species (sometimes also referred to as socio-ecological interactions), function as critical sources of selective pressure on an organism, and are considered to be key in explaining the evolution of extant fauna and their decision-making processes.

1.2.1. Collectives

In collective behavior, complex coordination and decision-making abilities in groups of “self-propelled particles” can emerge from simple localized rules of interaction (Vicsek et al. 1995, Watts and Strogatz 1998, Couzin et al. 2002). These particles can refer to individual entities at any level of biological organization, and recent models are capable of translating several collective behaviors with dynamic transitions between them, from small changes in individual responses escalating to large changes in group structure (Couzin et al. 2002, 2005, Ioannou et al. 2015). For example, the individual tendency of one individual to align with others in parallel minimizes collisions and facilitates information transfer. That is, in such highly parallel groups, if one individual makes a sharp turn, this information is quickly propagated to its (visual) neighbors and cascades through the whole group (Strandburg-Peshkin et al. 2013). On the other hand, if individuals exhibit strong attractions among them, the group will lose alignment and become a swarm or torus group type, i.e. either performing attraction-repulsion movements towards a center with low angular momentum, or exhibiting high angular momentum with individuals rotating at higher speeds around an empty core (Couzin et al. 2002).

Concordantly, groups of animals demonstrate intricate patterns of collective movement due to simple social interactions based on algorithmic attraction-repulsion rules (Couzin et al. 2005, Buhl et al. 2006, Ballerini et al. 2008, Figure 1.2). For example, individual fish changing in response to internal (individual internal states such as hunger) or external (e.g. predatory responses) stimuli to maximize individual fitness, lead to fish schools changing rapidly between the previously mentioned group types (Couzin et al. 2002). These individual-specific responses are thus critical, as differences in individual properties determine spatial positioning inside the group, the change of said positioning over time (e.g. depending on individual speed), and group movement. Moreover, the spatial structuring within groups has important ecological and evolutionary consequences, e.g. with individuals on the outskirts being more susceptible to predation (Krause and Ruxton 2002, Herbert-Read et al. 2017, Sosna et al. 2019).

Figure 1.2. Flock of birds exhibit complex group movement patterns based on simple local rules of interaction between individuals. Photo: Wikimedia Commons, Tanya Hart.

Despite the prevailing paradigm in collective movement studies considering every individual as an identical particle, such is rarely the case in nature. Biological systems have inherent intraspecific variation that can alter the “weight” of specific individual’s decision within a group (Couzin 2009, Nagy et al. 2013, Jolles et al. 2017, 2020). Consequently, how decisions are made in groups of animals can vary within a spectrum of a) consensus or shared decision-making, i.e. all individuals count as homogenous ‘particles’; and b) despotic decision-making, i.e. one or a subgroup of specific individuals being responsible for decisions (Conradt and Roper 2003). Recent evidence shows that sometimes differences in individual kinematics, such as speed, can decide leadership influence and the disparity between individual speeds determining how far along a group is in the shared-despotic leadership axis (Mishra et al. 2012, Pettit et al. 2015, Jolles et al. 2017). Social status in groups can also markedly affect the leadership status of individuals, albeit experiments show mixed results regarding socially dominant individuals acting as leaders. In baboons, there is evidence of despotic leadership in hierarchically superior individuals (King et al. 2008) - but see Strandburg-Peshkin et al. (2015) for evidence of shared decision-making in another baboon species -; concurrently, a cooperative breeding fish species reveals that submissive individuals can be more influential than dominant individuals due to less aggressive interactions and higher signal-to-noise ratio (Rodriguez-Santiago et al. 2020). Moreover, in flocks of birds, there are different hierarchies when the group is feeding ('social dominance') or when it is moving ('movement dominance') (Nagy et al. 2013).

According to game theory, despotic leadership can also be the result of producer-scrounger dynamics developing within the group, depending on differences in social interactions, behavioral traits, and knowledge of resources (Giraldeau and Beauchamp 1990, King et al. 2009, Guttal and Couzin 2010). In the literature, the underlying dynamics of this game or model are closely similar to the exploration-exploitation model (generally used when referring to behavioral choices for one individual) or the nuclear-follower model (generally used in heterospecific animal groups). In essence, producer (/explorer/nuclear) tactics result in leadership as these individuals explore new spatial locations for resources, which can be anything from food to shelter. Conversely, scroungers (/exploiters/followers) exploit the resources that the producer locates, limiting themselves to follower roles in terms of collective movement.

1.2.2. Complex cognition

So far, I have discussed relatively simple rules that influence how animals move and coordinate in a given environment, however social interactions can be intrinsically complex. In the last decades, the evolution of higher relative brain size has often been correlated with the demands of within-group social life (Barton and Dunbar 1997) (but see in Smaers et al. (2021) different pathways for species developing higher relative brain size as an end-product). Specifically, the Social Brain Hypothesis (or Social Intelligence Hypothesis) posits that prolonged interactions between individuals in groups, relating to social hierarchies and strategies for obtaining resources, favored larger brain-to-body ratios and higher cognitive functioning (Dunbar and Shultz 2007). For example, during cooperative interactions, partners rely on the ability to consider each other's behavior in their decision-making process (Lehmann and Keller 2006, Dugatkin 2014, Bshary et al. 2016). In such situations, maximizing personal benefits and avoiding exploitation involves understanding non-immediate rewards, evaluate costs and benefits (i.e. high neural processing energetic costs), and the potential use of partner control mechanisms (e.g. sanctions, punishment) (Bshary and Bergmüller 2008, Raihani et al. 2012). These assessments must occur recursively, as individuals can change their strategy over continuous iterations of the interactions (Axelrod and Hamilton 1984). Moreover, more than memorizing pairwise interactions, individuals may also have to incorporate information from subgroups of individuals (favoring cognitive development, e.g. of numerical discrimination), and interactions can additionally be eavesdropped by third parties intercepting social information intended for other individuals (which can also lead to 'audience effects') (Barton and Dunbar 1997, Krause and Ruxton 2002, Bshary and Grutter 2006, Taborsky and Oliveira 2012). All these potential factors combined also help explain why another presumptive property of this relationship between sociality and brain evolution is that relative brain (specifically neocortex) size could also correlate with group size if social interactions are maintained at the same complexity level, i.e. heuristic rules do not dominate the decision-making process (Dunbar 1992).

1.2.2.1. Social learning

Within processes requiring sophisticated cognition due to social interactions, a special case contemplates the memory of previously available social information and its application in the behavioral flexibility of posterior responses (Taborsky and Oliveira 2012). Learning can occur through self, asocial experiences with the surrounding environment on the basis of trial-and-error, allowing for adaptive behavior towards changes in the environment (Shettleworth 2001). A more complex form of adaptation is learning through the experience of others, a process termed social learning (Galef and Laland 2005). This vicarious process allows for a reduction of trial-and-error, and inherently optimizes the individual's behavior, by maximizing successful actions and/or minimizing energy expenditure. Social learning by observation can occur through several mechanisms with an increasing degree of cognitive complexity, namely local/stimulus enhancement, social facilitation, emulation, mimicry, and imitation (Olmstead and Kuhlmeier 2015). These different types of social learning vary in key cognitive requirements, from sensitization, to understanding object properties, end-state, and/or actions undertaken (Galef and Laland 2005, Olmstead and Kuhlmeier 2015).

Starting from the simplest mechanisms, stimulus/local enhancement is generally defined as observers being drawn quicker to a stimulus due to the presence of demonstrators. Additionally, social facilitation (or social enhancement) by itself predicts that in the presence of conspecifics, observers will enhance replication of the demonstrators' dominant response (Zentall and Levine

1973). Observational Pavlovian conditioning (through stimulus-stimulus learning) dictates that the same unconditioned response should be transferred from demonstrator to observer. Emulation through affordance learning is underpinned more on a “learning what” process instead of “learning how” (Galef and Laland 2005), dictating a focus on recognizing the properties of the object rather than the demonstrator’s actions. End-state emulation is the emulation of the final goal reached by the demonstrator by any other means. Complementary, using the same means as the demonstrator could be mimicry or imitation, depending if the purpose of the behavioral sequence is understood by the individual or not (Olmstead and Kuhlmeier 2015). Importantly, conspecifics are usually used as the source of information, but heterospecifics also provide opportunities for social learning when there is an overlap in niches/interests, such as food location or predatory evasion (Templeton and Greene 2007, Damas-Moreira et al. 2018, Oliveira and Bshary 2021).

1.2.2.2. Social tool use

First thought to be exclusive to humans and primates, it has become increasingly clear that tool use can occur across the tree of life in numerous forms (Seed and Byrne 2010). While still regarded as a hallmark of cognitive sophistication (Seed and Byrne 2010), another form of tool use involves using animated objects (i.e. live beings) instead of inanimate objects, and is defined as the ‘*physical and psychological manipulations of animate beings towards some goal*’ (Völter et al. 2015). Social tool use can be divided in 4 Levels, depending mostly on the different degrees of physical influence exerted by the user and the self-motivation of the social tool. In Level 1, the social tool is completely forced by the user in a manner analogous to an inanimate object (e.g. dolphins using sponges while hunting), while in Level 2 the social tool also exhibits self-behaviors while being manipulated physically (e.g. weaver ants use conspecifics to glue leaves with their secretions).

Thus, Levels 1 and 2 are viewed as coercive since the social tool is physically forced by the user. In contrast, Levels 3 and 4 are generally termed as cooperative since they are grounded on the social tool's self-motivation and communication, and are mostly distinguished through experimental manipulation (Bard 1990, Völter et al. 2015). Specifically, in Level 3, the user physically provides an object or item for the tool to use. For example, an orangutang providing a token to another orangutang to insert in an experimental apparatus thus providing food for both. In Level 4 there is no physical contact, with the tool being used strictly through communication and psychological effects, e.g. the same scenario as Level 3 but the token is picked up by the orangutang tool. Importantly, most species that have been identified as social tool users thus far have social/gregarious lifestyles characterized by large groups of conspecifics or prolonged co-existence in smalls groups of kin, such as hermit crabs (Ross 1983, Mann and Patterson 2013), social insects (see Pierce 1985), dolphins (Smolker et al. 1997, Krützen et al. 2005), and primates (Völter et al., 2015).

1.2.3. Heterospecific sociality

As discussed before, individuals can interact with conspecifics, but can also interact with heterospecifics in ecological contexts beyond predator-prey dynamics. The consistent aggregation of unrelated individuals across specific spatial and temporal segments is usually explained by shared interests regarding one or several traits of their life history (Clutton-Brock 2009, Smith et al. 2016, Sridhar and Guttal 2018, West et al. 2021). Shared or overlaying interests between species can arise across several specific contexts, such as territory defense, antipredator responses, mating, and parental care (Oliveira and Bshary 2021). In terms of collective movement, biological systems have inherent intraspecific variation that can alter the "weight" of specific individual's decision within a group (Couzin 2009, Nagy et al. 2013, Jolles et al. 2017, 2020). As a result different species-specific evolutionary histories, that variation is further magnified in interspecific groups.

Cooperation is said to generate benefits that are shared by all individuals, surpassing the costs associated with the presence of the other individuals (Clutton-Brock 2009), such as increased competition and/or activity matching (Sridhar and Guttal 2018). Under the theory of natural selection, stable social interactions provide immediate direct benefits and/or future indirect benefits to participating individuals (Lehmann and Keller 2006). In interspecific interactions, the lack of relatedness between individuals ensures that such benefits are personal, and interspecific cooperation is usually termed as a mutualism (Clutton-Brock 2009). As a side note, interspecific associations can also be parasitic, where one species benefits at the cost of the other, or more often commensalistic, where only one species benefits and the other does not incur in costs. In birds, some species eavesdrop on other's intraspecific alarm calls to gather information regarding dangers in the vicinity, such as predators (Templeton and Greene 2007) and parasites (Lawson et al. 2020). A classic example of parasitic heterospecific interactions regarding parental care are brood parasites such as cuckoos that lay eggs in other species' nests, with parents sometimes providing less food to their own chicks in favor of the more efficient parasite beggars (Davies 2011).

Heterospecific sociality can entail complex cognitive processes, as highlighted especially by cleaner-client interactions. In this system, complementary interests align, with cleaners finding a food source while clients get rid of parasites in their skin and other organs (Grutter 1999). Among other cognitive processes, cleaner fish have developed the ability of tactile stimulation to prevent aggression from less satiated clients (Grutter 2004). Conversely, clients learned to eavesdrop and image-score cleaner performance in order to choose the best service providers, which simultaneously promotes better performance and less cheating by cleaners in future interactions (Bshary and Grutter 2006). These repeated interactions, entailing complex feedback mechanisms between all parties involved, form a social context similar to conspecific interactions for the evolution of cognition (Oliveira and Bshary 2021).

1.2.3.1. Interspecific hunting

Within heterospecific sociality, interspecific hunting allows predators to find and target more numerous prey with greater efficiency than an individual can manage alone (Krause and Ruxton 2002, Farine et al. 2014). As such, for hunting groups to be stable, the benefits of hunting with other predators should surpass the costs associated with social grouping (Krause and Ruxton 2002, Lang and Farine 2017). The evolutionary history and ecology of different species shape species-specific hunting strategies and tactics, which can in its turn lower competition between partners and also more easily lead to coordination (Bshary and Bergmüller 2008). Nevertheless, like in other types of interactions between species, interspecific hunting groups can also be commensalistic, grounded on stable nuclear-follower ecological model or producer-scrounger games (Lang and Farine 2017). In these systems, one species serves as the nuclear predator creating prey opportunities for other species that accompany its movement, and opportunistically feed on prey or remains left by the nuclear predator. Sharks and remoras are a textbook example, with remoras literally attaching themselves to sharks and feeding on prey remains (Hubbs 1951). More complex examples can be found, for instance between Ethiopian wolves and herds of gelada monkeys (Venkataraman et al. 2015). In this system, the collective movement of monkey herds uncovers small rodents in the vegetation, which is taken advantage of by wolves that increase their prey capture rates. Additionally, it is noteworthy that wolves refrain from attacking monkey juveniles, avoiding retaliation and maintaining the benefits of following the monkey herds.

Interspecific and multispecific hunting can also be mutualistic, creating shared benefits for all species involved. In the marine realm, well-known examples are ‘bait balls’ of pelagic schooling fish and the multitude of predators interacting to feed on it. In essence, while fish, and sometimes marine mammals, drive fish to the surface in tighter and tighter formations, seabirds attack from the skies in a synchronized fashion. Several studies have shown shared benefits as a result of these multispecific associations and coordination, including for individual seabirds (Thiebault et al. 2016) and tuna (Hebshi et al. 2008). One of the most complex examples of interspecific collaborative hunting is the partnership between groupers and other species as hunting partners (predominantly moray eels) (Bshary et al. 2006). In these pairwise partnerships, groupers hunt for prey in the water column as per their usual strategy, and associate with one species that can reach for prey inside crevices. Groupers actively communicate with their partners, first using a horizontal shimming motion to recruit the partner, and then using the same shimming motion in a vertical stance, pointing to where there is hidden prey is (fulfilling the criteria for referential gesturing) (Vail et al. 2013). Hunting partners that have been targeted by these referential gestures are moray eels, napoleon wrasses, and octopuses. Groupers also evaluate their partners and are able to choose the one which in previous interactions has shown the best performance (Vail et al. 2014). As such, interspecific group hunting can be based on much of the same feedback and cognitive processes as hunting with conspecifics (Dugatkin and Wilson 1993, Bshary et al. 2014).

1.3. The lives of cephalopods

Sharing the last common ancestor with vertebrates around 500M years ago (Kocot et al. 2011), the members of the class Cephalopoda represent unique models for studying the underlying selective pressures that shape animal decision-making, as well as the independent (and convergent) evolution of complex behavior. The cephalopods refer to the class of extant animals that includes the nautilus and coleoid cephalopods, specifically octopuses, squid, and cuttlefish. In this dissertation, I will focus on coleoid cephalopods that evolved a more developed behavioral and cognitive repertoire (but see Crook and Basil (2008a, 2008b) for evidence of associative and spatial learning in nautilus). In the following sub-sections, I will detail the ecological and social landscapes across the life histories of the referred three main groups, as well as the current scientific understanding of their impact on cephalopod decision-making and behavior.

1.3.1. Ecological and heterospecific contexts

1.3.1.1. Antipredator tactics

Antipredator tactics are common to all three cephalopod groups (octopuses, squid, and cuttlefish), with some exceptions. The foremost defensive strategy of cephalopods is their ability to dynamically camouflage with the environment, a skill that is unmatched in the animal kingdom, and particularly developed in the benthic octopuses (Figure 1.3) and nektobenthic cuttlefish (Hanlon and Messenger 2018). Via direct neural connection to all skin cells containing chromatophores, iridophores, and leucophores, cephalopods can alter color, skin texture, and shape in milliseconds, mimicking rocks, algae, or the background itself, to go unnoticed to any nearby predators (Messenger 2001). Briefly, different local elements are used to form units of chromato-

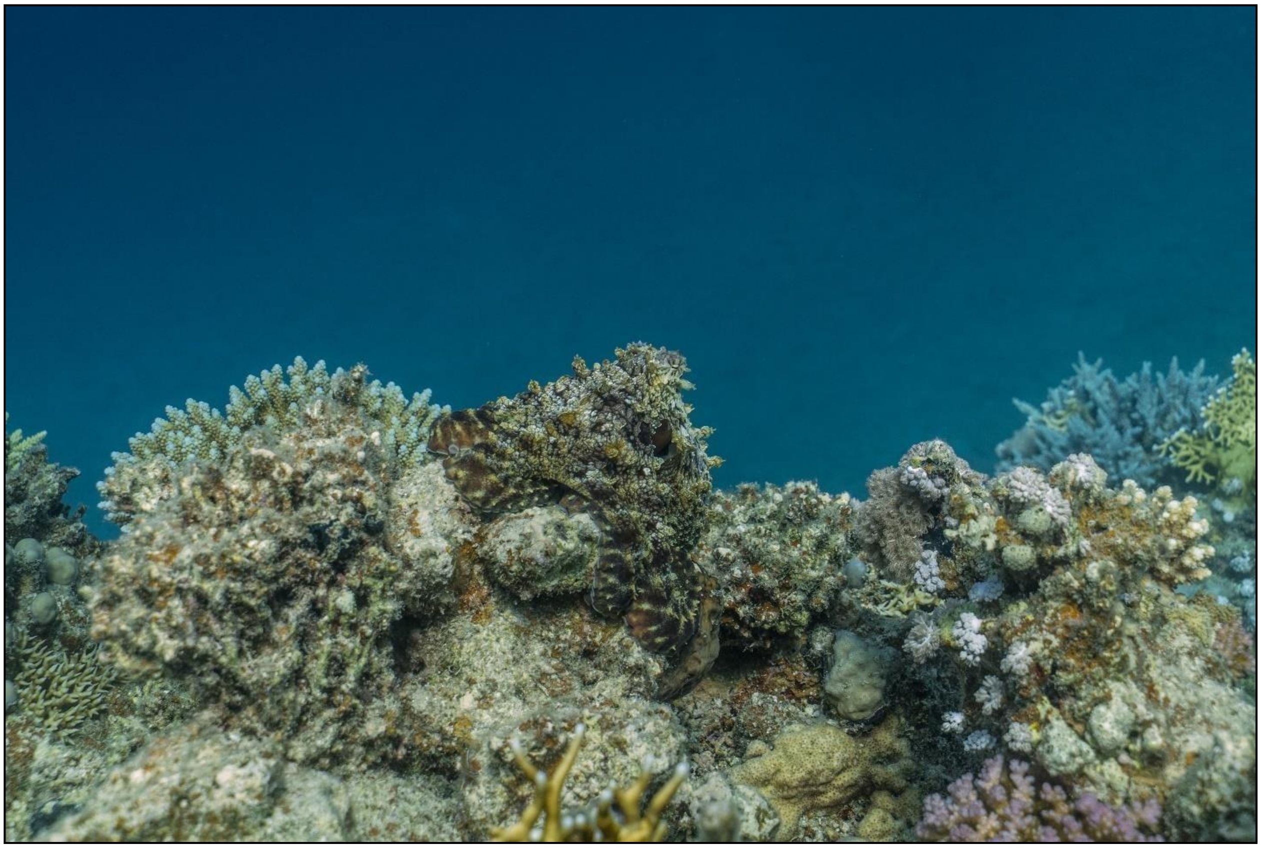

Figure 1.3. Example of an octopus using body shape, skin color and texture, to camouflage with the surrounding environment of rocks and corals. Photo: Eduardo Sampaio.

phore expansion (e.g. dark eye bar), as well as 3D skin texture. Multiple units are thus combined to form full body patterns, which are usually part of a specific behavior exhibited by the individual (Hanlon and Messenger, 2018, see also Reiter *et al.*, 2018 for detailed variation in individual chromatophores for *Sepia officinalis*). Given the highly connected and widely distributed neural junctions across skin cells, changes can occur over milliseconds enabling dynamic moving patterns in addition to static ones, e.g. the "Passing Cloud" pattern, resembling "waves" of alternate light/shade (Mather and Mather 2004, Borrelli et al. 2006, How et al. 2017).

In addition, the potential functions of sophisticated neural control of skin cells are not restricted only to camouflage and background matching. When detected, cephalopods can display high contrast and/or uniform patterns in order to try to appear bigger than what they are in reality, and deter the predator (Hanlon and Messenger 2018); or several disruptive patterns in order to ‘break’ their contours in the predator’s eyes either before or during jetting (Hanlon et al., 2009; Mather & O’Dor, 1991). Disruptive patterns are especially useful for deceiving and losing predators during pursuit while jetting in the water column, allowing for the octopus to adopt new camouflage patterns matching other close-by backgrounds. Cephalopods in general have a secondary anti-predatory response before or simultaneously with escaping, which is inking (Darmaillacq et al. 2014). Interestingly, ink squirts can have distinct features (Hanlon and Messenger 2018). Cephalopods appear to be capable of producing two different types of ink defense: one where a ‘curtain of smoke’ is formed and the animal jets away behind to safety, and another where the octopus squirts a conjoined ink ‘blob’, which can trick the predator to attack the bubble, providing time for the octopus to make its escape (Hanlon and Messenger 2018). Importantly, cephalopods can flexibly choose between these defensive tactics according to the speed, size, and type of predator that is hunting them (Mather 2010, Hanlon and Messenger 2018).

Impressively, as an additional anti-predatory response, octopuses in particular have been shown to use tools for alternate defensive strategies. For example, octopuses use rocks to make den entrances narrower (Mather 1994). In South Africa, *O. vulgaris* caught outside of their dens can use several separate mollusk shells scattered in the sea floor - i.e. composite tool use (Amodio et al., 2018; Finn et al., 2009) -, to create a physical barrier against predatory sharks. To accomplish this feat, the octopus uses its ability to control individual suckers to individually wield each of the dozens of shells picked up, and positions its arms facing outwards from all angles, around and over the head and mantle, thus creating a modular external physical armor against the shark’s biting attacks (Jeffs and Brownlow 2017).

After the description of antipredator tactics that are similar for the whole group of coleoid cephalopods, detailing other ecological contexts and challenges now requires particularizing the analysis for each group member.

1.3.1.2. Octopus

Figure 1.4. An *Octopus cyanea* sitting on top of coral structures in the Red Sea, Egypt. Photo: Martim Costa Seco.

Regarding foraging, when moving through the different habitats in which it resides, octopuses (Figure 1.4) usually adopt a cruise searching type of strategy, interspersed with pounces or jets towards specific locations where prey is likely to be located. Chemosensory receptors in its arms and suckers allow it to ‘taste’ the environment for prey (speculative hunting), while also scanning with vertebrate-like eyes (Hochner 2008) for prey and predators. Octopuses also possess the capability of bipedal locomotion, i.e. crawling or moving backwards grounded only on the most posterior pair of arms (IV) (Hernández-Urcera et al. 2020, Amodio et al. 2021). Because they are generalist predators, octopuses often feed on prey with a wide variety of defensive strategies, which seems to have further driven the octopus’ prey-handling and problem-solving skills (Amodio et al. 2018, Schnell et al. 2021). A textbook example of these skills is demonstrated on the priming of venom injection in different bivalve clams and gastropods (Ambrose and Nelson 1983, Fiorito and Gherardi 1999), as well as crab items (Villanueva et al. 2017). When pulling bivalve clams apart does not produce results, octopuses drill holes with their beak in species-specific locations to inject cephalotoxin (paralyzing neurotoxin) from their posterior salivary gland (Ghiretti 1959) into the clam’s adductor or heart muscles, thus gradually weakening muscle contraction and causing the bivalve to loosen its grip on the two shells, which facilitates the removal of physical armor. Octopuses have also been observed to leave the water to ambush and capture prey, e.g. hunting crabs near water in the intertidal zone (Indrisie 2015, Sampaio et al. 2018). These rare events showcase the behavioral flexibility of hunting strategies for which we still require a broader understanding.

Despite the lack of intraspecific interactions during most of their lifetime, octopuses interact with many other species within their natural environmental context, in relationships that differ from simple predator-prey interactions. Octopuses have been observed multiple times being cleaned by fish species that feed on parasites and dead skin, and engage in mutualistic relationships with other fish. Most of these events seem to be opportunistic, with cleaner fish taking advantage of the octopus' attention being focused on other stimuli (Johnson and Chase 1982, Hanlon and Messenger 2018, Guabiroba and Joyeux 2021), but a record of one octopus actively moving towards a cleaning station exists (Sazima et al. 2004), which opens up the possibility for more regular interactions. In other heterospecific associations, early reports detail common blanket octopus *Tremoctopus violaceus* individuals that have taken hold of Portuguese man-of-war *Physalia physalis* tentacles and the possibility of using these as 'weapons' (Jones 1963). Likewise, Rosa et al. (2019) report and discuss several reported associations between pelagic octopus species and cnidarians with different potential goals (including hijacking), although direct evidence is still absent. Most notably, octopuses are reported to be 'followed' by fish during foraging, among which *Serranus scriba*, *Epinephelus marginatus*, and *Halichoeres bivittatus* (Kayes 1973, Mather 1992, Machado and Barreiros 2008). These fish hover around the octopus while the latter is hunting; approaching or surrounding crevices and rocks that the octopus is targeting, to opportunistically corner and acquire fleeing prey. Furthermore, in the case of the big blue octopus *Octopus cyanea*, groupers actively signal locations using referential gestures, which provides more prey opportunities for the octopus (Vail et al. 2013).

1.3.1.3. Cuttlefish

Cuttlefish (Figure 1.5) are also generalist predators feeding on a wide range of mollusks, crustaceans, and fish (Boal et al. 2000, Hanlon and Messenger 2018). Less speculative and more reliant on precise attacks and ambush tactics than octopuses, cuttlefish usually lay on the seabed camouflaged and mainly use both visual and odor cues to detect prey. If a prey item passes in front of them, cuttlefish attack to acquire it (Nixon 1985). When a prey item is noticed at a larger distance, cuttlefish perform a three-stage attack sequence classically decomposed into attention, positioning, and seizure (Messenger 1968). In the first stage, cuttlefish fixate binocularly on prey, and raise their first arm pair while also changing body patterns. Secondly, they move toward the position of the prey item until reaching an attacking distance. Alternatively, if prey detects the cuttlefish previous to the seizure stage it may attempt to escape, and cuttlefish will initiate stalking. Notably, during this process a body pattern named ‘Passing Cloud’ can be exhibited, which creates the illusion of continuous movement in cuttlefish skin (Laan et al. 2014). It is still uncertain however how exactly this body pattern affects the psychophysics and decision-making of prey or if it increases successful capture of prey.

Figure 1.5. *Sepia officinalis* newborns reared at Laboratorio Maritimo da Guia. Photo: Eduardo Sampaio.

Lastly, cuttlefish can seize prey in two ways: either by directly shooting out their tentacles or by jumping on top of prey with their arms (Nixon 1985, Boal et al. 2000). Like octopuses, cuttlefish then use their beak to break the skin/armor of prey, and inject the cephalotoxin from its posterior salivary glands. Choosing between tentacle shooting and jumping/pouncing is influenced by the type of prey they are predating (e.g. they will generally tentacle shoot prawns and pounce on crabs), as well as its size (smaller animals will be tentacle shot, larges animals will be pounced on) (Hanlon and Messenger 2018). Moreover, cuttlefish also learn to attack specific body parts of crabs, preferring to attack the back of the animal and avoiding its pincers (Boal et al. 2000). It is also noteworthy that cuttlefish are able to use their siphon to clear grains of sand or sediment and look for prey in the substrate, such as prawns and shrimps (Hanlon and Messenger 2018).

1.3.1.4. Squid

Because they are pelagic, squid are the less studied cephalopods regarding foraging and most of the information comes from a small number of field studies performed. While relying less on ambushes (but see Hanlon and Messenger 2018 for some anecdotal reports), squid exhibit similar hunting flexibility as cuttlefish when actively searching or stalking prey. Usually hunting in the water column, squid can attack jet directly or backwards until reaching prey using its siphon and then turning to face it (Moynihan and Rodaniche 1982). Squid can also use their arms or their tentacles for capture, depending on the size and speed of prey (Hanlon and Messenger 2018). Moreover, like other cephalopods, squid can also disguise as other habitat features, e.g. sargassum leaves, and capture unaware prey (Moynihan and Rodaniche 1982). Similarly to cuttlefish, squid have been observed using their arms to churn sand and sediment and find prey that may be located underneath (Hanlon and Messenger 2018). Interestingly, there are indications of coordinated hunting in one squid species, namely jumbo squid (*Dosidicus gigas*), but more evidence is required to understand if, for example, the individual behavior is the same when hunting by itself or if individuals adjust behavior to the presence of others (Benoit-Bird and Gilly 2012).

Figure 1.6. Group of *Sepiotheutis lessoniana* individuals in the Red Sea, Egypt. Photo: Eduardo Sampaio.

1.3.2. Life histories and social contexts

Cephalopods in general, particularly those living in coastal areas, are semelparous and die after one spawning season (Morse and Huffard 2019). The only instances of parental care (or investment) behavior relate to egg stages, with females oxygenating and cleaning eggs until hatching in octopus species (Boyle and Rodhouse 2005, Robison et al. 2014), and females regularly revisiting egg-laying locations to clean their eggs in squid species (Moynihan and

Rodaniche 1982, Hanlon and Messenger 2018). In contrast, maternal care or investment behavior in cuttlefish, or behavioral paternal investment across all cephalopod families, have never been recorded (Morse and Huffard 2019). As such, transmission of information over generations is limited, severely so in the case of vertical transmission among kin (parent-offspring). Concurrently, given the exclusivity of maternal investment in egg care, the evolutionary dynamics of sexual selection seem relatively simple (Clutton-Brock 2007, Kokko and Jennions 2008), with males evolving solely to successfully mate and guard the female. Regarding horizontal transmission (offspring-offspring, or same generation) of social information, hatchlings from most squid and octopus species go through a planktonic paralarval stage where individuals can disperse widely across different habits and regions (Mangold 1987). Conversely, in cuttlefish clutches, individuals hatch in the same vicinity which in theory can facilitate social interactions between individuals with higher relatedness (Nixon and Mangold 1998).

Throughout the rest of their life, the three cephalopod groups generally make up different parts of a sociality continuum: solitary octopuses, semi-solitary (or semi-social) cuttlefish, and social squid. Starting from the most solitary, octopuses seem to rarely interact with conspecifics except during territory fighting and mating which often precedes death (Hanlon and Messenger 2018). There are however some exceptions, such as non-random aggregations of individuals of certain species under natural (see Scheel et al. 2015, 2017) and laboratory conditions (Edsinger and Dölen 2018, Edsinger et al. 2020). Remarkably, octopuses have been shown to learn by observing conspecifics (Fiorito and Scotto 1992), however it should be noted that some dispute has ensued regarding lack of controls and replication issues (Biederman et al. 1993, Schnell et al. 2021).

Cuttlefish also appear to be solitary most of their lives. However, hatching occurs in the vicinity of other individuals, and aggregations seem to occur more frequently in the wild compared to octopuses, particularly during sub-adult life stages (Yasumuro et al. 2015, Drerup and Cooke 2021). Moreover, during mating seasons, individuals can aggregate in groups reaching hundreds of thousands of individuals, and exhibit complex forms of interactions and intraspecific communication (Hanlon and Messenger 2018). Specifically, smaller males mimick females by adopting female-specific coloration and postures, and sneak by larger males to successfully copulate and pass on their genes (Hanlon et al. 2005). Furthermore, individuals can send unilateral signals towards a specific target and can perform double signaling, i.e. the simultaneous communication of two distinct messages to two different targets. For example, smaller males display courtship patterns to females on one side, while simultaneously exhibiting a female display to deceive larger male rivals on the other (Brown et al. 2012).

Like cuttlefish, squid also display several complex forms of social interactions during mating season, such as the presence of sneaker males, and unilateral or double signaling (Mather 2016, Hanlon and Messenger 2018). Moreover, beyond the myriad of sexual tactics present in this group (see review in Morse and Huffard (2019)), squid are usually the most social of the three cephalopod groups, with species frequently forming shoals and schools of hundreds of individuals. Surprisingly, very few studies have quantitatively assessed social behavior and how group living has shaped the evolution of decision-making in squid (Moynihan and Rodaniche 1982, Boal and Gonzalez 1998). For example, foraging jumbo squid individuals school and appear to coordinate to form multiple interwoven helices to catch prey (Benoit-Bird and Gilly 2012). Moreover, in coastal squid, there are indications of complex group dynamics and specific roles within groups, such as size-based dominance hierarchies and potential existence of group sentinels (Mather 2010, Hanlon and Messenger 2018, but see Boal 2006). However, fulfilling specific validating criteria and/or quantitative analyses are still missing to unequivocally assert these claims. Lastly, despite group living and complex mating tactics which can drive more intricate forms of sexual selection, like in other cephalopod groups, no paternal investment in egg caring has been reported (Morse and Huffard 2019).

1.3.3. Evolution of cephalopod behavior and cognition

One of the greatest lingering questions in biology is how did individuals that diverged from vertebrates more than 500M years ago evolve similar relative brain sizes - larger than most fish and reptiles, but smaller than birds and mammals (Maddock and Young 1987) -, similar cognitive complexity, and similar behavioral flexibility (Albertin et al. 2015, Roth 2015). This question becomes even more fundamental when one considers that other invertebrates showing complex cognitive skills are usually social insects with a lower relative brain size comparatively to the aforementioned groups (e.g. bees are capable of observational learning and innovation, Loukola et al. 2017). How did cephalopods evolve their cognitive and behavioral repertoires? And why?

The two main proposed pathways for the evolution of cognition are the Social Intelligence Hypothesis and the Ecological Intelligence Hypothesis. As mentioned before, the Social Intelligence Hypothesis (SIH) posits that complex cognition developed with group living and with the potential of more complex social interactions to occur, such as iteratively competing, cooperating, deceiving, and learning from others (Barton and Dunbar 1997, Dunbar and Shultz 2007). The continuously changing nature of these interactions thus hypothetically selected for behavioral and cognitive flexibility to respond to social stimuli. On the other hand, the Ecological Intelligence Hypothesis posits that complex cognition developed associated with challenges in finding and acquiring food during foraging (Rosati 2017). The supportive reasoning is that individuals with generalist diets and needing prey-specific strategies and extractive foraging techniques, developed flexibility in their behaviors and consequently cognitively complex processes. In other versions, the hypotheses relating to ecological challenges and intelligence are extended to also cover predator-prey interactions, from the individual's perspective of both sides of the equation (Navarrete et al. 2016, Dunbar and Shultz 2017, Amodio et al. 2018). Thus, again, the fact that individuals developed anti-predator and prey hunting strategies to deal with species- or functional-specific individuals theoretically increased flexibility of behaviors and tactics, which in itself would require higher cognitive processing.

While acknowledging that both these hypotheses are not mutually exclusive, recent consensus has favored a larger role of the Ecological Intelligence Hypothesis for the evolution of cognition in cephalopods, particularly for octopuses (Amodio et al. 2018, Schnell et al. 2021). Indeed, the social lives of cephalopods seem limited; more so in the case of octopuses, with only rare instances reported of aggregations and conspecific interactions outside of the mating season (Scheel et al. 2015, 2017). Theoretically, the most likely candidates among cephalopods to have had a larger degree of socially-driven evolution would be squid, as they mostly live in groups throughout their life. However, the number of cognitive tests performed with these animals as subjects is very limited, particularly on social capabilities (Ikeda 2009). In general, one social factor that may have helped the cognitive evolution of cephalopods is their mating system, which is characterized by instances of intense conflict and competition, and episodes of cannibalism (Brown et al. 2012, Hanlon and Messenger 2018, Morse and Huffard 2019). Particularly for squid and cuttlefish that can aggregate to the thousands, fast and accurate decision-making when given multiple choices during mating season could effectively result in successfully passing genes to the next generation. Therefore, cognitive capacities to flexibly change between mating strategies, adjusting to an extensive and changing enviroment, could have been selected (Schnell et al. 2021).

Nevertheless, the general consensus is that the “leap” in cephalopod cognitive complexity had its key point when coleoid cephalopods lost or internalized their protective outer shells, common to all other molluscean families including the nautilus (Amodio et al. 2018, Schnell et al. 2021). Although it is disputed the order of occurrence between losing the outer shell and developing more complex intelligence (Amodio et al. 2019, Mollo et al. 2019), theoretically, by losing their physical armor cephalopods became more exposed to predators given their soft skin and migrated to locations with lower predator pressure such as the open ocean (Amodio et al. 2019). After several generations, cephalopods would have migrated back to coastal areas and, as a response to high vulnerability to predators, naturally evolved predator-specific strategies of escape and their unparalleled capacity to regulate skin color and texture (Packard 1972, Grasso and Basil 2009). Thus, to survive with their soft-bodies in a vertebrate-dominated world, cephalopods also evolved a unique capability to modulate skin color in the light spectrum visible to fishes despite being presumably color blind (Packard 1972, Shashar and Cronin 1996, Hanlon et al. 2013, Imarazene et al. 2017). Moreover, since coastal areas are more complex habitats than the deep ocean, the increasing challenges of spatial navigation while foraging for temporally-limited food resources, and developing species-specific strategies to prey on these resources, would have further driven the sophistication of cephalopod cognition (Amodio et al. 2018, Schnell et al. 2021).

Given the proposed evolutionary context mainly driven by predator-prey dynamics and competition, could there be a relevant role of social interactions in shaping the evolution of behavior, decision-making, and cognition of cephalopods?

1.4. Objectives and outline

The aim of this dissertation was to understand how social information, interactions and heterospecific contexts shape the behavior and decision-making of cephalopods. Taking advantage of the wide range of social phenotypes within this animal group, I organized this dissertation in two parts and investigated social understanding and information use in conspecific-wise social and asocial species.

In **Part I**, I examined conspecific "Social behavior and decision-making in social and semi-social species" using squid and cuttlefish as model systems; and, in **Part II**, I evaluated "Heterospecific interactions in non-social species" using the octopus as the model system. Specifically assessing conspecific interactions, **Part I** encompasses:

Chapter 2, entitled *"Location probing by males complicates sexual dynamics and successful mate-guarding in squid groups"*, shows the existence of a new sexual behavior by consort males that adds complexity to courtship and alternative tactics in the wild. Moreover, it is shown that this behavior can have consequences for reproductive success of intervening participants.

Next, in ***Chapter 3***, it is demonstrated that *"Neurally underdeveloped cuttlefish newborns exhibit social learning"*. Here, we test the social capabilities of 5-days old cuttlefish in learning inhibition or stimulation of predatory behavior by observing others. Furthermore, simpler learning mechanisms are ruled out and putative affordance learning is discussed.

Part II starts with ***Chapter 4***, where it is presented the first *"Evidence of social tool use by octopuses"* from fieldwork observations of octopuses using sea cucumbers as den covers and vantage points. This is the first reported case of social tool use in a non-social or gregarious species.

Chapter 5 was focused on interspecific hunting groups and shows that *"Octopuses punch fishes during interspecific collaborative hunting events"*. Such punches are used as partner control mechanisms by octopuses during collective hunting, and multiple putative underlying feedbacks are discussed.

In **Chapter 6**, we deep dive into the underlying and organizing principles of interspecific groups, and demonstrate *"Shared decision making and hierarchical dynamics in interspecific collective hunting"*. We combine our understanding of physical components, such as individual speed and position within the group, with biologically relevant differences provided by dissimilar evolutionary histories to explain the movement hierarchies in these groups.

Lastly, **Chapter 7** provides an overview of the previous chapters in support of social (conspecific and heterospecific) interactions shaping decision-making in cephalopods and discusses a more comprehensive framework to understand the evolution of cephalopod behavior and cognition.

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PART I

2

Location probing by males complicates sexual dynamics and successful mate-guarding in squid groups

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Fitness is defined by the successful passing of an individual's genes to the next generation. Cephalopods that mate in groups, in particular coastal squid and cuttlefish species, have evolved complex sexual tactics in order to maximize reproductive success (Morse and Huffard 2019). Through dynamic skin patterning accompanied by typical body postures, individuals send visual signals in the form of displays to potential mates and rival competitors, often engaging in escalating conflicts (Moynihan and Rodaniche 1982, Mather 2016). Moreover, males can simultaneously exhibit receptive color patterns to females and agonistic patterns or even deceptive female patterns to rival males (Brown et al. 2012, Hanlon and Messenger 2018). Thus, given the diverse number of tactics used to both attract a mate and deter competitors, effective female mate-guarding by consorting males is critical to reproductive success in group-mating cephalopod species. Indeed, literature on coastal squids has described mate guarding as the pre- and post-copulatory role of males, who are generally observed positioned around the female while she lays eggs in crevices, as well as exhibiting agonistic displays to ward-off rivals in order to prevent insemination by other males' sperm (Moynihan and Rodaniche 1982, Mather 2016, Hanlon and Messenger 2018, Morse and Huffard 2019).

The bigfin reef squid *Sepioteuthis lessoniana*, like the closely related *S. sepioidea,* is a group-living species that forms shoals and/or schools, and exhibits complex social and sexual behaviors (Moynihan and Rodaniche 1982, Boal and Gonzalez 1998, Sugimoto and Ikeda 2012), including the presence of sneaker males or 'female mimics' (Wada et al. 2005). However, thus far it has not been the focus of detailed behavioral research in the wild. In this study, we observed *S. lessoniana* behavior during mating seasons in two distant geographical locations, and found new behavioral elements in the sexual courtship of *S. lessoniana*. These elements add complexity to existing social interactions and impact sexual strategic choices faced by individuals, with potential implications for the reproductive fitness of both males and females.

Using SCUBA, and after allowing an initial habituation period to the divers, we recorded interactions of individuals and groups in Indonesia (Lembeh, 1.4249° N, 125.2258° E); total 1 hour of recording) and Egypt (El Quseir, 26.1014° N, 34.2803° E; total 2 hours of recording), between July-September 2013 and May-July 2019, respectively. The focal squids (consort male, female, and main rival male) were distinguished by size, strategy, prominent markings potentially left by attacks, or typical displays (Mather et al. 2010). During filming, we observed that besides females entering crevices to lay eggs as previously described for loliginid squid species (Morse and Huffard 2019), the male also displayed the same behavior (Video S1). Within this context, we annotated when males entered the same crevices where females laid eggs, as well as other notable occurrences, such as visual displays, male-male attacks, and copulations.

Figure 2.1. Some typical patterns and displays by *S. lessoniana* from the Red Sea. **A.** the Stripe display, with a noticeable white stripe across the centre of the mantle. **B.** Female displaying sexual receptivity with an almost uniform purple color. **C.** Double signalling by the consort males, exhibiting sexual courting pattern to the female (bright purple) and an agonistic pattern to the competing male (dark brown and white flashes, see Video S1).

We observed that visual displays, in terms of skin and body patterns, were mostly similar to the ones described for *S. sepioidea* (Moynihan and Rodaniche 1982, Mather 2016, Hanlon and Messenger 2018), with some degree of variation (see Lin et al. 2017). For example, the male Stripe display (Figure 2.1A, also featured in 2.1C), and females exhibiting an almost uniform purple display when sexually receptive (Figure 2.1B). Dominant males also used double signalling, exhibiting an agonistic pattern (brown with white flashes) on one side to competing male(s), and a sexual courting pattern to the female on the other (bright purple, Figure 2.1C). Notably, during agonistic bouts, we also identified an undescribed display, performed particularly by the dominant male (Figure 2.2). During this display, white flashing is often visible (Figure 2.2A), complemented sometimes by a Zebra color pattern (Figure 2.2B, Video S2). Interestingly, the tentacles were markedly exposed and set on a downward direction, with the arms splayed around the mouth or also facing downwards (Tentacle Exhibition display, Figure 2.2). This conspicuous behavior makes the squid highly visible, deterring potential rivals (e.g. Video S1).

We also consistently observed a unique behavior across both sites during egg laying. Of the 15 individual events where females laid eggs (once or multiple times), 13 were preceded by males entering the same crevice (~87% probability of laying eggs after male probing; 2-tailed exact binomial test: $p = 0.005$; Figure 2.3A). The remaining 2 events occurred less than 90 seconds after filming started, which makes it possible that the male could have previously entered the location beforehand. Moreover, as only once did a competitor instead of the consort male enter after the female, events are therefore probabilistically dependent as reversing their order would markedly change the probability of occurrence (to ~6%). When performing this behavior, the male not only hovered close to the crevice, but effectively entered more than half-body length inside and remained for a period of seconds, withdrawing afterwards to give place to the female (Video S1).

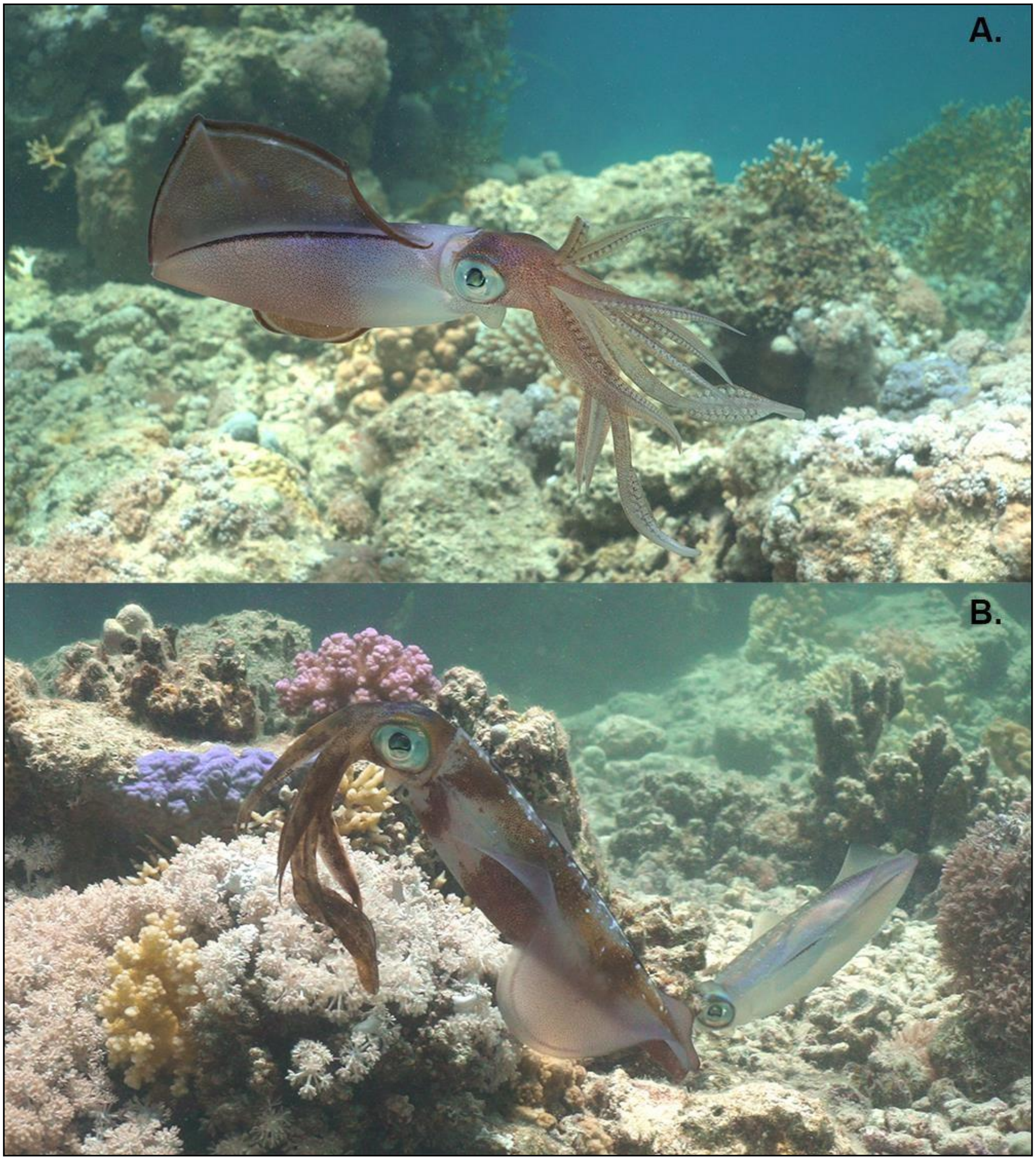

Figure 2.2. Males performing the Tentacle Exhibition display, with variations: **A.** white flashing and arms spread around the mouth. **B.** with Zebra color pattern, and arms down with tentacles (and the female laying eggs in the background, see Video S2).

Each sex exhibited specific skin patterns during burrowing and laying eggs, both assuming a generally white appearance, but the female with an iridescent green/yellow across the centre of the mantle (Figure 2.3B), and the male with brown spots around the mantle and a clear blue line (Figure 2.3C). In some occasions, sand and debris was seen coming out of the crevice when males entered (second event in Video S1). However, it is not clear if this ejection was performed intentionally with the arms/funnel, or if it was an unintentional by-product stemming from fin movement. It is thus uncertain what the purpose of this location probing behavior is, e.g.: if the male is inspecting the crevice for potential predators that could feed on the eggs, marking a specific site within the location/crevice for the female to lay the eggs, or cleaning the location for the female to deposit the eggs on a stable surface.

Pre-probing locations carries a clear risk for the male, as he abdicates from mate-guarding momentarily and leaves the female vulnerable to the approaches of competing males. When in groups, the main competing male would attempt to seize these opportunities to copulate with the female (with the consort male rushing to position himself between the two, often attacking the competitor, e.g. Video S1), successfully doing so in at least one occasion (Video S3). Evidence suggests that female *S. lessoniana* can store sperm and may have cryptic female choice of sperm (Morse and Huffard 2019), which could lead to the complete ejection of the consort male's sperm and thus greatly reduce his reproductive fitness. On the other hand, the opportunity of copulating with another male while the male consort is absent may increase the reproductive success of the female (and the competing male), by providing a more diverse gene pool with which to fertilize her eggs.

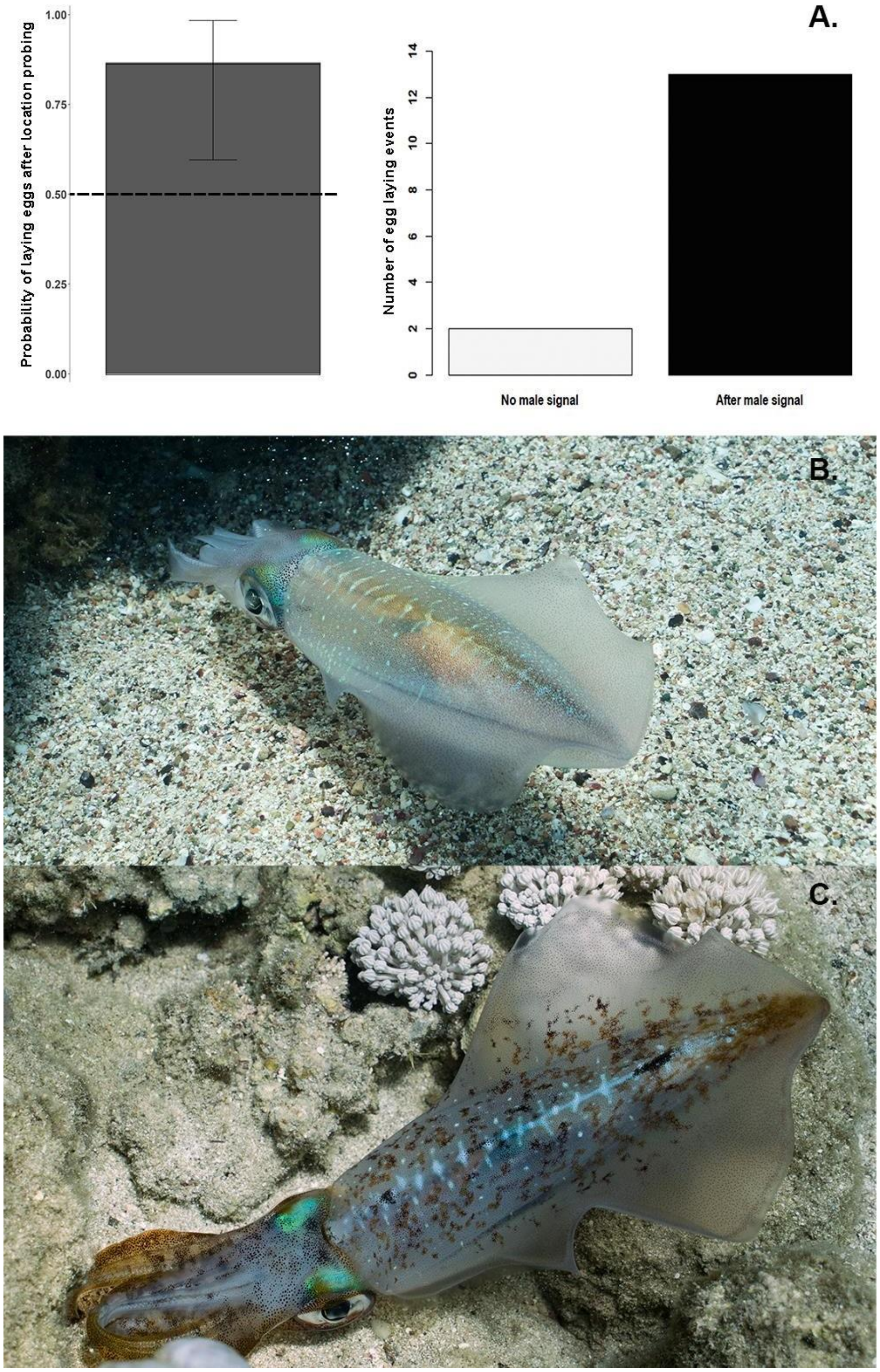

Figure 2.3. Location probing data and displays (see also Video S1). **A.** Data and exact binomial estimated probability of egg laying occurring with or without male location probing **B.** Typical female **s**kin pattern while laying eggs, bright iridescent tones in a mostly white body concentrated around the eyes and the dorsal mantle. **C.** Typical male **s**kin pattern while probing the location, also characterized by a mostly white body but with a blue line across the mantle intersected by smaller blue lines, and darker tones of brown in the arms as well as in the tip and across the mantle.

S. lessoniana is considered to be a species complex composed by several different cryptic species across the Indian and Pacific Oceans, many with overlapping ranges (Cheng et al. 2014). However, distinguishing between cryptic species in the wild is difficult because, while these species are genetically distinct, they are hard to distinguish morphologically. Notably, individuals from considerably distant geographic regions (~10,000 km between the Red Sea and Indonesia) both show the Tentacle Exhibition display and the probing behavior. This suggests that either: a) the individuals observed are the same species, or b) these behaviors are shared across cryptic species, either as a conserved trait or as a product of convergent evolution. The occurrence of location probing behaviors may also be dependent on habitat features. *S. lessoniana* have been observed to lay eggs on multiple different types of substrate, from within branching corals to seagrass beds, on mooring lines, and on open sandy substrate (Cheng, pers. obs). In ‘open environments’ where the substrate is visible, such as sandy substrates, location probing may not be present; however, further observations of egg laying are required to understand the potential role habitat structure may have in shaping male and female behaviors. While its purpose is still unknown, location probing by males seems to be a frequent and important aspect of mating and egg laying in crevices for these populations. From an evolutionary perspective - as this behavior carries potentially high costs to the consort male due to abandoning mate-guarding -, there may be significant selective advantages, such as both partners avoiding egg predation. Moreover, location probing by consort males could also be selected by females, as it additionally provides the female with the opportunity to attain spermatophores from other males. Further observations and studies will help us better understand the ecological (geographical distribution, and habitat specificity) and evolutionary (phylogeny, development, and purpose) drivers behind these behaviors, as well as how they shape individual decision-making in complex social and ecological contexts.

Supplemental Materials

Supplemental videos are available at https://doi.org/10.1002/ecy.3529.

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3

Neurally underdeveloped cuttlefish newborns exhibit social learning

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Abstract

Learning can occur through self-experience with the environment, or through the observation of others. The latter allows for adaptive behaviour without trial-and-error, thus maximizing individual fitness. Perhaps given their mostly solitary lifestyle, cuttlefish have seldomly been tested under observational learning scenarios. Here we used a multi-treatment design to disentangle if and how neurally-immature cuttlefish *Sepia officinalis* hatchlings (up to 5 days) incorporate social information into their decision-making, when performing a task where inhibition of predatory behaviour is learned. In the classical social learning treatment using pre-trained demonstrators, observers did not register any predatory behaviour. In the inhibition by social learning treatment, using naïve (or sham) demonstrators, more observers than demonstrators learned the task, while also reaching learning criterion in fewer trials, and performing less number of attacks per trial. Moreover, the performance of demonstrator-observer pairs was highly correlated, indicating that the mere presence of conspecifics did not explain our results by itself. Additionally, observers always reported higher latency time to attack during trials, a trend that was reversed in the positive controls. Lastly, pre-exposure to the stimulus did not improve learning rates. Our findings reveal the vicarious capacity of these invertebrate newborns to learn modulation (inhibition) of predatory behaviour, potentially through emulation (i.e. affordance learning). Despite ongoing changes on neural organization during early ontogeny, cognitively-demanding forms of learning are already present in cuttlefish newborns, facilitating behavioural adaptation at a critical life stage, and potentially improving individual fitness in the environment.

Introduction

Learning can occur through self, asocial experiences with the surrounding environment, on the basis of trial-and-error, allowing for adaptive behaviour towards changes in the environment (Shettleworth 2001). A more complex form of adaption is learning through the experience of others, a process termed social learning (Galef and Laland 2005, Heyes 2012). This vicarious process allows for a reduction of trial and error, and inherently optimizes the individual's behaviour, by maximizing successful actions, and/or minimizing costly actions and energy expenditure. Social learning by observation can occur through several mechanisms with an increasing degree of cognitive complexity, namely local/stimulus enhancement, social facilitation, emulation, mimicry, and imitation (see Olmstead and Kuhlmeier 2015 for review). These different mechanisms entail distinct information-processing dynamics, from sensitization/habituation to stimulus, to understanding object properties, end-state, and/or actions undertaken; each with specific cognitive requirements (Galef and Laland 2005, Olmstead and Kuhlmeier 2015).

Cognitive comparisons between invertebrates (especially cephalopods) and vertebrates with similar/superior relative brain size (fishes, reptiles and mammals), are still under intense debate (Amodio et al. 2018, Schnell and Clayton 2019). Arguably due to the lack of physical defenses and intense predatory pressure, cephalopods evolved the most sophisticated nervous systems among invertebrates (Albertin et al. 2015, Liscovitch-Brauer et al. 2017), exhibiting cognitive and behavioural capabilities that rival those of vertebrates (Amodio et al. 2018), such as episodic-like memory (Jozet-Alves et al. 2013). Despite showing social tolerance in early stages during laboratory rearing (Boal 1996, Boal et al. 2000), and some species reporting occasional schooling in the field (Yasumuro et al. 2015), cuttlefish are mostly solitary (or semi-solitary) animals throughout their life (Boal et al. 1999, Boal 2006). Even though learning through others has been shown in other non-social animals - e.g. sharks (Vila Pouca et al. 2020) -, perhaps due to the predominantly solitary lifestyle of cephalopods (excluding squids), literature on sociality and social learning is scarce in this group. Notably, *Octopus vulgaris* individuals increase successful choices in a discriminative learning task, after observing a conspecific performing said task (Fiorito and Scotto 1992). However, cuttlefish (*Sepia officinalis*) were not able to improve predation techniques after watching conspecifics prey on crabs (Boal et al. 2000), and a subsequent study testing social learning of danger avoidance provided unclear results (Huang and Chiao 2013).

After hatching, cuttlefish externally already resemble adults (Hanlon and Messenger 1988, Nixon and Mangold 1998), however neural organization is not fully matured until 10 days post hatching, or reaching the juvenile stage at 30 days post-hatching for specific brain areas (Dickel et al. 1997, Liu et al. 2017). Among these, are the optic lobes responsible for visual integration (Liu et al. 2017), and the vertical lobe (Dickel et al. 1997) that is highly associated with both short and long term memory potentiation (Agin et al. 2001, Shomrat et al. 2008), and proven to hamper learning when still underdevelopment (Dickel et al. 1997). An extensively used test for the assessment of learning and memory in cuttlefish is the "prawn-in-the-tube" procedure, where prey within a tube are presented to an individual, which must then learn to inhibit predatory behaviour (Messenger 1973). In this test, cuttlefish associatively learn to inhibit predation towards prey, by detecting the glass tube through multimodal sensorial integration (both visual and tactile exploration were shown to be important), rather than negative 'pain' reinforcement (i.e. provoked by tentacles striking against the glass tube) (Agin et al. 2006, Purdy et al. 2006, Cartron et al. 2013).

Here we modified both the nature of the tasks and resulting end-states expected in previous studies, by using the "prawn-in-the-tube" procedure to gauge if *S. officinalis* can associatively learn to inhibit predatory behaviour by observing conspecifics. Moreover, we added a series of experimental conditions, in an effort to control for potential confounding variables and disentangle the mechanisms underpinning social learning. In gauging social learning potential before neural maturation (newly hatched cuttlefish), we aimed to further explore the neural plasticity of invertebrates that evolved complex neural systems, on which they heavily rely to navigate the world.

Materials & Methods

Collection, husbandry and maintenance conditions

Cuttlefish (*S. officinalis*) eggs from different clutches were collected directly from the wild (n ~ 300), in the Sado River (38°28'40.1"N, 8°47'35.2"W), and transported to Laboratório Marítimo da Guia, Cascais, Portugal. Eggs were randomly assigned to six nurseries (sand bottom area, 49 × 24 x 20 cm; volume = 2.2 L), within a larger 400 L tank with seawater flowing-through the nurseries. The latter was a closed system with a sump tank below, equipped with mechanical (net and cotton mesh) and biological (bio-balls and protein skimmers ReefSkimPro 850, TMC) filtering, as well as UV sterilization (300 L/h UV Vecton, TMC). Water was maintained at 18 ºC and pH at 8.0, and nitrate, nitrite and ammonia were kept at minimum levels, during the 40 experimental days. Once cuttlefish started hatching, prey was introduced (amphipod *Gammarus locusta*) in the nurseries. After 1-3 days of hatching, most cuttlefish would attack and eat the live prey. Cuttlefish used on the experiment were fed for 2 consecutive days (i.e. subjects were 3-5 days old hatchlings at the time of testing), and were fasted for 8 hours prior to the experiment, to prevent hunger level-related biases. From the initial multi-clutch pool of eggs retrieved, we managed to successfully rear ~150 *S. officinalis* hatchlings.

General experimental setup and procedure

Our experimental setup was based on the prawn-in-the-tube procedure (Messenger 1973, Agin et al. 2006, Purdy et al. 2006, Cartron et al. 2013), with one additional arena in parallel, so that one test subject could observe (observer) the other performing the task (demonstrator) (see setup photo in Supplemental Figure 1). For each session (e.g. in the social learning condition), cuttlefish subjects (usually, 1 demonstrator and 1 observer) were taken from their nurseries and placed separately in the two adjacent arenas (20 × 5 × 15 cm), both filled up to ~7 cm with water from the original 400 L tank. After 1 hour of acclimation, the stimulus, a glass cylindrical tube (2 × 10 cm) containing two prey items (amphipod *Gammarus locusta*) were introduced in the middle of one of the aquariums, starting a 10-minute trial (timeframe based on our own preliminary tests of cuttlefish activity). The demonstrator was randomly chosen between the two subjects, to control for arena side and eventual relative size difference between subjects. After the first trial, subsequent trials were spaced by 10 min between them, and this was repeated for a maximum of 10 trials. The criterion for successful learning was defined as 3 consecutive trials where the cuttlefish displayed no predatory behaviour (i.e. performed no attacks). Contrarily, after 10 trials without reaching learning criterion, it was considered that the animal was unable to learn the task. After testing the demonstrator and 30 minutes had passed, the tube with prey was introduced in the observer arena, and the same trial protocol was followed. All sessions were video recorded, and at the end of the session the pair was placed in a nursery separated from the untested subjects.

Study design

To disentangle the mechanisms underpinning potential social learning through observation, and simultaneously control for the existence of several confounding factors, we designed a series of different experimental controls:

Treatment 1. Classical social learning test - where an observer (T1-O) was paired with a demonstrator (T1-D) that already learned the task, i.e. did not elicit predatory behaviour (classical demonstrator). Given our inhibition learning based on social cues task, this procedure served ultimately as a confirmation of end-state acquisition by T1-O.

Treatment 2. Inhibition by social learning test - where one demonstrator (T2-D) learns the task (i.e. naïve individual or sham demonstrator), while another subject observed (T2-O). Given the nature of our task, in this test we observed the learning rates until behaviour and end-state acquisition.

Treatment 3. Stimulus pre-exposure test - where subjects were exposed to the stimulus, demonstrator-free, on the adjacent arena 5 times prior to their test, to control for learning by pre-exposure to the stimulus alone (T3-P). We reasoned 5 trials of pre-exposure, since this was the average number of trials that T2-O observed T2-D (see Figure 1A).

Treatment 4. Tube control test - where no prey was in the tube, to control for unwarranted elicitation of predatory behaviour by the glass tube used for stimulus presentation (e.g. due to reflection), in either demonstrators (T4-D) or observers (T4-O).

Treatment 5. Positive control (prey reward) test - where demonstrators (T5-D) and observers (T5-O) were immediately rewarded on the first time attacking the stimulus, to control for fear-related and potential inactivity biases in the rest of the treatments.

Overall, we performed: 9 sessions for T1 (n = 9 for T1-O and T1-D), 13 sessions for T2 (n = 13 for T2-O and T2-D, total n = 26), 14 sessions for T3 (n = 14 subjects, i.e. T3-P), 7 sessions for T4 (n = 7 for T4-O and T4-D, total n = 14), and 16 sessions for T5 (i.e. positive control, n = 16 for T5-O and T5-D, total n = 32), in a total of 59 sessions and 95 cuttlefish individuals.

Statistics

Analyzing presence/absence of successful learning and learning rates

First, we wanted to know if the number of naïve subjects reaching learning criterion (role played by T2-D), was different from the number of respective observers that successfully completed the task (T2-O). To that end, we used a chi-squared test on a 2x2 matrix with the number of subjects that learned and did not learn the task, in T2-D and T2-O. Moreover, we were also interested in investigating a potential correlation in the performance of T2-D/T2-O pairs, and used a Pearson correlation test to that end. After, we were interested in comparing learning rates among all treatments (excluding T5) to compare between all different conditions. For that, we used time-to-event analyses, i.e. cox proportional hazards tests, to gauge differences in the number of trials required for learning between subjects, i.e. T1-D, T1-O, T2-D, T2-O, T3-P, T4-D, and T4-O. The proportional hazards assumption was confirmed for all cases.

Number of attacks during trials

Here, we were interested in verifying if the learning rates patterns emerging from different treatments were also consistent with the number of attacks over trials. Since we registered three different response variables (between arm, tentacle, and total number of strikes), we first checked for correlation to verify collinearity in the data using Pearson correlation tests. We found that total number of attacks was highly correlated with both arm (Pearson correlation = 0.8615, t = 19.933, df = 138, p < 0.0001) and tentacle attacks (Pearson correlation = 0.8788, t = 21.633, df = 138, p < 0.0001). Considering it the most inclusive parameter, total number of attacks was used as representative explanatory variable. To detect significant differences in total number of attacks over trials among T2-D, T2-O, and T3-O, we fitted a negative binomial Generalized Linear Mixed Model (GLMM) with subject and trial number as fixed effects. We also computed trial and session number (which served as cuttlefish ID) as random slope and intercept, to account for dependency of cuttlefish identity over trials. Details on model choice and validation are presented at the end of the section.

Latency attack time in T2 and T5

With the inhibition task (T2) and the positive control test (T5), we were interested in checking if observers would be slower than demonstrators on the former (T2-O vs T2-D), but would be quicker than demonstrators on the latter (T5-O vs T5-D), i.e. reporting an opposite trends. To measure time differences in T2 and T5 between observers and demonstrators, we fitted two gaussian GLMMs with the same aforementioned fixed (T5 only Subject) and random effects for each treatment, with time latency as the response variable.

For all GLMMs, structure was chosen from an initial full model, decayed using the Akaike Information Criteria, and posteriorly validated depending on model family, by checking for overdispersion, normality, predicted and fitted structure, homogeneity of variances, and non-existence of influential values (see Script). When pertinent, in both multi-level cox proportional hazard models and GLMMs, subject/condition factors were releved to obtain pairwise comparisons between all levels. All statistical analyses were performed in R 3.5.2 (R Development Core Team 2018).

Results

Analyzing presence/absence of successful learning and learning rates

In T2, all observers learned the task (T2-O, 13/13), whereas only 70% of demonstrators (T2-D, 9/13) were able to reach learning criterion after 10 trials ($X^2 = 4.7273$, $p = 0.02969$, Figure 3.1A). Individual T2-O learning rates were directly correlated with its respective T2-D pair's learning rates (Pearson correlation = 0.8526, $t = 6.7272$, $p < 0.0001$). Accordingly, time-to-event analysis also reported faster learning rates of predatory behaviour inhibition in T2-O than in T2-D (Table 1, Figure 3.1A). In fact, compared to any individual T2-D, all T2-O required fewer trials to learn (CI 95%, Figure 3.1A), with 77% of individuals (10/13) starting to reach learning criterion from the first trial, and the remaining reporting a maximum of 4 trials to do so (Figure 3.1A).

Time-to-event pairwise analysis considering all treatments (except T5) (Table 1, Figure 3.1B), disclosed significant differences across treatments. First, it is worth noting that observers and demonstrators in T1 (T1-O and T1-D) and T4 (T4-O and T4-D), reported no attacks, and were pooled together by treatment for formal analyses (Table 1). T2-D showed slower learning rates when compared to: T2-O, T1, and T4 (Reference T2-D, Table 1, Figure 3.1B). T2-D learning rates did not differ from T3-P, i.e. subjects which had been pre-exposed to the stimulus (Table 1, Figure 3.1B). Conversely, T2-O learned significantly faster than both T2-D and T3-P, whereas no differences were found compared to T1 and T4 (Reference T2-O, Table 1). Additionally, T3-P subjects reported significantly lower learning rates than T1 and T4 (Reference T3-P, Table 1).

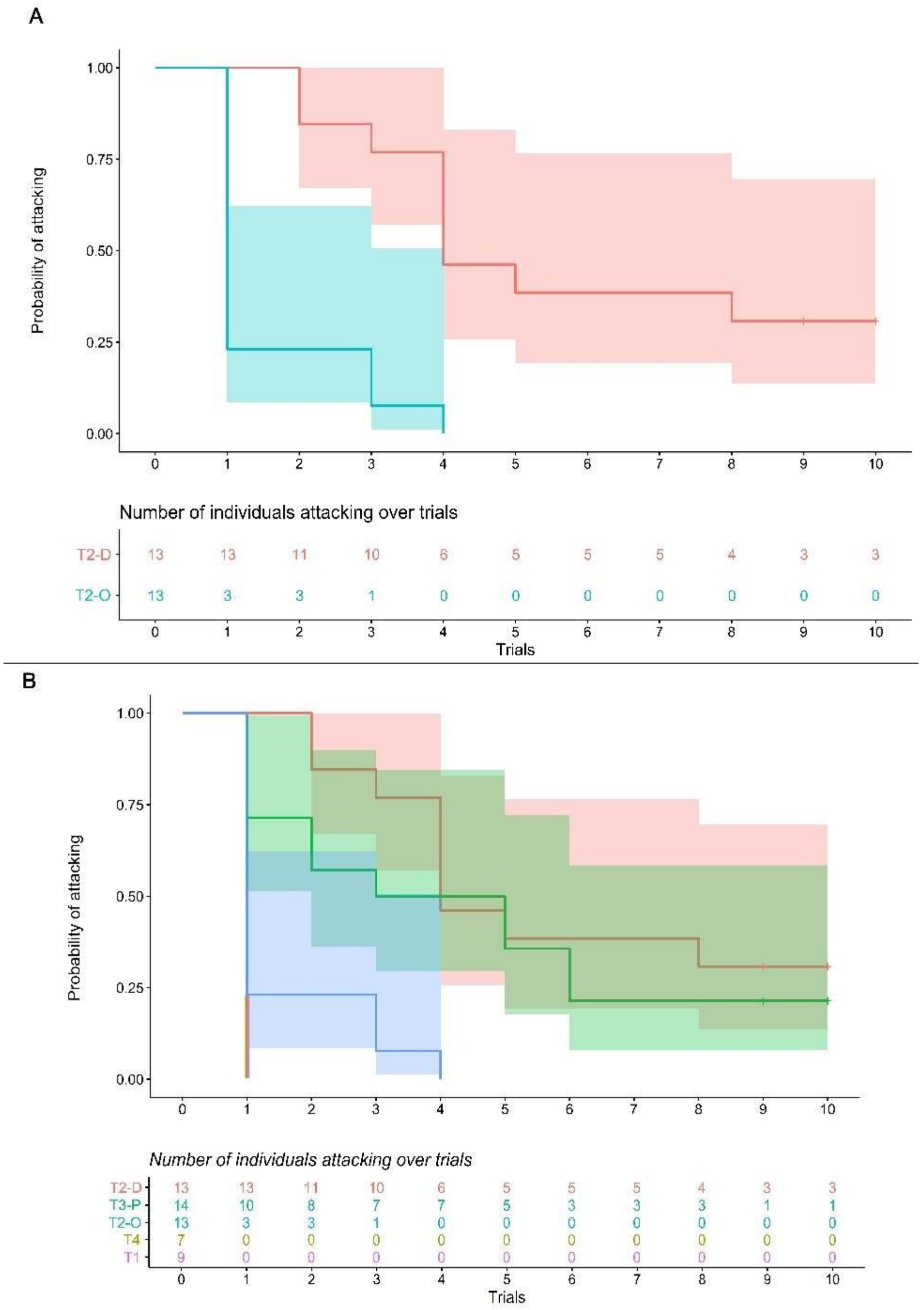

Figure 3.1. Time-to-event analyses on the probability of attacking the stimulus (i.e. not reaching learning criterion) across trials, for **A.** inhibition by social learning test (T2); and for **B.** all different treatments (excluding T5). T2 demonstrators (T2-D) and observers (T2-O), T3 pre-exposed to stimulus (T3-P), and both subject conditions (observers and demonstrators) of T1 and T4, which are pooled per treatment since probability of attacking was the same (i.e. zero). Table of numbers depicts sample progression across number of trials.

Cox Proportional Hazards		Est.	Std. Err	z value	p value
Inhibition social learning (T2):	(global assumption: $X^2 = 2.14$, p = 0.144)				
Surv(Trial event, right censor) ~ Subject	Subject	2.038	0.521	3.910	< 0.0001
All treatment conditions:	(global assumption: $X^2 = 3.00$, p = 0.557)				
Surv(Trial event, right censor) ~ Condition	T4	2.704	0.596	4.536	< 0.0001
	T3-P	0.359	0.451	0.797	0.4260
Reference: T2-D	T2-O	1.956	0.478	4.091	< 0.0001
	T1	2.704	0.569	4.754	< 0.0001
	T3-P	-1.597	0.460	-3.471	0.0005
Reference: T2-O	T2-D	-1.956	0.478	-4.091	< 0.0001
	T4	0.748	0.496	1.507	0.1318
	T1	0.748	0.463	1.615	0.1063
	T2-D	-0.359	0.451	-0.797	0.4257
Reference: T3-P	T4	2.345	0.579	4.052	< 0.0001
	T2-O	1.597	0.460	3.471	0.0005
	T1	2.345	0.551	4.259	< 0.0001
	T2-O	-0.747	0.496	-1.507	0.1320
Reference: T4	T3-P	-2.345	0.578	-4.052	0.0001
	T2-D	-2.704	0.596	-4.536	< 0.0001
	T1	0.000	0.504	0.000	1.0000
	T4	0.000	0.504	0.000	1.0000
Reference: T1	T2-O	-0.747	0.463	-1.615	0.1060
	T3-P	-2.345	0.550	-4.259	< 0.0001
	T2-D	-2.704	0.568	-4.754	< 0.0001

Table 1. Cox proportional hazard models and pairwise comparisons between all experimental treatments. T2 demonstrators (T2-D) and observers (T2-O), T3 pre-exposed to stimulus (T3-P), and both subject conditions (observers and demonstrators) of T1 and T4, which are pooled per treatment since probability of attacking was the same (i.e. zero).

Number of attacks during trials

As mentioned above, predatory behaviour (i.e. attack stimulus) was not registered in both T1 and T4, for either demonstrators or observers (Figure 3.1B). As such, only demonstrators and observers from the inhibition by social learning test (T2-D and T2-O, respectively), and subjects pre-exposed to stimulus (T3-P) were analyzed statistically for significant differences in the number of attacks per trial (Negative Binomial GLMM, Table 2, Figure 3.2). Concordantly to what was verified in learning rates, T2-D and T3-P were shown to not differ in the number of attacks performed per trial (Reference T2-D, Table 2). Furthermore, T2-O also performed significantly less attacks per trial than both T2-D and T3-P (Reference T2-O, Table 2), thus fully confirming the pattern registered for learning rates. As expected, a general decreasing trend on the number of attacks over trials was reported for all three treatment conditions (Trial, Table 2, Figure 3.2).

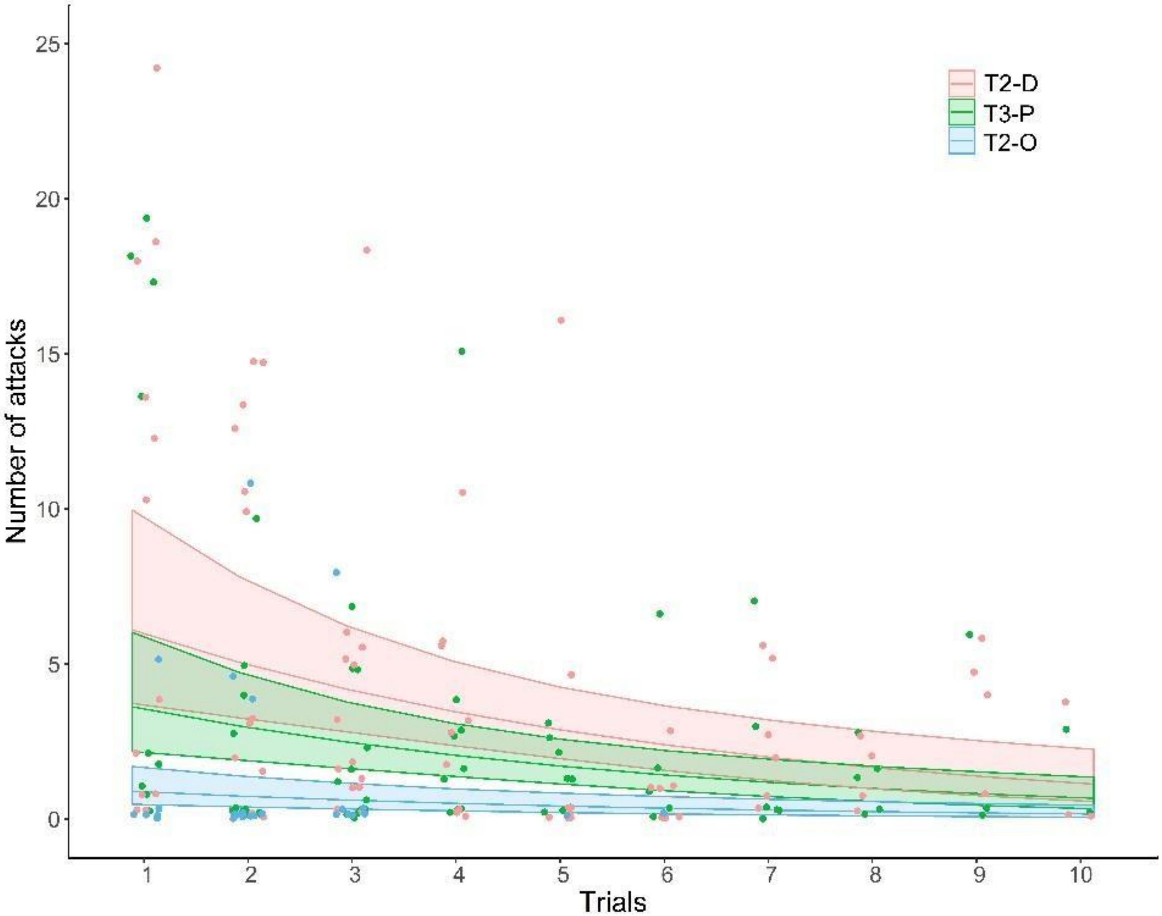

Figure 3.2. Mean number of attacks per trial (model and data points) by different treatment subjects: T2 demonstrators (T2-D) and observers (T2-O), and T3 pre-exposed to stimulus (T3-P).

Negative Binomial Mixed Model		Est.	Std. Err	z value	p value
Attacks ~ Condition + Trial + (Session | Trial)					
	(Intercept)	1.995	0.283	7.041	< 0.0001
Reference: T2-D	T3-P	-0.520	0.281	-1.853	0.0639
	T2-O	-1.912	0.382	-5.001	< 0.0001
	Trial	-0.187	0.050	-3.761	0.0002
	(Intercept)	0.082	0.335	0.246	0.8054
Reference: T2-O	T2-D	1.912	0.382	5.001	< 0.0001
	T3-P	1.392	0.388	3.590	0.0003
	Trial	-0.187	0.050	-3.761	0.0002
	(Intercept)	1.474	0.291	5.074	< 0.0001
Reference: T3-P	T2-D	0.520	0.281	1.853	0.0639
	T2-O	-1.392	0.388	-3.590	0.0003
	Trial	-0.187	0.050	-3.761	0.0002

Table 2. Negative binomial mixed model and pairwise comparisons between all experimental conditions which reported attacking behaviours: T2 demonstrators (T2-D) and observers (T2-O), and T3 pre-exposed to stimulus (T3-P).

Latency attack time in T2 and T5

Lastly, mean latency time to elicit predatory behaviour (i.e. attack stimulus) was not significantly different between trials in T2, but was significantly higher for T2-O than for T2-D (GLMM, T2, Table 3, Figure 3.3A). Conversely, in T5 (i.e. prey reward as positive control) the trend registered in T2 was reversed, with observers (T5-O) now exhibiting significantly lower latency time responding to stimulus presentation than demonstrators (T5-D) (GLMM, T5, Table 3, Figure 3.3B).

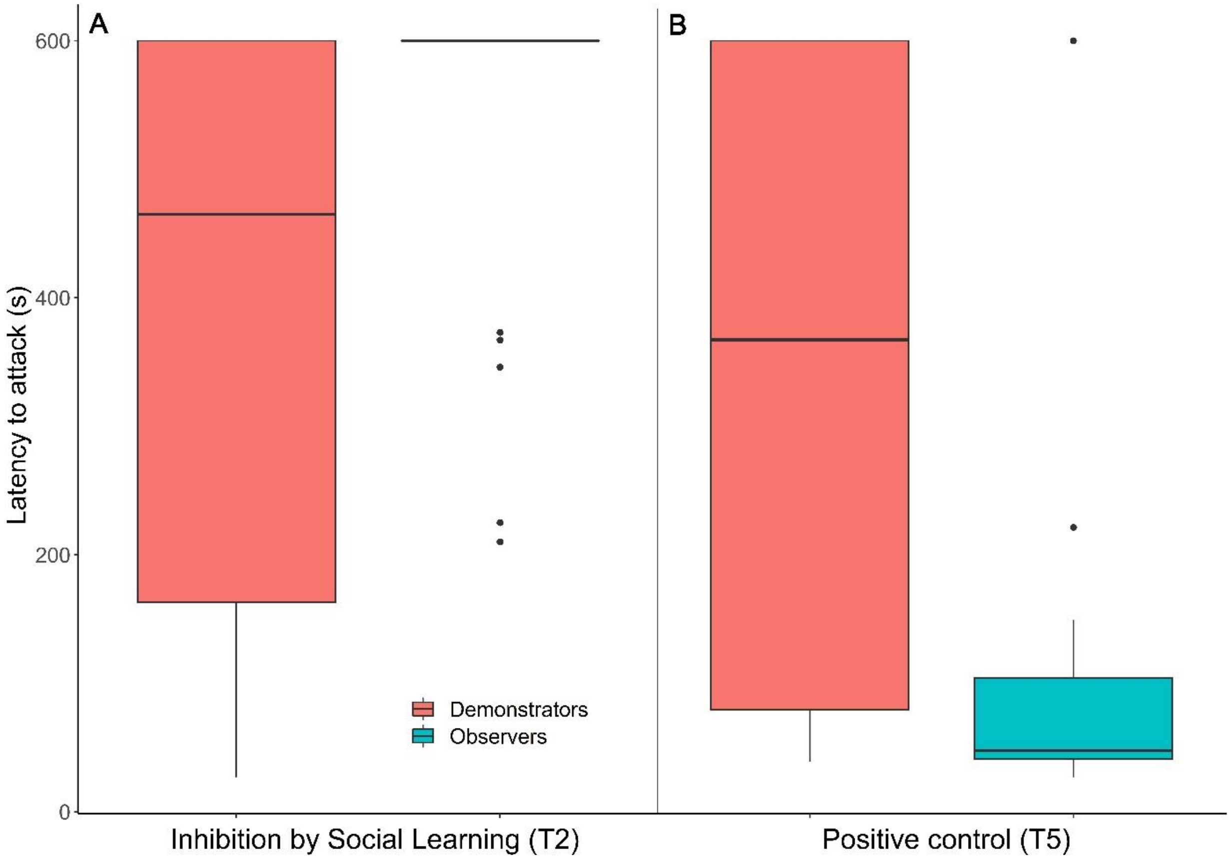

Figure 3.3. Mean latency time (seconds) to attack the stimulus by demonstrators (D) and observers (O), in **A.** the inhibition by social learning test (T2-D and T2-O), and **B.** in the positive control test (T5-D and T5-O).

Generalized Linear Mixed Model		Est.	Std. Err	df	t value	p value
Inhibition Social learning (T2):	(Intercept)	308.081	47.471	5.651	6.490	0.0008
Latency ~ Subject + Trial + (Session | Trial)	Subject	212.038	35.201	126.591	6.024	< 0.0001
	Trial	16.112	8.515	8.165	1.892	0.0944
Positive Control (T5):	(Intercept)	281.473	62.905	1.826	4.475	0.0548
	Subject	-225.886	74.207	32.91	-3.044	0.0046
Latency ~ Subject + (Session | Trial)						

Table 3. General linear mixed models for measuring differences in T2 (T2-O vs T2-D) and T5 (T5-D vs T5-O) of mean latency response time.

Discussion

We show that cuttlefish (up to 5 days post hatching) use social information and can learn to inhibit (or modulate) predatory behaviour, as a result of observing other individuals attempting to retrieve prey behind a glass tube. In addition to the significant reduction of predatory behaviour in observers of naïve individuals, we highlight that the: i) correlation between number of trials taken to learn by pairs of demonstrators and observers (which simultaneously shows that potential inactivity did not bias results); ii) non-differentiation between pre-exposed and naïve individuals (i.e. non-experienced demonstrators), indicating that knowing the stimulus beforehand did not improve learning rates; iii) non-elicitation of predatory behaviour when paired with an experienced demonstrator; and iv) the inversed time latency response pattern in the positive control (again controlling for inactivity biases), corroborate our reasoning.

Previous studies with adult/juvenile cuttlefish reported no clear evidence of social transmission of correct predatory behaviour (Boal et al. 2000), and different individual responses in the improvement of evading behaviour (Huang and Chiao 2013). Since *S. officinalis* hatch within close vicinity to others in nature, hatchlings may be more gregarious or socially tolerant - further hinted by laboratory rearing (Boal 1996) - than the later life stages used in the previous studies, which may have facilitated learning of the task. Moreover, different methodologies (e.g. in Boal et al. 2000, multiple cuttlefish simultaneously observed one cuttlefish performing the test, which may have led to unwarranted third-party audience effects), as well as distinct end-state and learning mechanisms underlying different tasks, are likely to be another of the causes for discrepant results among studies. By adopting a test measuring higher-level parameters (attack/not attack, number of attacks), we simplified previous experimental designs that entailed higher intraspecific variation (i.e. assessing choices of attack/defense strategies), which provided more consistent results.

Social learning theoretical frameworks are continuously being updated, and not always unanimous on specific applicable nomenclature and underlying mechanisms (see Biederman et al. 1993; Heyes 1994; Galef and Laland 2005; Olmstead and Kuhlmeier 2015). Taking that into account, and moving from more heuristic to theoretically more cognitively-complex mechanisms, stimulus enhancement is generally defined as observers being drawn quicker to a stimulus, and individually learning how to perform the task through trial-and-error (Galef and Laland 2005, Olmstead and Kuhlmeier 2015). Vicariously learning the “prawn-in-the-tube” procedure appears to go beyond stimulus enhancement, since only a small portion of observers (30%) required trial-and-error learning. Social facilitation (or social enhancement) predicts that the mere presence of conspecifics, regardless of their performance, will make observers perform better in the task (Klein and Zentall 2003). However, our results show a marked correlation between the number of trials taken for a naïve demonstrator to learn, and the number required by its respective observer. Moreover, observers of trained demonstrators did not produce one single attack on the stimulus, indicating that learning is mediated by the performance/behaviour of the demonstrator, and not only by its presence. Observational Pavlovian conditioning (through stimulus-stimulus learning) predicts that the same unconditioned response should be transferred from demonstrator to observer, which does not seem to be the case here, as seen by observers that learned the task despite being paired with demonstrators that did not learn. Thus, the occurrence of emulation appears to be the most likely social learning process, underpinned more on a “learning what” process instead of “learning how” (i.e. affordance learning, see Galef and Laland 2005; Olmstead and Kuhlmeier 2015). Rather than concentrating on the demonstrator’s actions *per se,* emulation through affordance learning dictates a focus on recognizing the properties of the object (i.e. there is a glass tube) through the actions of the demonstrator. Accordingly, the presence of the conspecific performing the task is key for learning, which explains why individuals pre-exposed to the stimulus did not perform better than naïve demonstrators, when observers did (individuals that observed the stimulus and a conspecific performing the task). Moreover, affordance learning as the underlying social learning mechanism aligns with how cuttlefish self-learn this procedure (Heyes 2012), i.e. by recognizing the existence of the glass tube through multimodal sensorial integration (Cartron et al. 2013). However, end-state or goal emulation, i.e. emulation/mimicry of the final demonstrator behaviour after trying to get the prey, are other possible mechanisms that could not be disentangled through the used experimental conditions.

Cuttlefish eggs do not receive any parental care (Nixon and Mangold 1998), but individuals can gather information about existing predators and prey through vision and olfaction from within the egg (Darmaillacq et al. 2008, Guibé et al. 2012), showing that cuttlefish hatchlings are capable of individual learning albeit learning rates improve over ontogeny (Dickel et al. 1997). However, despite the still immature state of key brain areas (Dickel et al. 1997, Liu et al. 2017), we found that cuttlefish newborns are additionally able to learn through emulation and perform much more cognitively-demanding tasks (i.e. incorporating information from observing conspecifics, and inhibit natural behaviour), effectively improving the efficiency of their actions towards new stimulus. In the wild, the potential advantages of inhibiting predatory behaviour following actions of conspecifics, can go from preventing meaningless energy expenditure, to predatory avoidance associated with disengaged camouflage when attacking (Hanlon and Messenger 1988). Moreover, this occurs at a critical life stage, where the ability to circumvent trial-and-error for acquiring knowledge from the environment can signify avoiding extremely costly actions, conferring an evolutionary advantage to hatchlings that incorporate information provided by conspecifics.

Recent studies have highlighted the comparatively slower conditions of cephalopod genome evolution linked to higher RNA editing capabilities (Liscovitch-Brauer et al. 2017), together with the presence of serotonergic neurotransmission and its conserved role underpinning social behaviour in the same solitary octopus species (Edsinger and Dölen 2018). Considering the ~600-million-year gap in the evolutionary pathways of vertebrate and invertebrate neural systems (Hochner 2008, Amodio et al. 2018), further investigation of cephalopod social behaviour and learning, outputted behavioural plasticity, and respective neural correlates, can shed light on universal mechanisms shared between the two distinct branches, and deepen our knowledge on the evolution of complex learning and cognition. As a model case-study of convergent evolution (Darmaillacq et al. 2014, Amodio et al. 2018), cephalopods are known for their vertebrate-like cognitive abilities, and our findings further reveal the potential for neuroplasticity and behavioural flexibility of these invertebrate brains (Schnell and Clayton 2019). These highly responsive phenotypic features are instrumental for quickly adapting to changes in the environment, by minimizing costs and maximizing individual fitness in nature.

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Author contributions

ES, VM, and RR conceived the study. ES, VM, CSR, BBLM, BM, AML, and EM reared animals, performed the experiment, and collected the data. ES and CSR analysed the data. All authors contributed to the manuscript, agree to be held accountable for the content therein, and approved the final version of the manuscript.

Ethics approval

Research was conducted under approval of Faculdade de Ciências da Universidade de Lisboa animal welfare body (ORBEA) and Direção-Geral de Alimentação e Veterinária (DGAV), within the framework of project VALPRAD (MAR-01.04.02-FEAMP-0007), in accordance with the requirements imposed by the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

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PART II

4

Evidence of social tool use byoctopuses

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Keywords: Tool use, social, heterospecific, manipulation, octopus.

Under review

Abstract

Tool use, the manipulation of objects for a specific goal, is relatively widespread across the animal kingdom. Social tool use is a rarer phenomenon entailing the physical/psychological manipulation of another animated object. We show direct observational evidence that octopuses (*Octopus* cyanea) can manipulate animated objects for their own goals. Two different natural events were recorded two weeks apart, where *O. cyanea* use a nearby sea cucumber (*Holothuria atra*) as den cover and vantage viewpoint, thus physically coercing the animated object and using it as an inanimate object (social tool use Level 1). To our knowledge, this study constitutes the first proof of social tool use in non-social or gregarious animals (i.e. absence of conspecific interactions), and we suggest that complex heterospecific/ecological environments may enable its occurrence. Our findings extend the defensive behavioral repertoire and the nature of tools usable by octopuses, and open the possibility of social tool use occurring in a wider range of phylogenies across the tree of life.

Introduction

First thought to be exclusive to humans and primates, it has become increasingly clear that tool use can occur across the tree of life in numerous forms (Seed and Byrne 2010). Based on Shumaker et al. (2011), Mann & Patterson (2013) define tool use as '*the conditional external employment of an unattached or manipulable attached environmental object to alter more efficiently the form, position, or condition of another object, another organism, or the user itself, when the user holds and directly manipulates the tool during or prior to use and is responsible for the proper and effective orientation of the tool'*. Therefore, this definition rules out shelters or objects perpetually attached to the body, but includes, for instance, objects immediately used in shaping shelters or manipulated in specific ecological contexts (e.g. deter a predator).

Another form of tool use involves using animated objects (i.e. live beings) instead of inanimate objects. Völter et al. (2015) define social tool use as '*physical and psychological manipulations of animate beings towards some goal*'. Social tool use can be divided in 4 Levels, depending mostly on the different degrees of physical influence exerted by the user and the self-motivation of the social tool. While in Level 1 the social tool is completely forced by the user in a manner analogous to an inanimate object, in Level 2 the social tool may also exhibit self-behaviors while being manipulated physically. Thus, Levels 1 and 2 are viewed as coercive since the social tool is physically forced by the user. Complementary, Levels 3 and 4 are generally termed as cooperative since they are grounded on the social tool's self-motivation and communication (see Völter et al. 2015 for examples).

Over the years octopuses have been shown to use various tools for defensive strategies. Primarily, and most frequently, several species make immediate use of rocks to modify shelters and block the entrance to their den to incoming predators (Mather 1994, Hanlon and Messenger

2018). Increasing in complexity, some octopus species have also been shown to be capable of composite tool use (Amodio et al. 2018). Outside its den, the veined octopus *Amphioctopus marginatus* can carry two coconut halves and use these to form a 'mobile den' in which it can hide in case of approaching danger (Finn et al. 2009). Additionally, the common octopus *Octopus vulgaris* (South Africa) can use individual suckers to pick up multiple empty clam shells, envelope its whole body with the arms externally-rotated so that shells face outward, thus forming a spherical physical armor to deter predators (Jeffs and Brownlow 2017).

While there are indicative signs of flexibility regarding the nature of the tools used (e.g. rocks, coconuts), octopuses have never been directly observed using 'animate objects' as tools, i.e. social tool use, despite several suggestive observations. Early reports detail common blanket octopus *Tremoctopus violaceus* individuals that have taken hold of Portuguese man-of-war *Physalia physalis* tentacles and the possibility of using these as 'weapons' (Jones 1963). Likewise, (Rosa et al. 2019) reports and discusses several reported associations between pelagic octopus species and cnidarians with different potential goals (including hijacking), but hitherto we lack *in loco* observations providing direct evidence of tentacle/body usage for defense, prey capture or other purposes. Here we report the first direct observations of octopus (*Octopus cyanea*) individuals using and manipulating live heterospecifics for a self-serving end.

Methods

While filming interspecific hunting between octopus and fishes via SCUBA diving (Sampaio et al. 2020), we recorded 2 interactions of *Octopus cyanea* with nearby black sea cucumbers *Holothuria atra*. The footage (approximately 30 seconds each) was recorded in the same location in Eilat (Israel) with Sony Alpha II cameras, at approximately 1-2 m depth, in 2 different days (11-

10-2018 and 27-10-2018) corresponding to 2 different events (n = 2). Event 1 was filmed with one camera view, whereas Event 2 was filmed with two camera views (all videos collated in Video S1). We annotated the main behavioral motifs and respective time stamps in each event to analyze the interspecific interactions between *O. cyanea* and *H. atra*.

Results

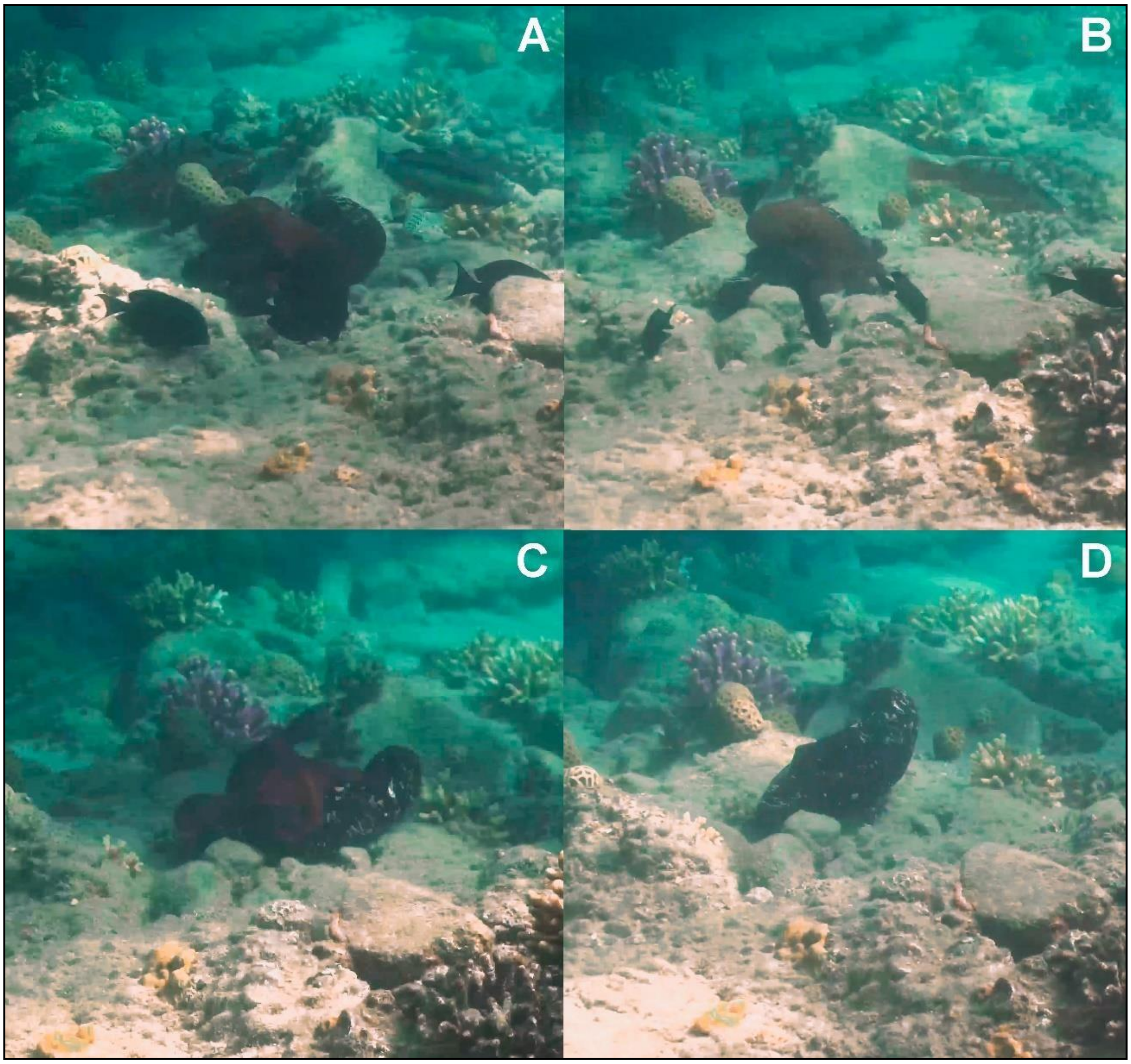

Figure 4.1. Zoomed in snapshots taken from Video S1 (Event 1). **A.** *O. cyanea* (red/brown) lunges towards an *H. atra* individual, **B.** sitting on top and grabbing it with the arms and suckers. Afterwards, **C.** the octopus rolls/pulls the sea cucumber towards itself, **D.** ultimately placing it as a den cover.

In the first event (11-10-2018, Event 1 - Video S1), the octopus lunges forward towards the *H. atra* individual (Figure 4.1A, 00:04-00:09), afterwards grabbing hold of it using its arms and suckers (Figure 4.1B), and rolling the sea cucumber back and on top of itself (Figure 4.1C, 00:22-00:30) to serve as den cover (Figure 4.1D). In the second event (27-10-2018, Event 2 - Video S1), the octopus lifts the sea cucumber mainly with its right arms II and III, and enters the den (00:53-01:00) (Figure 4.2A). It then attempts to further manipulate/displace the sea cucumber twice with the upper regions of right arms I and II (01:05-01:15), ultimately seizing it via arm suckers (exposing a noticeably pink area under the arms as in Event 1, Video S1), and using *H. atra* as both a den cover and a vantage viewpoint (01:18-onwards, Figure 4.2B). On both occasions, no noticeable self-motivated movement or self-serving behavior *by H. atra* was discerned.

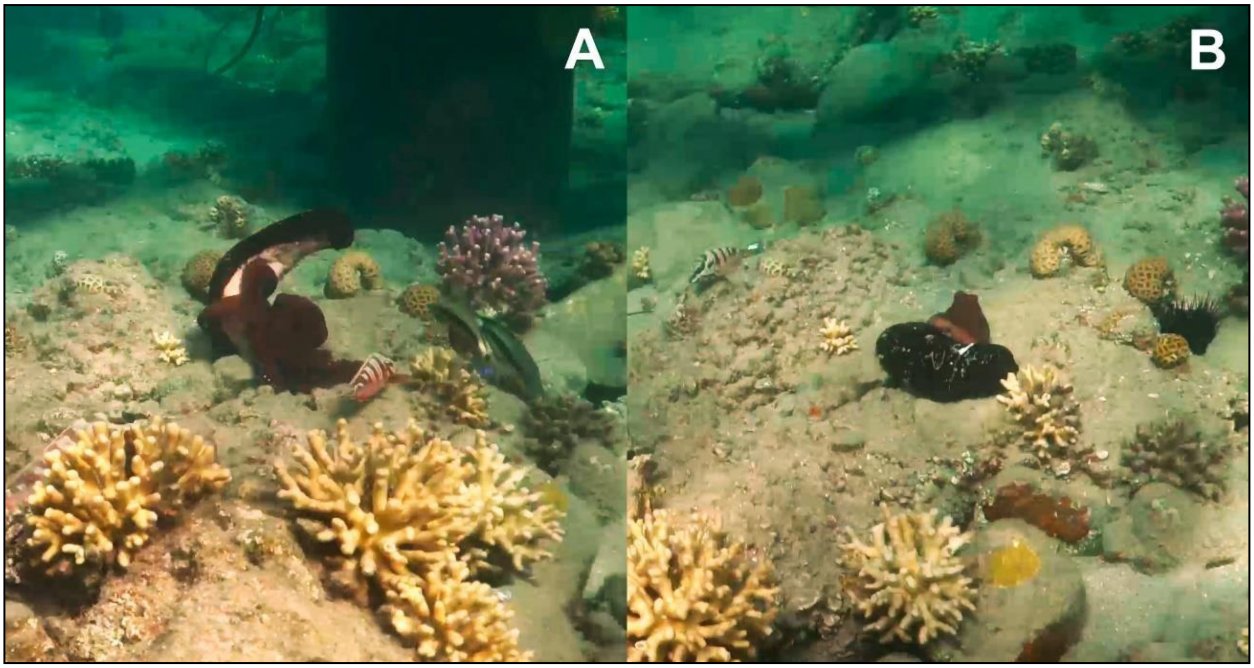

Figure 4.2. Zoomed in snapshots taken from Video S1 (Event 2). **A.** *O. cyanea* lifts up the *H. atra* individual, **B.** proceeding to use it both as den cover and vantage point to observe the environment.

Discussion

The behavioral actions by the user in both events are similar to defensive tool use with rocks, where an octopus blocks the den's entrance by pulling nearby rocks to enclose itself (Mather 1994). As an *H. atra* individual is near the den in both events, the octopus reaches and pulls the sea cucumber (Figure 4.1, Event 1, Video S1), also using it as a den cover and as a vantage point to inspect the surrounding environment (Figure 4.2; Event 2, Video S1). These observations show that *O. cyanea* can coercively use *H. atra* as a tool, with no discernable self-motivated movement by the sea cucumber. Following the definitions by Völter et al. (2015), the filmed interspecific interactions reveal that octopuses are capable of social tool use Level 1, i.e. using an animated object as an inanimate object to fulfil an action solely motivated by the user.

Comparatively to other cases of social tool use in the animal kingdom, this use of an animated object is analogous to an orangutang (actor) pulling the arm of another orangutang (social tool) to access a piece of fruit in the latter's hand (Völter et al. 2015). That is, the social tool is entirely coerced, and in that action performs no self-motivated behavior, being effectively used as an inanimate object. Another analogous example in the marine realm is the social tool use of sponges by dolphins, while foraging for bottom-dwelling prey (Patterson and Mann 2011). Dolphins use sponges as inanimate objects to churn the seabed/protection, and no 'live' properties of the tool are taken advantage of. This sponging behavior is found in specific pods showing that it is socially transmitted between related conspecifics (Krützen et al. 2005). In our observations, since both events occurred in the same location with a ~2-week time gap and the filmed octopus seems to be of similar size, it is likely that it is the same individual.

Importantly, most species that have been identified as social tool users thus far have social/gregarious lifestyles, usually characterized by large groups of conspecifics or prolonged co-existence in smalls groups of kin. For example, other animal groups showing social tool use as defined by (Völter et al. 2015) include: i) several species of hermit crabs carrying anemones as protection from predators (Ross 1983, Mann and Patterson 2013) - which would be analogous to a potential proof that the octopus *T. violaceus* uses Physalia tentacles to deter predators (Jones 1963); ii) social insects, e.g. weaver ants (genus *Oecophylla*) using conspecifics as means to glue leaves (see (Pierce 1985); and iii) primates, which often rely on forms of social tool use based on self-motivation and initiative by the tool, e.g. mother orangutangs hand tools to progeny, which can then use said tools to provide food for both [4]. To our knowledge, *O. cyanea* is the first species where individuals that have a solitary life history conspecific-wise (the only exception is their mating season, shortly after which both males and females perish (Hanlon and Messenger 2018)), are capable of manipulating and coercively use animated objects (in this case, heterospecifics) for their own self-serving ends. Evolutionarily, this finding indicates that conspecific sociality is not strictly necessary for animals to learn how to use other animated objects, i.e. for social tool use to occur. We suggest that, in its stand, heterospecific environments involving complex interactions beyond predator-prey relationships (e.g. interspecific collective hunting (Sampaio et al. 2020)) may provide similar ecological/cognitive conditions for behaviors exploring other individuals to arise (see (Oliveira and Bshary 2021) for a review on heterospecific sociality).

Taken together with previous signs of potential social tool use (Jones 1963, Rosa et al. 2019), the *in loco* evidence presented here warrants further observations and potential experimental manipulations to better ascertain the frequency (opportunistic or consistent) and variety of social tool use in octopuses. Cephalopods are known for being exceptionally adaptable to their surrounding environment, exhibiting broad behavioral flexibility and a notable capacity to develop defensive strategies involving inanimate objects (Amodio et al. 2018). Our findings extend the nature of tools usable by octopuses, which can include other animals (i.e. animated objects). Hence, as in other (albeit social) species (Patterson and Mann 2011, Fujii et al. 2015), the occurrence and diversity of object manipulation and tool use by octopuses appears connected to the heterospecific environment and ecological contexts. These direct observations open new paths for research exploring adaptive ecological strategies, social tool use, and defensive behavior flexibility, in a highly-cognitive marine invertebrate, as well as in other less studied non-social (conspecific-wise) phyla of the tree of life.

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Supplemental Materials

Video S1. *Octopus cyanea* uses *Holoturia atra* to close den entrance and as a vantage viewpoint. 2 separate events (n = 2), with 1 view on the 1st event (11-10-2018), and 2 different views on the 2nd event (27-10-2018). (www.dropbox.com/s/ax39rbe393112vt/VideoS1_1K.mp4?dl=0)

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5

Octopuses punch fishes during collaborative interspecific hunting events

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Cooperation is ubiquitous in nature, and occurs at all levels of biological complexity, providing immediate direct benefits and/or future indirect benefits to participating partners (Lehmann and Keller 2006, Bshary and Bergmüller 2008). In interspecific interactions, the lack of relatedness between individuals ensures that the underlying dynamics and potential feedback mechanisms can be interpreted in terms of direct, personal benefits. Moreover, in multi-specific collaborative hunting groups, ecology and game theory are implicitly linked, as the life history and evolved hunting strategy of each species often leads to specialized roles within a group (asymmetric conditions), which facilities coordination (Bshary et al. 2006, Bshary and Bergmüller 2008). For instance, collaborative hunting between moray eels and groupers provides one of the most elaborate examples on how different species with complementary hunting techniques (groupers hunt in the water column and eels enter rock crevices) can join forces and increase their predation success (Bshary et al. 2006, Vail et al. 2013, 2014). Involving active recruitment and referential gestures, the nature of this relationship is mutually beneficial (byproduct mutualism), i.e. both can increase their hunting success rate from the presence of the other species, which likely played an important role in the emergence of complex interactions between groupers and eels.

Concurrently, groupers and various other species of coral reef fishes are also known to form hunting associations with octopuses (Figure 5.1), often involving numerous partners from several species at the same time (Diamant and Shpigel 1985, Forsythe and Hanlon 1997, Bayley and Rose 2020). These events can last over one hour, with octopuses pursuing prey within rock and coral crevices (identically to the moray eel), while other fishes search the sea floor around a larger perimeter (bottom-feeders, e.g. yellow-saddle goatfish *Parupeneus cyclostomus*) and others guard the water column (semi-benthic predators, e.g. smooth cornetfish *Fistularia commersonii*) (Video

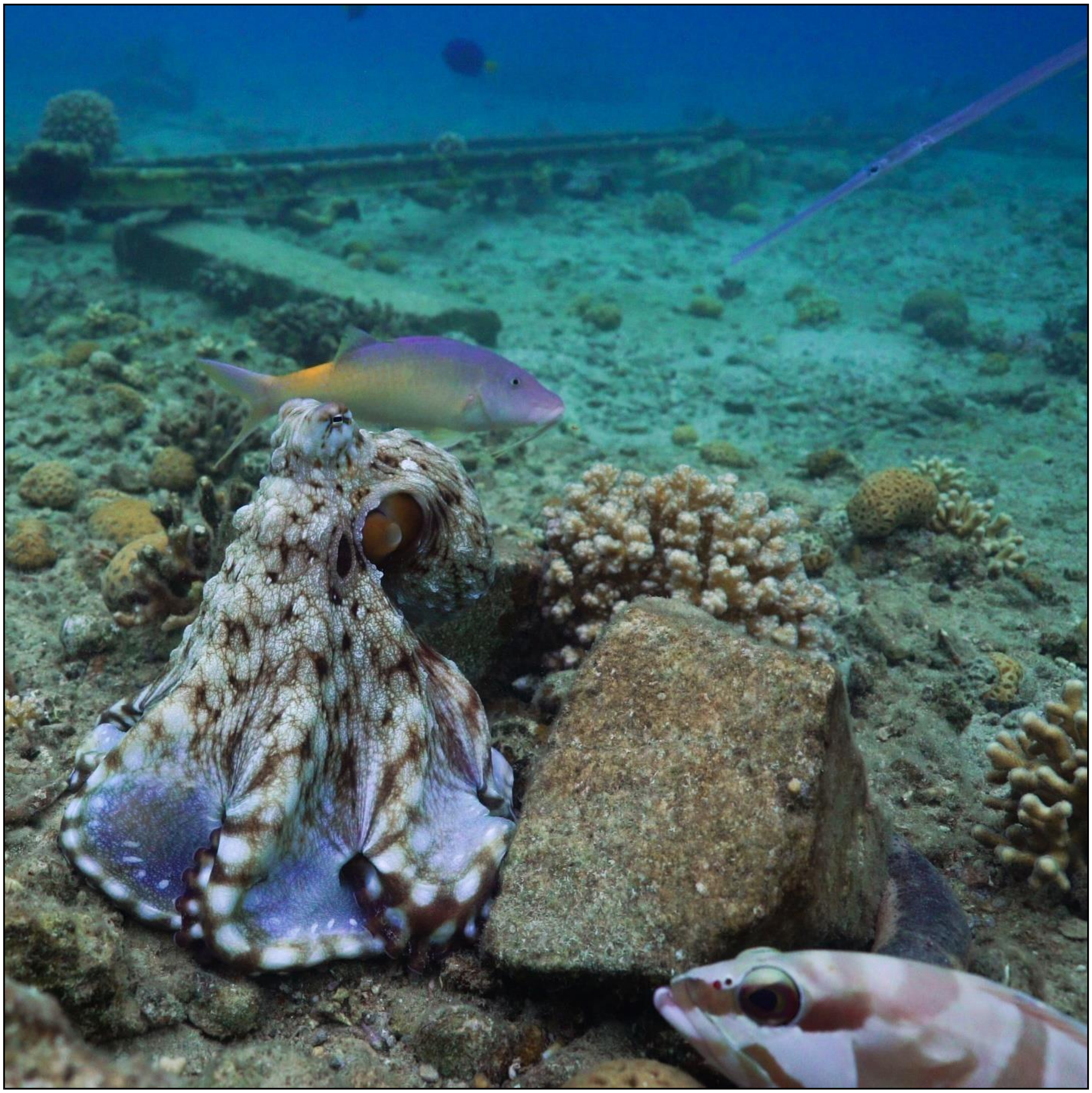

Figure 5.1. Example of a multi-specific hunting group composed by a day octopus *Octopus cyanea*, a yellow-saddle goatfish *Parupeneus cyclostomus*, a smooth cornetfish *Fistularia commersonii*, and a blacktip grouper *Epinephelus fasciatus* (Video S1).

S1). While the octopus plays a central role, some of its followers are opportunistic predators that join the group, and do not actively seek prey (e.g. tailspot squirrelfish *Sargocentron caudimaculatum*). With these species, interspecific interactions may be commensalistic or even parasitic (Diamant and Shpigel 1985). However, octopuses also follow fish partners for more prey opportunities, namely: groupers, that use referential gestures to signal prey locations to octopus in the same way as they do to moray eels (Vail et al. 2013); and goatfishes, which also scour the sea bottom and crevices (Video S2, see also Bayley and Rose 2020). These observations suggest that with certain species of fish partners, interspecific interactions can be mutualistically beneficial (Bshary and Bergmüller 2008). As a result, in heterogeneous multi-specific groups, conflicts between partners can arise over the level of investment or the distribution of payoffs (Lang and Farine 2017). Thus, in this complex social network of interactions, partner control mechanisms might emerge in order to prevent exploitation and ensure collaboration (Raihani et al. 2012).

Here we report a series of events, dating between 01-10-2018 and 01-11-2018 (29.5577°N, 34.9519°E, Eilat, Israel), and 10-05-2019 and 10-07-2019 (26.2032° N, 34.2165° E, El Quseir, Egypt), where different *Octopus cyanea* individuals engage in active displacement of partner fish during collaborative hunting. To this end, the octopus performs a swift, explosive motion with one arm directed at a specific fish partner, which we refer to as "punching" (Figure 5.2). We recorded punches (n=8 events, see video S2) targeting different fish species: tailspot squirrelfish (*S. caudimaculatum,* Event 1), blacktip (*Epinephelus fasciatus,* Events 2 and 3) and lyretail (*Variola louti*, Event 4) groupers, yellow-saddle (*P. cyclostomus,* Event 5 and 6) and Red Sea goatfishes (*Parupeneus forsskali,* Event 7), and halfspotted hind (*Cephalopholis hemistiktos,* Event 8). These multiple observations involving different octopuses in different locations suggest that punching serves a concrete purpose in interspecific interactions. From an ecological perspective, actively punching a fish partner entails a small energetic cost for the actor (i.e. octopus), and simultaneously imposes a cost on the targeted fish partner. From the fish's standpoint, this cost can take several forms, such as: subtraction of an immediate opportunity to catch prey (e.g. Events 3 and 8), relocation to a more external or less advantageous location in the group (e.g. Event 5), or even permanent eviction (e.g. Event 1). Thus, from the octopus' perspective, punching serves as a partner control mechanism, the nature of which is dependent on the ecological context of the interaction, and on how the octopus benefits from inflicting costs on fish partners (Clutton-Brock and Parker 1995, Bshary and Bergmüller 2008).

Figure 5.2. Image sequence depicting the behavioural action of *O. cyanea* "punching" (white arrows) a yellow saddle goatfish (*P. cyclostomus*) partner during interspecific multi-collaborative hunting (see Video S3).

In cases where continuous interactions over time and collaboration are not evident - *S. caudimaculatum* has an opportunistic hunting strategy and is not reported to be commonly included in these interspecific hunting groups (Diamant and Shpigel 1985) -, simple competition for similar food resources can explain the punching behavior (Event 1) (Raihani et al. 2012). In situations where collaboration does exist, and the octopus punches a specific partner to gain direct access to prey (performing a web-over immediately after punching, e.g. towards *E. fasciatus* or *C. hemistiktos* in Events 3 and 8 respectively), immediate benefits are yielded from that aggressive action. That is to say, in this scenario the octopus performs a self-serving behavior (displacing a fish to access prey), which despite a small energetic cost produces immediate benefits. This action simultaneously imposes a cost to the partner (subtraction of prey opportunity) and can promote cooperation in future interactions. This mechanism is a form of direct negative pseudo-reciprocity, i.e. sanctions (Raihani et al. 2012). Thus, when the octopus punches and obtains immediate benefits from that action, the underlying mechanisms and ecological role are relatively simple and direct.

However, other events show that punching is not always followed by an attempt to retrieve prey (e.g. Events 5 and 6), indicating it also occurs in the absence of immediate benefits. In a collaborative context, such as with the yellow-saddle goatfish *P. cyclostomus* (Bayley and Rose 2020, Video S2) or with certain species of groupers (Vail et al. 2013), other mechanisms might explain punching. In these cases, two different theoretical scenarios are possible. In the first one, benefits are disregarded entirely by the octopus, and punching is a spiteful behaviour, used to impose a cost on the fish regardless of self-cost, e.g. after defection (stealing prey) by a usually collaborative partner (Clutton-Brock and Parker 1995). In the other theoretical scenario, punching may be a form of aggression with delayed benefits (i.e. direct negative reciprocity or punishment), where the octopus pays a small cost to impose a heavier one on the misbehaving partner, in an effort to promote collaborative behavior in the following interactions (Clutton-Brock and Parker 1995). In other words, punching might impose an immediate cost to both partners, but since hunting groups promote additional subsequent interactions, such negative feedback can yield an overall higher benefit for players in the long run (Raihani et al. 2012). Documented cases of consistent change in partner behaviour after negative feedback are rare in non-human species (Raihani et al. 2012), making its potential use by octopuses during collaborative hunting worthy of further investigation. However, in order to disentangle between the numerous mechanisms that may underly punching behaviour, careful studies of (subsequent) interactions between the octopus and the targeted fish, within the changing dynamics of the group, are warranted.

Comparatively to the paired structure of the grouper-moray eel system (Bshary et al. 2006), the existence of direct negative feedback mechanisms when octopus and multiple fish partners hunt together, indicates that additional rules shape these ecological relationships. Thus, the multi-layered network of interactions suggests that the underpinnings of these interspecific groups are significantly more complex than what both pairwise collaborative associations, or group nuclear-follower ecological models, describe (Diamant and Shpigel 1985, Vail et al. 2013). Detailed quantitative analyses of these multi-specific hunting events can explore several other important ecological questions, such as the potential existence of privileged relationships between octopuses and specific fish partners (e.g. are some species or individuals more punched than others?), and how individual dynamics are modulated by the network of social interactions (e.g. do fishes also provide feedback to each other?).

Further work on this severely understudied system can shed light on costs, benefits, and control mechanisms in underlying game structures (Bshary and Bergmüller 2008, Raihani et al. 2012), unexplored cognitive processes (Vail et al. 2013, 2014), particularly for an otherwise-solitary marine invertebrate (Schnell and Clayton 2019), as well as the ecological role and conditions promoting the emergence of multi-specific cooperation (Lehmann and Keller 2006, Lang and Farine 2017).

Supplemental Material

Supplemental Videos available at https://doi.org/10.1002/ecy.3266.

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6

Shared decision-making and hierarchical species dynamics in multispecific collective hunts between octopus and fishes

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In preparation

Abstract

Group decision-making is defined across a despotic-shared leadership axis, i.e. ranging from one individual leader to fully distributed leadership. Multispecific hunting groups provide complex interaction scenarios, where distinct species-specific hunting strategies entails distinguishing among social information sources differing in morphology, behavior, and cognition. We performed field observations of interspecific hunting events between the otherwise-solitary *Octopus cyanea* and multiple fish partners using a mobile stereo-camera system. We then reconstructed both the habitat and the animal movement tracks in 3D. While previous studies have mostly described these interactions as a nuclear-follower system centered around the octopus, our results show that decision-making in octopus-fish groups is not despotic, but shared. Moreover, leadership status is not random or equal between individuals: goatfishes (particularly adult yellowsaddles) are the main effective leaders hierarchically, while other species tend to have more follower roles. Conversely, octopuses (and black tip groupers) are the main hierarchical anchorers in interspecific groups. Respectively, we show that these roles are also linked to movement kinematics and group characteristics, namely individual speed and distance to centroid. Our findings show the influence of higher order biological traits (morphological, behavioral, and cognitive) in collective movement and decision-making in these heterogenous groups, and that heterospecific sociality promotes the use of social information and drives decision-making in a cognitively evolved and "solitary" cephalopod.

Introduction

In collective behavior, complex coordination and decision-making abilities in groups can emerge from simple localized rules of interaction (Vicsek et al. 1995, Watts and Strogatz 1998, Couzin et al. 2002). Concordantly, groups of monospecific individuals demonstrate intricate patterns of movement due to simple social interactions based on algorithmic attraction-repulsion rules (Couzin et al. 2005, Buhl et al. 2006, Ballerini et al. 2008). However, despite the prevailing paradigm in collective movement studies considering every individual as an identical "self-propelled" particle, such is rarely the case in nature. Biological systems have inherent intraspecific variation that can alter the "weight" of an individual's influence within a group (Couzin 2009, Nagy et al. 2013, Jolles et al. 2017, 2020); variation which is further magnified in interspecific groups. How collective movement predicted by physics is modulated by heterogeneous morphology, behavior and/or cognition-based processes is still understudied (Lang and Farine 2017, Sridhar and Guttal 2018).

Under the theory of natural selection, stable social interactions provide immediate direct benefits and/or future indirect benefits to participating individuals. Also, in interspecific interactions, the lack of relatedness between individuals ensures that such benefits are personal, i.e. non-kin related (Lehmann and Keller 2006, Clutton-Brock 2009). The general mechanism promoting the occurrence of grouping behavior between different species is the existence of shared interests (Clutton-Brock 2009, West et al. 2021), which can relate to several aspects of an organism's life history and ecology, such as reproduction, predatory defense, or prey acquisition. Multispecific groups of animals hunting together largely increase the probability of locating and acquiring prey (Krause and Ruxton 2002, Bshary and Bergmüller 2008, Lang and Farine 2017), and the evolved hunting strategy of each species often leads to specialized roles within a group, facilitating coordination (Bshary et al. 2006).

Generally described as asocial in the literature (Hanlon and Messenger 2018), coastal octopuses are known to be accompanied by several fish species when hunting similar prey (i.e. foraging for small crustaceans, mollusks, and even other fish) (Diamant and Shpigel 1985, Forsythe and Hanlon 1997, Sampaio et al. 2020) (see Supplemental Figure 2). In these multispecific hunting events, octopuses (*Octopus cyanea*) pursue prey within rock and coral crevices, while fish search the seafloor (bottom feeders, e.g. Red sea goatfish *Parupeneus forsskali* and yellowsaddle goatfish *Parupeneus cyclostomus*) and others hunt from the water column (bentho-pelagic predators, e.g. lyretail grouper *Variola louti*). Complementary, other species, particularly the black tip grouper *E. marginatus*, rely on an ambush predation style, by laying immobile on the seabed until striking prey that comes in sight (Diamant and Shpigel 1985, Sampaio et al. 2020). Until recently, these groups were thought of as purely producer-scrounger associations, which in movement dynamics would translate into despotic group leadership by the octopus, with fish exclusively following and exploiting the octopus (Giraldeau and Beauchamp 1990, King et al. 2008, 2009). However, recent qualitative observations suggest a more complexly-organized system, with leadership dynamically changing amongst different individuals (Bayley and Rose 2020, Sampaio et al. 2020), but quantitative evidence to define the true nature of these groups is still lacking.

In multispecific groups, the existence of one octopus and multiple fish partners brings into question how individuals process, and potentially discriminate, simultaneous social information from various heterogeneous sources (social network scenarios) that differ in size, morphology and behavior. Moreover, pairwise hunting associations between *Octopus cyanea* and groupers can entail referential gestures on prey locations by the latter (lyretail grouper, Video S1), indicating the occurrence of cognitively-complex behaviors (Vail et al. 2013, Bshary et al. 2014). Thus, using these multispecific groups, we aimed to evaluate how social contexts can shape the behavior and decision-making of otherwise-solitary octopuses, and gauge the underpinnings of collective movement and leader-follower dynamics in groups of differently-specialized individuals.

Methods

Location and recording procedures

Fieldwork spanned one month, between 01-10-2018 and 01-11-2018 (29.5577°N, 34.9519°E, Eilat, Israel) in a total of ~60h of SCUBA diving. Dives were performed 2/3 dives/day, at relatively shallow depths (8-15m) allowing for ~2-3h underwater/day, complying with local scientific diving regulations. We recorded interspecific hunting events between Octopus cyanea and multiple partners, and, given that these hunts are not stationary, we adopted a search-and-follow procedure while maintaining a distance of >5 m to minimize disturbing natural interactions. We used two full-frame Sony Alpha 7SII with Zeiss f/2 25mm wide lenses mounted on an aluminum structure, as a stereocamera setup (hereafter ‘Stereocamera Rig’, see Supplemental Figure 3). A third full-frame Sony Alpha 7SII with Sony f/4 24-70mm lens served as focal camera for the octopus (hereafter ‘Zoom Camera’). Videos were all filmed at 25 fps with 4k resolution, and cameras were synchronized in Adobe Premiere via the timestamp of an underwater horn at the start of all recordings.

3D scene reconstructions

Following the pipeline established by Francisco et al. (2020), we took advantage of stereopsis provided by the overlap of the two cameras’ field views (the Stereocamera Rig), which allowed for reliable and accurate 3D-tracking of overall group collective movement and 3D reconstruction of habitat features.

First, using computer-vision methods, videos were run through a Structure-from-Motion and Multi-View Stereo open-source pipeline named *colmap* (Schönberger and Frahm 2016). The concept of Structure-from-Motion allows for the retrieval of 3-dimensional information from 2-dimensional images, by matching key points of a stationary background over several video frames. The lack of depth that could regardless be a problem with stationary cameras can then be solved by using multiple camera views (Multi-View Stereo), i.e. by adding different camera positions over time, which then allows for key points to be triangulated in 3D space and decreases camera projection errors. In addition, *colmap* also performs intrinsic camera calibration, undistorting images due to different frame or lens types; and extrinsic calibration by calculating the position of one camera relative to the other per time frame. This extrinsic calibration per time frame yields camera positions relative to the reconstructed habitat at all time frames, allowing us to recreate the path taken by the cameras while filming (see example in Supplemental Figure 4), along with a high-resolution 3D spatial reconstruction where all habitat features across hunting events are detailed.

We manually tracked individuals in the videos using the software Computer Vision Annotation Tool (CVAT, https://github.com/openvinotoolkit/cvat). CVAT is a freeware online annotation tool for computer vision which allows for several annotators to work simultaneously in joint tasks. A Docker-contained server was booted on a workstation to provide a continuous open internet connection for the first author and paid volunteers to annotate images and perform quality control several times along the process. We annotated 3 frames per second, which yielded a time resolution of 0.33 seconds for animal movement. Combining both camera views, this sampling effort represents a total ~500,000 individual annotations. We annotated all individuals in a collective hunt, specifically the left eye of the octopus and the end of the rostrum of each fish, ensuring consistency between different annotators. Individual tracks from each camera were then downloaded as .csv files and prepared to enter the reconstruction pipeline.

We then used software purposefully developed to incorporate the *colmap* habitat models and camera paths together with the previously tracked animals in each camera, namely *multiviewtracks* or *mvt* (Francisco et al. 2020). Similarly to how key points were triangulated in the habitat reconstruction phase, individual positions were triangulated from the Stereocamera Rig's known camera relative positions, and their movements reconstructed in 3D from the entire camera path (and therefore nullifying camera motion). Next, we specified the known world distance between the two cameras for scale (1.2 m) and obtained accurate individual trajectories in real xyz coordinates. To increase the accuracy of the 3D individual positions, we only retained positions of individuals that were tracked simultaneously in both cameras at a given time, thus dispensing depth interpolation of single camera views. Taking advantage of knowing the real-world distance between cameras, we were also able to calculate reconstruction accuracy and reprojection camera errors. Reconstructions from *mvt* had a remarkable Median accuracy of 0.0001 m (i.e. 0.1 mm) and a 3-sigma limit of 0.01 m (3 * Standard Deviation error, i.e. ~99.7% of the data). In total, 3.5 h of collective hunting were reconstructed (example in Supplemental Figure 5), in a total of 13 different scenes representing different groups of interspecific hunting.

Finally, to further account for potential jittering arising from manual tracking, we searched and removed position outliers (x, y, or z coordinate values diverging three times the standard deviation from the last 3 frames), linearly interpolated missing values (up to 12 frames, or 4 seconds), and applied a Savitzky-Golay filter to smoothen data encompassing a time window of

19 frames (package *SciPy*). From these clean tracked positions, we calculated 3D descriptive kinematics for each individual (time resolution = 0.33 seconds), such as individual:

$$\sqrt{(x_t - x_{t'})^2 + (y_t - y_{t'})^2 + (z_t - z_{t'})^2 / \text{time r}}\text{esolution}$$

where t and t’ are a given frame and the next frame immediately after. And the Euclidean distance of individuals (i) relatively to the mean of the positions of all individuals present in a given frame t, i.e. group centroid (c), computed as:

$$\sqrt{(x_c - x_i)^2 + (y_c - y_i)^2 + (z_c - z_i)^2.}$$

Pull-and-anchor analysis

The main analytical methodology used to quantify leadership was the pull-and-anchor analysis from Strandburg-Peshkin et al. (2015). In this paper, GPS tracks were in 2D since animals moved almost exclusively in a 2D horizontal plane (i.e. baboons moving over XY coordinates), so the code was adapted to include the Z coordinate and the vertical movements of the octopus and fishes in the water column (XYZ coordinates). Comparatively to other metrics (e.g. directional or speed correlations) pull-and-anchor analysis are particularly suited for analyzing groups that show erratic movement patterns and frequently change between tight to sparse formations, as well as for analyzing movement sequences over short or long time-scales (Strandburg-Peshkin et al. 2015).

Pull-and-anchor analysis focuses on assessing variation in dyadic distance between two individuals (i and j), and extracting successful and unsuccessful leadership events (pulls and anchors, respectively). In essence, we look for minimum and maximum values of dyadic distance, until a minimum (t1)-maximum(t2)-minimum(t3) sequence is formed. For each of these interactions there is one leader and one follower: between t1-t2, the individual increasing the distance relating to the other (let it be i in this example) is the attempted leader and the other individual (let it be j) is the candidate follower. After reaching the maximum dyadic distance (t2), the individual that then shortens dyadic distance dictates if this sequence was a successful leading event (i.e. pull) or an unsuccessful leading event (i.e. anchor). Following the example above, if the tentative leader i is the one shortening dyadic distance to j, then this sequence is classified as an anchor event. If on the other hand, j is the one shortening the dyadic distance, that means that j followed i and it is classified as a successful leading event (i.e. pull).

Following the original methodology, several steps were taken to ensure that small-scale variations were not included as pull-anchor events by filtering candidate sequences using disparity, strength, and noise thresholds. Before candidate sequences were identified *per se*, to prevent jittering interference and small body part movements to be considered as potential events, a noise threshold of 0.1 m was set as the minimum dyadic distance change between i and j. Note that this value is 1-2 orders of magnitude above the error calculated for the reconstruction, and approximately represents 0.5-1 body size of the individuals present in the hunts. Disparity was calculated via the difference of covered distance by i and j across each time segment in relation to each other. Complementary, strength was calculated as the relative change in dyadic distance between each time segment. Both these parameters range between 0 and 1, where, in the case of disparity, values near 0 depict an interaction where both i and j moved similar distances during each time segment (thus making classification of the event ambiguous), and values near 1 indicate that either i or j performed the majority of movement in each time segment. In the case of strength, values near 0 indicate that the relative change in dyadic distance in each time segment was negligible (i.e. i and j were always close together), whereas values near 1 indicate that the distance markedly changed among t1,t2, and t3 (i.e. individuals were close, then far apart, then close again). As per (Strandburg-Peshkin et al. 2015), we defined both thresholds at a minimum of 0.1.

Using this methodology, we extracted 611 pulls and 735 anchors from the dataset, in a total of 1346 events. To account for differences in time spent as part of the hunting groups, we divided all scenes in blocks of 5 minutes and quantified pull-anchor events within our dataset as:

a) Anchored per minute - number of anchors (i.e. unsuccessful pulls) over time spent (i.e. 5 minutes block) within the interspecific group;
b) Anchors per minute – number of anchors as follower (i.e. anchoring a tentative leader) over time;
c) Pulled per minute – number of follows performed when solicited by a tentative leader over time;
d) Pulls per minute – number of pulls (i.e. successful leading) over time.

Moreover, we also quantified ratios between leader-follower categories in pull-anchor events by measuring:

i) Pulling ratio – number of successful leading events divided by the total number of pulls attempted;
ii) Following ratio – number of times following a tentative leader divided by the total number of solicited by a tentative leader;
iii) Leading attempts-Following ratio – number of times an individual attempted to lead divided by the number of times it followed when pulled;

iv) Pulls-Following ratio – number of times effectively leading divided by the number of times effectively following when pulled.

Hierarchical Social Networks

Social network analysis has been widely used in the fields of sociality and group behaviour over the last decade (Gokcekus et al. 2021). This type of analyses comprises a number of different tools that allows to characterize specific individual roles within groups as well as the structure and characteristics of their social interactions with other members of the group (Farine et al. 2012). Following Nagy et al. (2010, 2013), we adapted this approach by including directed social networks (i.e. the influence of individual i over individual j, instead of 'dyadic influence' by itself), and by constructing a rank order which yields hierarchical influence in groups.

To find hierarchical structures within groups we evaluated relationships and ranks in different pull-anchor event parameters - i.e. leadership ratio (Pulls-Anchors/Pulls+Anchors), effective anchoring (normalized values of anchors over time) and effective leadership (normalized values of pulls over time) -, by creating adjacency matrixes where pairwise comparisons between i and j were performed on both sides of the leader-follower axis (i.e. as leader and as follower). From these matrices (see Supplemental Figures 9-11), we used directed social networks to find underlying network hierarchies for each parameter. First, edge direction (i.e. arrow) was determined by the highest positive number, signifying for example that in a given interaction, individual i was more likely to pull the other. Next, edge width was calculated as the difference between the scores of individuals i and j in pulling each other. In other words, if individual i had a much higher pulling score than j, the edge will be thicker and directed to i. We then used weighted degree centrality (i.e. the sum of all edge's widths around a specific node, irrespective of direction) to color each species node, with brighter and darker colors respectively indicating higher (more 'one-way' interactions) and lower (more 'divided' interactions) weighted degree centrality.

Lastly, we used a given species' average parameter score, e.g. the sum of all species-specific pulling scores for the octopus as the leader, divided by the number of species it interacted with (calculated in the 'Sum' row on the adjacency matrixes), to rank individuals according to the parameter analyzed in each network structure. We used rank with continuous data in the y axis and an integer-based rank in the x axis, to make hierarchical relationships clearer. All hierarchical social networks were built with package *NetworkX* in Python.

Statistics

All statistical analysis were performed in R (R Development Core Team 2018). To analyze variations in speed and distance from group centroid according to different species (6 levels: BTG – blacktip grouper, GG – green goatfish 'Red Sea goatfish', LTG – lyretail grouper, OCT – octopus, YG – yellowsaddle goatfish, YGJ – yellowsaddle goatfish juvenile), we used auto-regressive generalized linear mixed models (GLMM) available in the package *glmmTMB*. Specifically, we used a nested structure of random effects with individual identity nested within different scenes (i.e. different collective hunts). The auto-regressive structure was specified as the frame number grouped within each collective hunt, thus establishing temporal auto-correlation for each scene. Speed was transformed using the natural logarithm and a gaussian(link = identity) model family was used, whereas distance from centroid was modelled with a gamma (link=log) distribution.

Differences in pull-anchor quantitative data over time across species were analyzed via GLMM with Poisson distribution with time as offset, and with a two random effects structure, the first being individual identity nested within each scene, and the second ‘block’ (representing differently numbered time blocks across all scenes, n = 104). Differences in pull-anchor ratios across species based on binary outcomes (e.g. successful, unsuccessful) were modelled using the binomial family distribution (link=logit) with the same random effects structure as detailed for quantitative data over time. Lastly, differences in pull-anchor ratios across species, based on quantitative data (e.g. pulls/follows), were modelled using negative binomial distributions with the numerator as independent variable (e.g. pulls) and the denominator as offset (e.g. follows). The structure of random effects used was the same as above. Lastly, we assessed how speed of pull-anchor events varied according to species and type of event (2 levels: pull and anchor) in the totality of the dataset (i.e. all species), and with the octopus in the follower role, using GLMMs with gamma (link=log) distribution, with individual identity nested within each scene.

When more than one factor was used as dependent variable, our criteria for best model selection were the lowest Akaike information criterion (AIC) and the Bayesian information criterion (BIC). All models were validated by checking homogeneity of variances, residuals normality, normality of random effects, as well as overdispersion and collinearity when required (e.g. with count data and when multiple factors were tested). To this end, we used the *check_model* function within package’s *performance* and *see*, or the package *DHARMa*. Pairwise comparisons between levels within significant factors were analyzed via Tukey Honest Significant Differences (HSD) with Tukey multiplicity adjustments, using the package *emmeans*. Using the spearman rank-based correlation test, we also performed a correlation analysis between anchors over time and distance to centroid. Results were plotted using *ggplot2*.

Results

In terms of physical parameters, using an auto-regressive one-dimensional model (gaussian auto-regressive GLMM, Figure 6.1A; see also Supplemental Figure 6 for the profiles of the natural logarithm of speed), we found differences in speed profiles across species, specifically black tip groupers were significantly slower than both lyretail groupers ($p < 0.01$) and yellowsaddle goatfishes ($p < 0.0001$, Tukey HSD, Table S1). Moreover, also with an auto-regressive one-dimensional model (gamma auto-regressive GLMM, Figure 6.1B), we found differences in the average distance to group centroid across species (Table S2). Specifically, octopuses and green goatfish were significantly closer to the group's centroid when compared to black tip groupers (both $p < 0.01$, Tukey HSD, Table S2), and lyretail groupers ($p < 0.05$, Tukey HSD, Table S2). The profiles of average distance to group centroid and the mode-based rank order are described in Supplemental Figure 7. The yielded species rank is 1) octopus (0.15m from centroid), 2) green goatfish (0.18m from centroid), 3) black tip grouper (0.311m from centroid), 4) yellowsaddle goatfish (0.427m from centroid), 5) lyretail grouper (0.612m from centroid), and 6) juvenile yellowsaddle goatfish (0.867m from centroid).

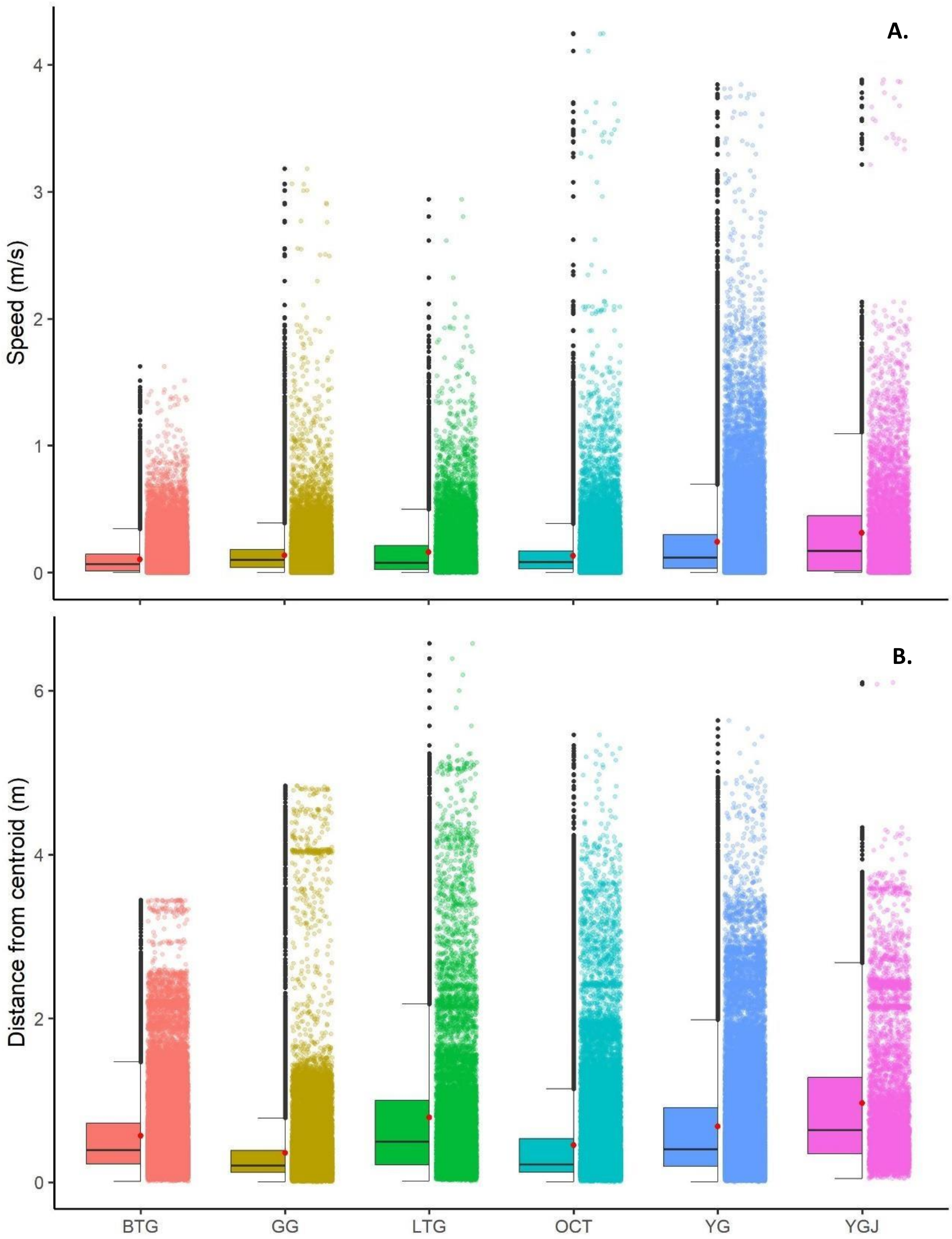

Figure 6.1. Boxplot displaying variations in **A.** speed, and **B.** distance to group centroid for species present in collective interspecific hunts between octopus and fishes. BTG - Black tip grouper, GG - Green Goatfish, LGT - Lyretail grouper, OCT - Octopus, YG - yellowsaddle goatfish, YGJ - yellowsaddle goatfish juvenile.

Concerning the pull-anchor analyses, the average number of times anchored per minute when attempting to lead significantly varied according to species (Figure 6.2A, Table S3). We found that both black tip groupers and octopuses were anchored less often than yellowsaddle ($p < 0.01$) and green goatfishes ($p < 0.05$, Table S3, Tukey HSD). Moreover, lyretail groupers were also less frequently anchored than yellowsaddle goatfish ($p < 0.05$, Table S3, Tukey HSD).

We again found significant differences in the average number of anchors per minute (i.e. anchoring a potential leader) according to species (Figure 6.2B, Table S4). We found that octopuses anchored individuals more often than yellowsaddle goatfishes, adults ($p < 0.0001$) and juveniles ($p < 0.001$), as well as greengoatfish and lyretail groupers (both $p < 0.01$, Table S4, Tukey HSD). Moreover, black tip groupers also anchored individuals more often than adult and juvenile yellowsaddle goatfishes (respectively $p < 0.01$ and $p < 0.05$, Table S4, Tukey HSD).

However, we found no significant differences between species regarding the average number of times following (i.e. being pulled) per minute when another individual attempted to lead, according to species (Figure 6.2C, Table S5). Importantly, the average number of pulls per minute significantly varied according to species (Figure 6.2D, Table S6). We found that yellowsaddle goatfishes executed more pulls per minute compared to black tip groupers, lyretail groupers and octopuses (all $p < 0.001$, Table S6). Moreover, we found that greengoatfish also executed significantly more pulls per minute than blacktip groupers ($p < 0.05$, Table S6). These patterns were repeated when data was restricted to the occasions when the octopus assumed the role of follower (Supplemental Figure 8, Table S7).

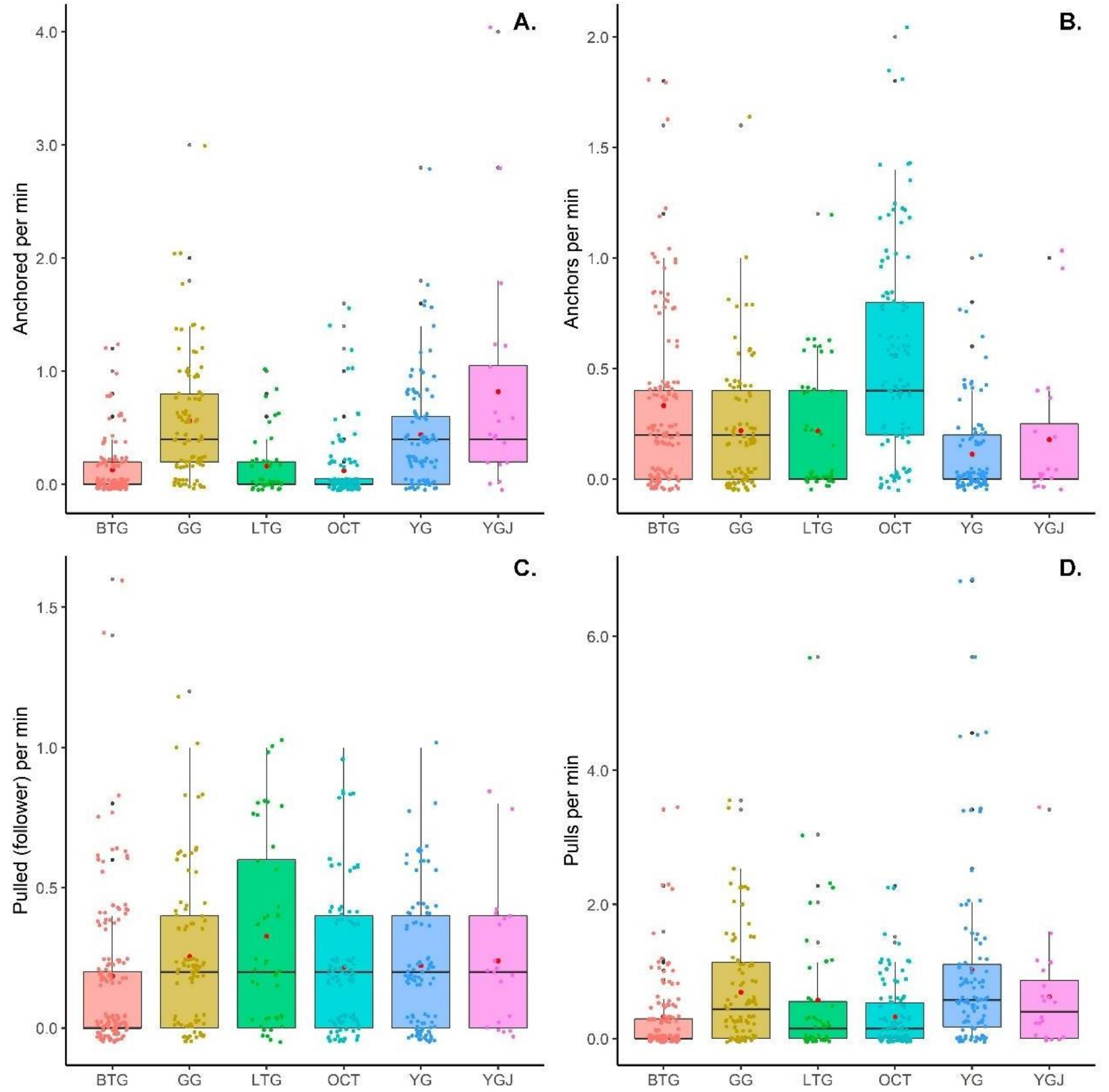

Figure 6.2. Boxplots and datapoints showing variations in **A.** times anchored per minute, **B.** times anchoring other individuals per minute, **C.** times being pulled as a follower per minute, and **D.** number of pulls per minute, according to species. Red dots indicate the mean value for each species.

Regarding leader-follower ratios, we found significant differences across species on the rates of successfully pulling (i.e. leading) when attempts were made (Figure 6.3A, Table S8). Pairwise comparisons showed that juvenile yellowsaddle goatfish were less likely to be followed than blacktip groupers and octopuses (both $p < 0.01$, Table S8, Tukey HSD). Conversely, from a follower perspective, we also noted significant differences on the rates of following or anchoring the potential leader, according to species (Figure 6.3B, Table S9). Indeed, octopuses were more likely to anchor when solicited by potential leaders than both greengoatfish and yellowsaddle goatfish (respectively $p < 0.01$ and $p < 0.001$, Table S9, Tukey HSD).

Interestingly, we noted significant differences on the ratio of leading attempts (i.e. both pulling and being anchored) to following events, according to species (Figure 6.3C, Table S10). Yellowsaddle goatfish exhibited higher leading attempt ratios when compared to octopuses, and black tip and lyretail groupers (all $p < 0.001$, Table S10, Tukey HSD). In addition, green goatfish also exhibited higher leading attempt-follower ratios than octopuses ($p < 0.05$) and black tip groupers ($p < 0.01$). Black tip groupers, lyretail groupers and octopuses were on or below the attempted leading threshold (i.e. red dashed line in Figures 6.3C-D), thus acting more often or as often as followers than attempting to be leaders. Conversely, goatfishes (green, and both juvenile and adult yellowsaddle) markedly attempt leading more often than they follow in interspecific groups (Figure 6.3C).

Likewise, we noted significant differences on the ratio of effective leading (i.e. successful pulling) and following, according to species (Figure 6.3D, Table S11). Yellowsaddle goatfish again exhibited higher leading ratios when compared to octopuses ($p < 0.01$), as well as black tip and lyretail groupers (both $p < 0.001$, Table S11, Tukey HSD). In addition, green goatfish exhibited higher leading ratio on average than lyretail groupers ($p < 0.05$). More markedly even, black tip groupers, lyretail groupers and octopuses were on average below the effective leading-following threshold (i.e. red dashed line in Figures 6.3C-D), thus acting more often as followers than leaders. Conversely, all goatfishes (green, and both juvenile and adult yellowsaddle) were more often effective leaders than followers in the interspecific groups (Figure 6.3D).

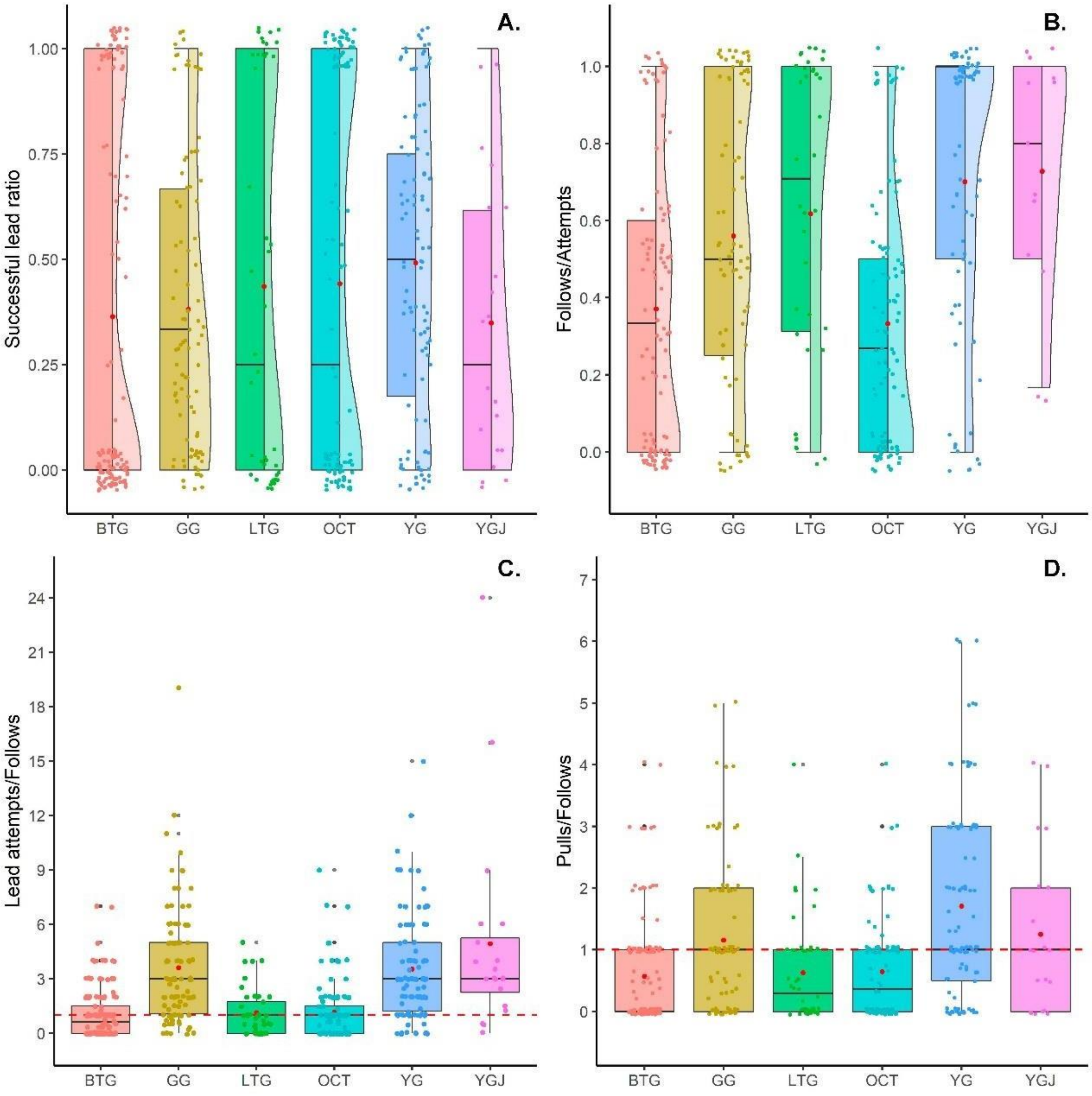

Figure 6.3. Boxplots, violin plots, and data points representing ratio variations in **A.** successful pulls divided by total number of leading attempts, **B.** times following other individuals divided by number of targeted leading attempts, **C.** leading attempts divided follower events, and **D.** successful pulls divided by follower events, according to species. Red dots indicate the mean value for each species. The red dashed line in **C.** and **D.** represents a leader(attempts)-follower threshold, where values >1 indicate a stronger leadership role and values <1 indicate a stronger role in the context of the group's movement.

We also measured how pull-anchor kinematics, specifically the mean speed of pull-anchor events, and found significant differences according to species (Figure 6.4A) and the outcome (successful vs unsuccessful) of said events (Figure 6.4B, Table S12). Specifically, we found that yellowsaddle goatfishes were significantly faster than octopuses in leading attempts ($p < 0.01$, Table S12, Tukey HSD). Moreover, when attempting to lead, we found that successful pulls were significantly faster than anchors (i.e. unsuccessful pulls, $p < 0.01$, Table S12).

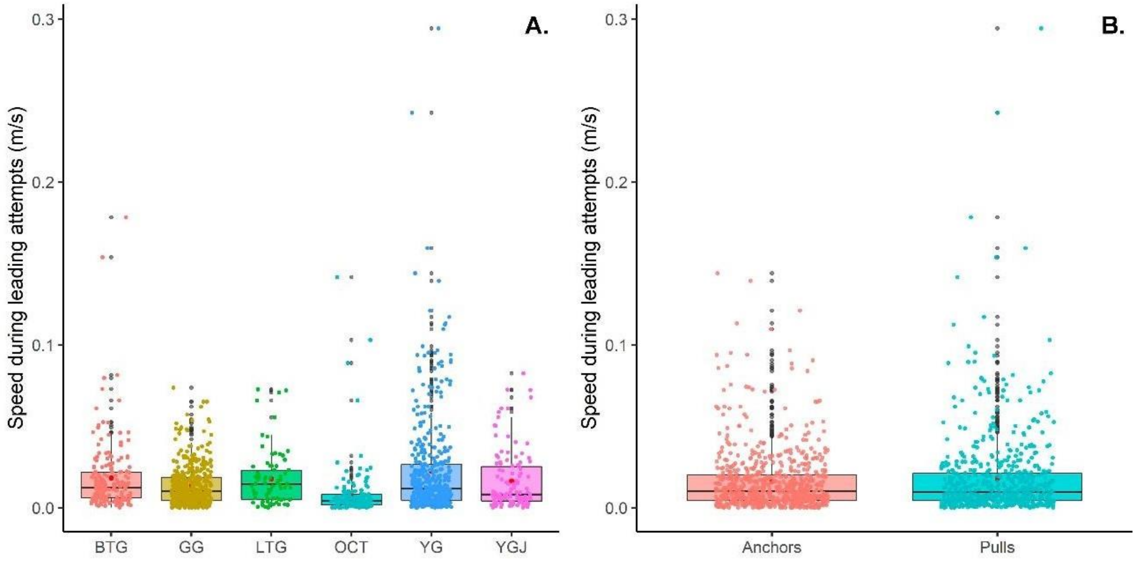

Figure 6.4. Boxplots and datapoints showing variations in the leader's mean speed during leading attempts, depending on **A.** species, and **B.** outcome of the leading attempt (Pull or Anchor). Red dots indicate the mean value for each species.

From the perspective of the octopus as follower, the differential patterns seen for all species become more evident and further differences are found regarding speed of pull-anchor events, according to species (Figure 6.5A) and the outcome of said events (Figure 6.5B, Table S13). Specifically, we found that both adults and juvenile yellowsaddle goatfishes were significantly faster than black tip groupers and green goatfish when attempting to pull octopuses (see Table S13, Tukey HSD). As for all species, when attempting to lead octopuses, we again found that successful pulls were significantly faster than anchors ($p < 0.05$, Table S13).

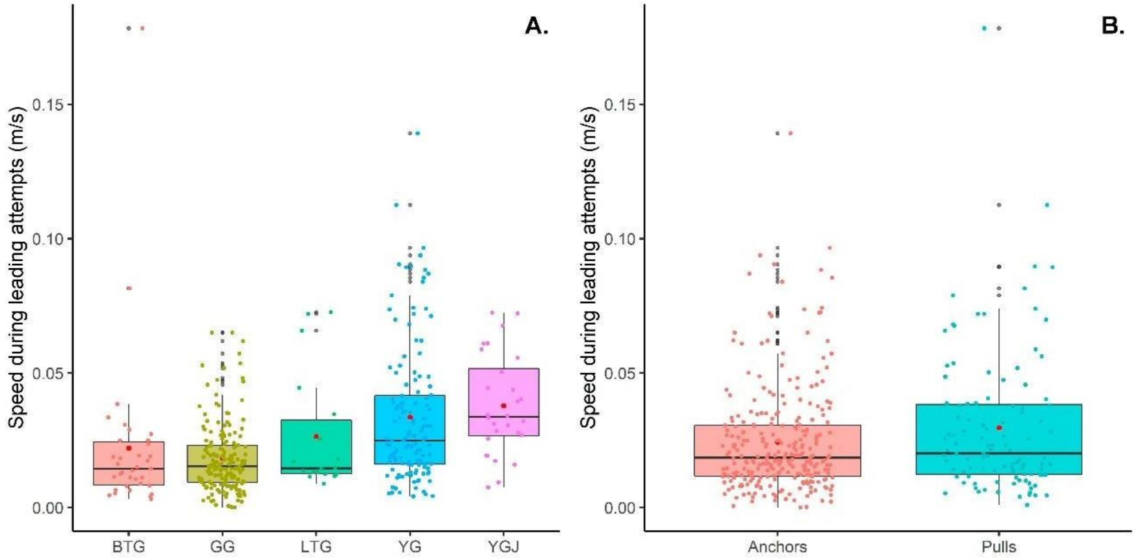

Figure 6.5. Boxplots and datapoints showing variations in the leader's speed during leading attempts, specifically with the octopus as follower, depending on **A.** species, and **B.** outcome of the leading attempt (Pull or Anchor). Red dots indicate the mean value for each species.

Taking the perspective of each species as both pullers and followers, we built an adjacency matrix based on the positive or negative relationship of pulls and anchors divided by the total numbers of attempts made, i.e. pulls and anchors summed (Supplemental Figure 9). Both octopuses and black tip groupers are more often leaders by ratio compared to other species, but black tip groupers also pull octopuses more often than they are pulled. Nevertheless, directed social networks ordered by the strength of leading ratio ranks, reveal that in the overall hierarchy of these ratio leadership relationships, octopuses are first seconded by black tip groupers (despite leading octopuses in pairwise interactions), with all other species showing similar leadership ratio scores (Figure 6.6).

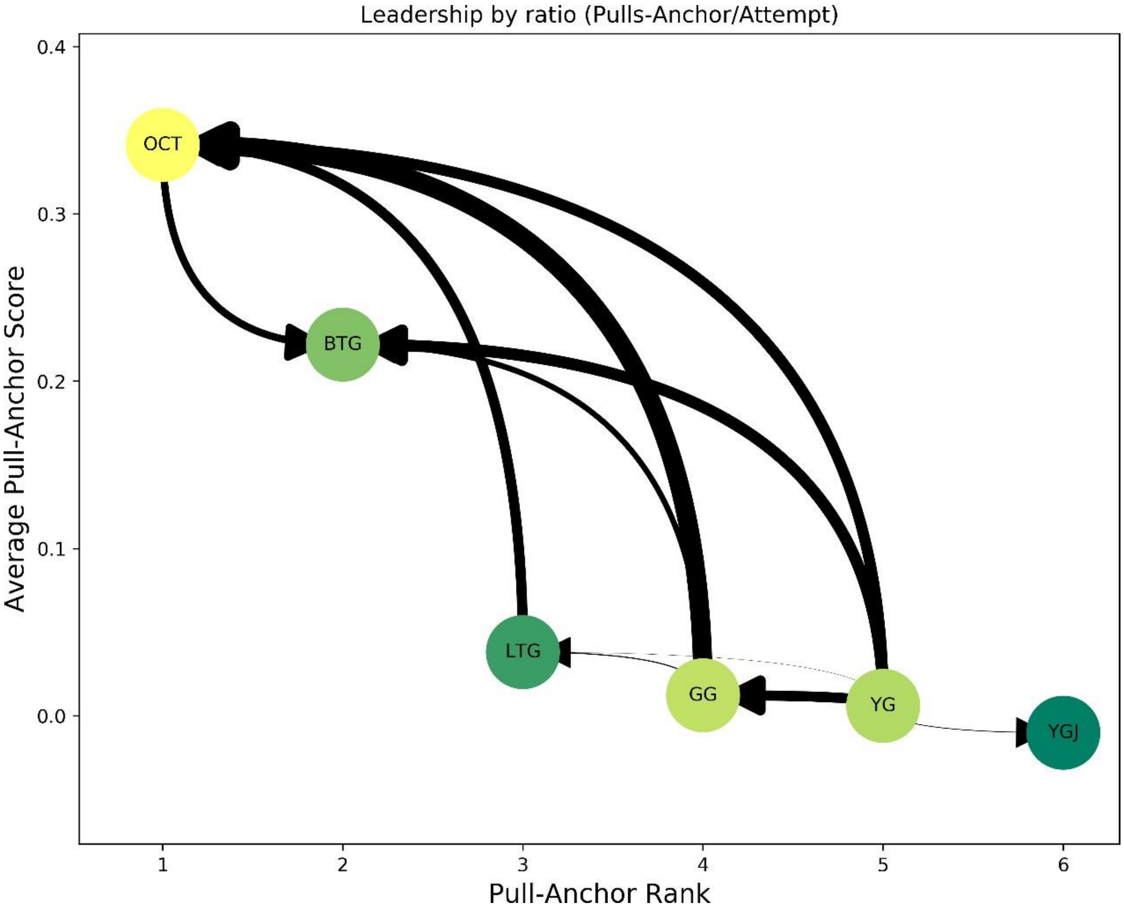

Figure 6.6. Directed social networks based on pairwise pulls and anchors divided by the total numbers of attempts, posteriorly ordered in the y-axis by the continuous value dictated by the score index in Supplemental Figure 9. On the horizontal, the x-axis is ordered as the rank-transformed value of the same index. Direction of arrows indicates which species has the highest positive score in the sum of pairwise interactions, while arrow (i.e. edge) wideness indicates how large the difference between the two scores is (i.e. wider arrows indicate larger differences). Lastly, brighter colors indicate a higher weighted degree centrality (i.e. the sum of all arrows' wideness around a specific node, irrespective of direction) and darker colors indicate lower weighted degree centrality.

In more detail, taking the perspective of each species as both pullers and followers regarding anchors per minute (Supplemental Figure 10), a similar pattern to leadership ratio emerges in species hierarchy (Figure 6.7). Ranking species according to the sum of all species-specific ratios as an index score, octopuses reach the highest rank of anchors across time with black tip groupers in second place, which again anchor octopuses more often. Interestingly, the order of the anchoring frequency rank is inversely correlated with each species' mode of the distribution of distance to group centroid (spearman correlation = -0.8857, p = 0.0188), i.e. individuals in the center of the group anchor other individuals more often than individuals on the periphery.

Finally, taking the perspective of each species as both pullers (columns) and followers (rows) regarding pulls per minute (adjacency matrix in Supplemental Figure 11), shows leadership ranks in real time (i.e. effective leadership). Here, according to the sum of all species-specific ratios and dividing for the number of species interacted with as the index score, we find that yellowsaddle goatfishes take the highest rank of effective leadership (Figure 6.8). This result arises simultaneously from pulling all other species in pairwise interactions, as well as doing so with a stronger difference on average (i.e. species i pulling species j, markedly more than j pulls i). Interestingly, effective leadership ranks place green goatfish in second place, and blacktip groupers in third, closely followed by octopuses. Lastly, lyretail groupers and juvenile yellowsaddle goatfish are reported as the lowest ranking effective leader species.

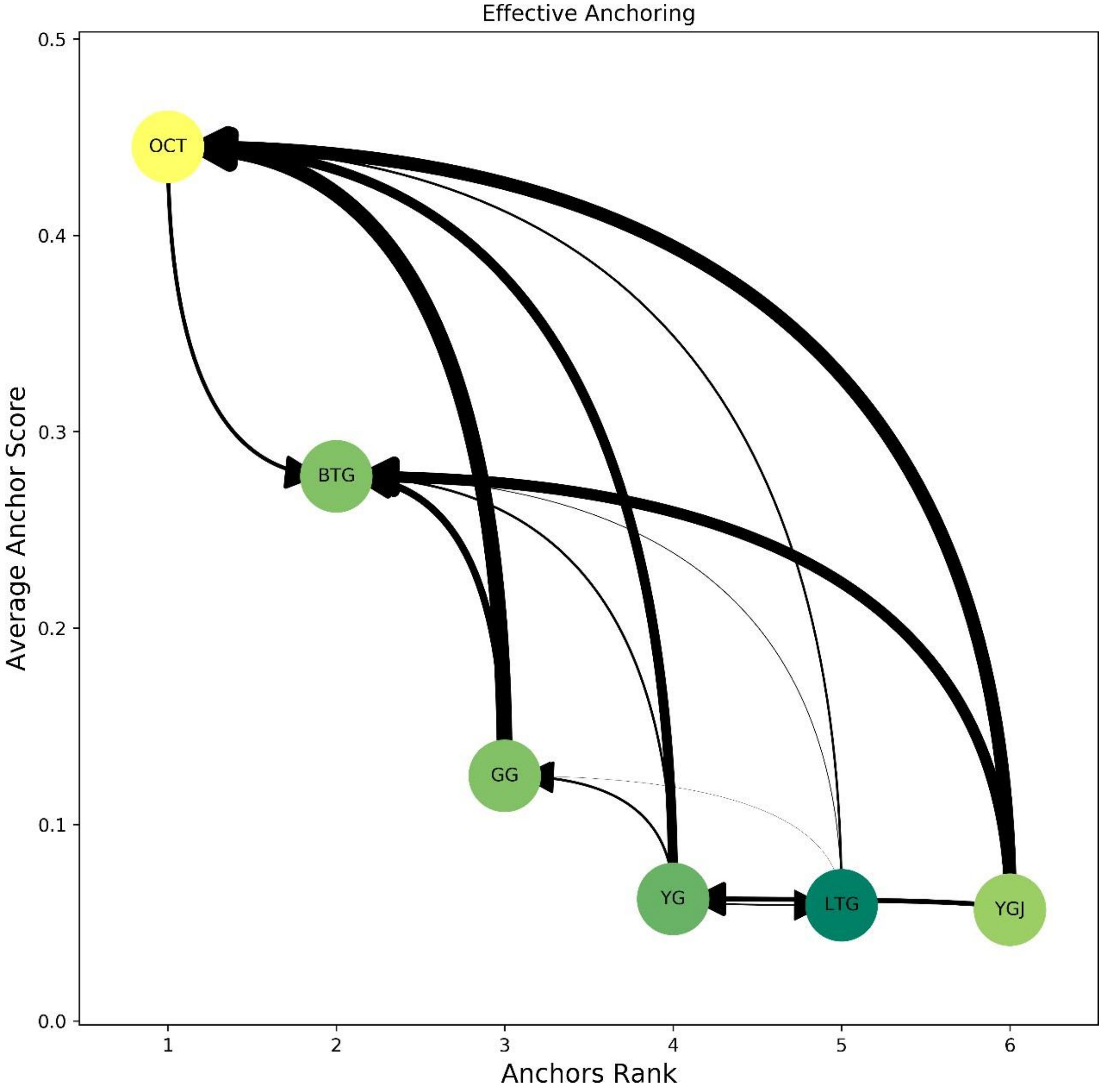

Figure 6.7. Directed social networks based on pairwise anchor interactions per minute, posteriorly ordered in the y-axis by the continuous value dictated by the score index in Supplemental Figure 10. Below, x-axis is ordered as the rank-transformed value of the same index. Direction of arrows indicates which species has the highest positive score in the sum of pairwise interactions, while arrow (i.e. edge) wideness indicates how large the difference between the two scores is (i.e. wider arrows indicate larger differences). Lastly, brighter colors indicate a higher weighted degree centrality (i.e. the sum of all arrows' wideness around a specific node, irrespective of direction) and darker colors indicate lower weighted degree centrality.

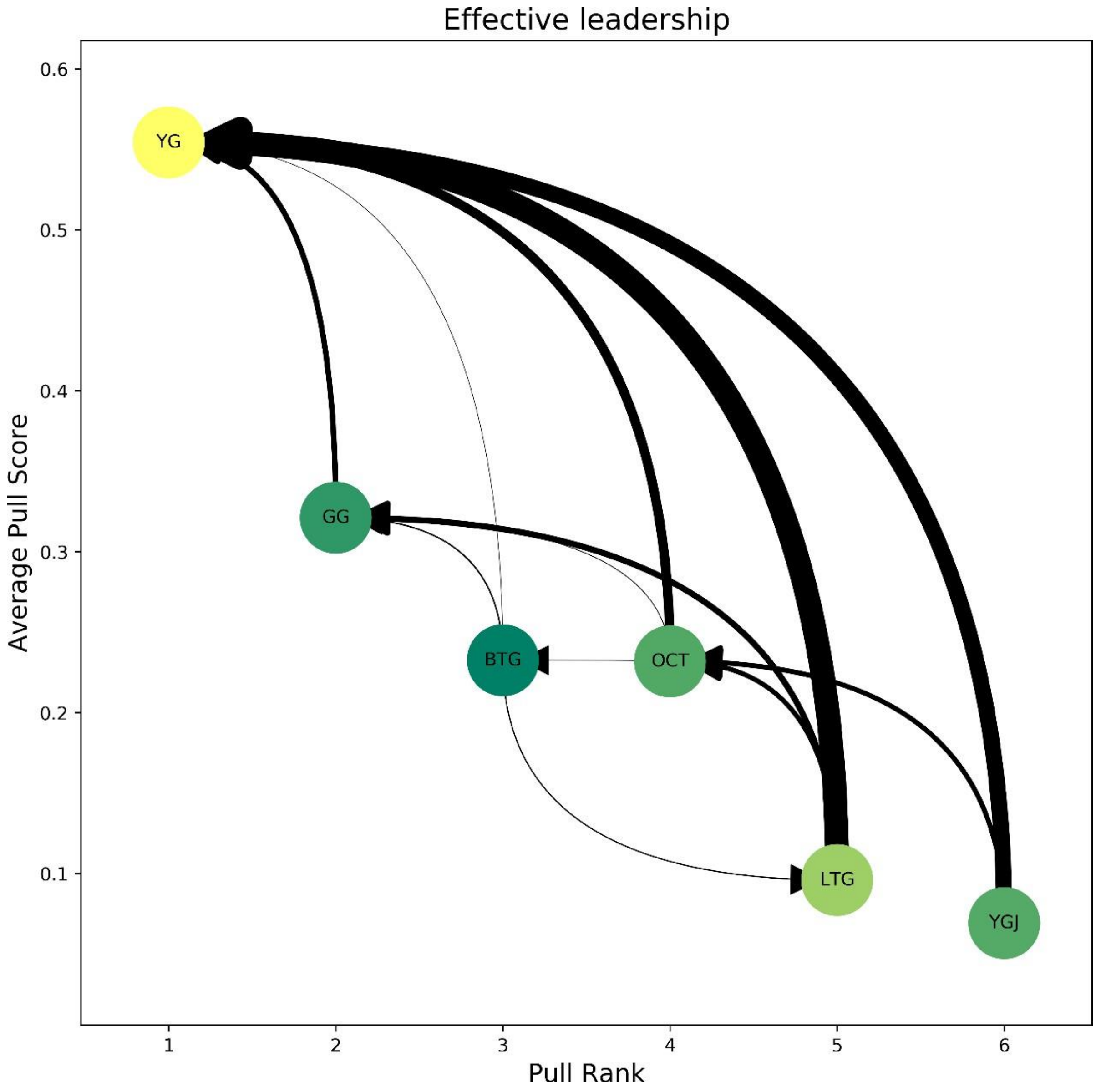

Figure 6.8. Directed social networks based on pairwise pull interactions per minute, posteriorly ordered in the y-axis by the continuous value dictated by the score index in Supplemental Figure 11. Below, the x-axis is ordered as the rank-transformed value of the same index. Direction of arrows indicate which species has the highest positive score in the sum of pairwise interactions, while arrow (i.e. edge) wideness indicates how large the difference between the two scores is (i.e. wider arrows indicate larger differences). Lastly, brighter colors indicate a higher weighted degree centrality (i.e. the sum of all arrows' wideness around a specific node, irrespective of direction) and darker colors indicate lower weighted degree centrality.

Discussion

Our quantitative results show that decision-making in multirspecific groups composed by one octopus and several fish(es) is demonstrably not despotic, but shared. That is, contrary to what would be predicted by nuclear-follower models, the group's collective movement is led by different individuals across time, and does not rely only on a single individual. However, our results also show that individuals do not have identical leadership status; their species is highly relevant to determine leader-follower roles during interspecific hunting. Contrary to nuclear-follower predictions where fish would follow the octopus (nuclear predator), our results show that goatfishes (particularly adult yellowsaddles) are the main drivers of group movement in real time, i.e. effective leadership, while other species tend to have more follower roles on average. Conversely to effective leadership, octopuses and black tip groupers are the main anchorers in interspecific groups, with these species having a role of 'ratio leadership' or a higher signal-to-noise ratio. In terms of collective movement, one could translate this apparent dual nature of leadership (effective and ratio) into other words: goatfishes decide where the group goes, octopuses and black tip groupers decide if the group goes.

Simple interaction rules from collective behavior play a significant role in the collective movement of these interspecific groups. Specifically, in animal groups, faster individuals tend to lead more often as they assume a frontal position in the group (Couzin et al. 2002, Mishra et al. 2012, Ioannou et al. 2015), which has been demonstrated in multiple animal collectives, such as bird flocks (Pettit et al. 2015) and fish shoals (Jolles et al. 2017). Here, the existence of this rule is supported by goatfishes being in general relatively fast and mobile species, and by successful pulls being usually performed over higher speeds than unsuccessful ones. However, there are some significant deviations from that rule in the analyzed hunts. For instance, both lyretail groupers and juvenile yellowsaddle goatfishes tend to be faster relatively to most of the other species, but are not better leaders in general. On the other hand, octopuses are relatively slow pullers, but highly successful in their leading attempts. Concurrently, closeness to centroid is directly correlated to effective anchoring in the group, which is likely due to the group's movement being made across the habitat while also rotating around a central axis, i.e. the octopus. There are nevertheless exceptions here as well, with green goatfishes performing very little anchoring, despite being often near the centroid. Thus, taken together, evidence suggests that additional factors and mechanisms further influence the collective movement dynamics of multispecific groups.

Key aspects that shape leader-follower roles played and movement by each species can be interpreted from their own species-specific hunting strategies, which are generally maintained during interspecific groups (Bshary et al. 2006, Lang and Farine 2017). It is interesting to note that because blacktip groupers rely more on ambush predation (Diamant and Shpigel 1985), we had anticipated that this species would play little more than a follower role in these groups. However, our analysis shows that when individuals from this species move, they are usually followed by others (leadership ratio second only to the octopus itself). We hypothesize that this could be due to a higher signal-to-noise ratio relatively to other members (as seen in Rodriguez-Santiago et al. 2020), since blacktip groupers are most often immobile contrary to other fish species that are constantly in motion. Therefore, when they actively move during the hunt, it may be a more salient signal for other group members that prey is nearby. We also predicted that lyretail groupers would be preferred partners for the octopus to hunt with, given previous descriptions of pairwise interactions entailing complex communication and elaborate signaling (Vail et al. 2013) (see Video S1 for a filmed example). However, we found that lyretail groupers have in fact reduced leadership potential for the octopus and the other fish species (both effectively and by ratio). Multispecific groups can be highly dynamical (e.g. see Video S1 in Sampaio et al. 2020), with up to 10 individuals moving with different strategies, which creates larger amounts of statistical noise than smaller groups (Mann 2020, Rahmani et al. 2020). Given that these grouper species tend to use more elaborate and prolonged forms of communication, one possibility is that due to the inherently higher noise stemming from the presence of multiple individuals in the group, simpler and faster forms of signaling become comparatively more salient to recipient(s). Lastly, it is noteworthy that intraspecific variations are highlighted by the performance difference between juvenile and adult yellowsaddle goatfish. While yellowsaddle goatfish are known to hunt collaboratively with same-species individuals (Steinegger et al. 2018), the improvement in movement/leadership parameters across the two ontogenic stages suggests that hunting behavior is refined over time with increasing experience (Ortiz et al. 2019, Lagogiannis et al. 2020).

From the octopus perspective, we show that individuals actively follow fish, maintain hunts with partners for more than one hour, and specifically discriminate among social information sources when choosing whom to follow. In social interactions, maximizing personal benefits and avoiding exploitation involves re-evaluating costs and benefits (i.e. high neural processing energetic costs), and using partner control mechanisms (e.g. sanctions, punishment) (Lehmann and Keller 2006, Clutton-Brock 2009). Comparatively neglected to conspecific sociality (Sridhar and Guttal 2018), heterospecific sociality can also lead to cognitively complex processes, as highlighted in cleaner-client systems (Grutter 1999), where e.g. cleaner fish perform tactile stimulation to prevent aggression from less satiated clients (Grutter 2004), while clients eavesdrop and image-score the cleaners performance which simultaneously promotes cooperation by cleaners in future interactions (Bshary and Grutter 2006). Parallelly, despite initial simplistic descriptions, the octopus-fish system is characterized by an intricate network of interactions where each individual assesses the other partners' specific position, characteristics, and behavior. Moreover, this system involves coordinated movement, species-specific social preferences, interspecific referential gestures, and partner control mechanisms (Sampaio et al. 2020). These interspecific relationships form complex social contexts, akin to the demands of intraspecific cooperation and intermittent group living (Oliveira and Bshary 2021), and we hypothesize that they may have played a role in the evolution of behavioral flexibility and cognition in octopuses (Navarrete et al. 2016, Dunbar and Shultz 2017, Amodio et al. 2018).

Both from the biology and physics standpoints, interspecific collective behavior is exceptionally understudied (Couzin et al. 2005, Couzin 2009, Farine et al. 2014, Jolles et al. 2017). Studying these hierarchically-organized and complex social systems can provide new insights into the fields of cephalopod and fish behavior, convergent evolution, and selective factors underpinning the rise of complex cognition in the tree of life (Amodio et al. 2018, Oliveira and Bshary 2021, Schnell et al. 2021). Moreover, by quantifying the building blocks of complex interactions between multiple differently-specialized individuals, further work can advance our current understanding of interspecific collective coordination and cognition (Couzin 2009, Berdahl et al. 2013), and inspire future theoretical developments in overarching research fields, such as animal movement, swarm intelligence, and physics.

Supplemental Materials

Supplemental Figures and Tables are made available in the Annex section. Video S1 is available at https://www.dropbox.com/s/aaqjt1g26w306ch/referential_gestures.mp4?dl=0.

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7

General Discussion

The purpose of the present dissertation was to understand how social information in the natural environment and interactions with other individuals (both within conspecific and heterospecific contexts) can shape decision-making and behavioral dynamics in cephalopods. Taking advantage of the wide range of social phenotypes within this animal group, I organized this dissertation in two parts and investigated social understanding and information use in both social (**Part I**) and asocial (**Part II**) animal species belonging to divergent orders within the family of cephalopods.

In **Part I**, I examined how the presence of conspecifics can shape the behavior of individual squid and cuttlefish. ***Chapter 2*** describes the existence of new courtship dynamics and sexual strategies in squid groups during mating season. The findings show that competent behavior and adequate strategy choice during mating can be even more complex to achieve than previously thought (Wada et al. 2005, Morse and Huffard 2019). The work extends the hypothetical sexual strategies in, at least, one further option branch for each of the individuals involved. Specifically, consort males may or may not perform location probing; females may or may not lay their eggs after location probing and may or may not enforce said location probing by withholding egg-laying; and rival males may now wait until consort males perform location-probing to copulate with the female, together with the previously known options of fighting the consort male or 'sneaking' as a female mimick. Importantly, the present work may have also provided the first evidence of paternal investment in cephalopods (discussed further in the next section).

Afterwards, in ***Chapter 3*** it was shown that cuttlefish learn how to modulate predatory behavior from observing conspecifics. In the wild, the potential advantages of inhibiting predatory behavior following actions of conspecifics, can go from preventing meaningless energy expenditure to predatory avoidance associated with disengaged camouflage when attacking (Hanlon and Messenger 1988). Moreover, this occurs at a critical life stage, where the ability to circumvent trial-and-error for acquiring knowledge from the environment can signify avoiding extremely costly actions, conferring an evolutionary advantage to hatchlings that incorporate information provided by conspecifics. Together with previous findings for *Sepia latimanus* (Yasumuro et al. 2015), recent reports have also shown that *Sepia officinalis* aggregations are more common than previously thought in the wild (Drerup and Cooke 2021). Interestingly, both previous studies report that aggregations are composed of sub-adult cuttlefish, with Drerup and Cooke (2021) hypothesizing that shoal formation could serve as group protection while migrating to deeper waters. Collective animal migration has several potential advantages, such as the availability of social information regarding prey, predators, and group movement (Couzin 2018). Effectively taking advantage of such public information entails forms of social/local enhancement and social learning (Krause and Ruxton 2002, Dugatkin 2014). After hatching all in the same vicinity, it is possible that cuttlefish species remain more socially engaged than previously thought (particularly during sub-adult stages), and that a link exists between the capability of learning through others and the (now multiple times) reported group formations in the wild, as development of one could lead to development/improvement of the other.

In **Part II,** I evaluated interspecific or heterospecific interactions between asocial octopuses and other individuals that usually co-occur in the same habitat and functional domain. In ***Chapter 4***, it was showed that octopuses are able to physically take advantage of heterospecifics, by using nearby sea cucumbers both as a den cover and vantage point from which to keep vigilance of the environment. As explained in the same chapter, this report constitutes the first evidence of social tool use (Völter et al. 2015) in a non-social or aggregating animal, as *Octopus cyanea* individuals are not known to aggregate, with exception to mating pairs near their life cycle. Albeit a simple use is made of the sea cucumber (analogous to how one would use an inanimate object), the occurrence of social tool use suggests that the complexity of the heterospecific enviroment can be sufficient for individuals to learn and exploit the properties of other individuals (or 'live objects') to their own advantage.

Finally, in ***Chapter 5*** and ***Chapter 6***, I further explore the heterospecific environment of *O. cyanea*, specifically the underlying dynamics and mechanisms of the hunting groups formed with several species of fish. While varied species-specific dynamics and feedbacks exist as demonstrated in the latter chapter, here the focus was on how collective hunts shape decision-making and behavior of octopuses. From the octopus perspective, it is showed that individuals actively follow fish, maintain hunts with the same partners for more than one hour, and specifically discriminate among social information sources when choosing whom to follow. In social interactions, partners rely on the ability to consider each other's behavior in their decision-making process (Axelrod and Hamilton 1984, Lehmann and Keller 2006). In such situations, maximizing personal benefits and avoiding exploitation involves evaluating costs and benefits and using partner control mechanisms (Raihani et al. 2012). Importantly, the octopus-fish system does not fit nuclear-follower models or descriptions, as group movement stems mostly from shared decision-making. In fact, individuals from at least two fish species are more often leaders in these groups than the octopus itself, indicating that the latter interprets and frequently uses the social information made available by fish partners. As such, this system creates an intricate network of social interactions where each individual differentially takes into account multiple partner's specific position, kinematic characteristics, and behavior, to decide on their own actions.

7.1. Are cephalopods social?

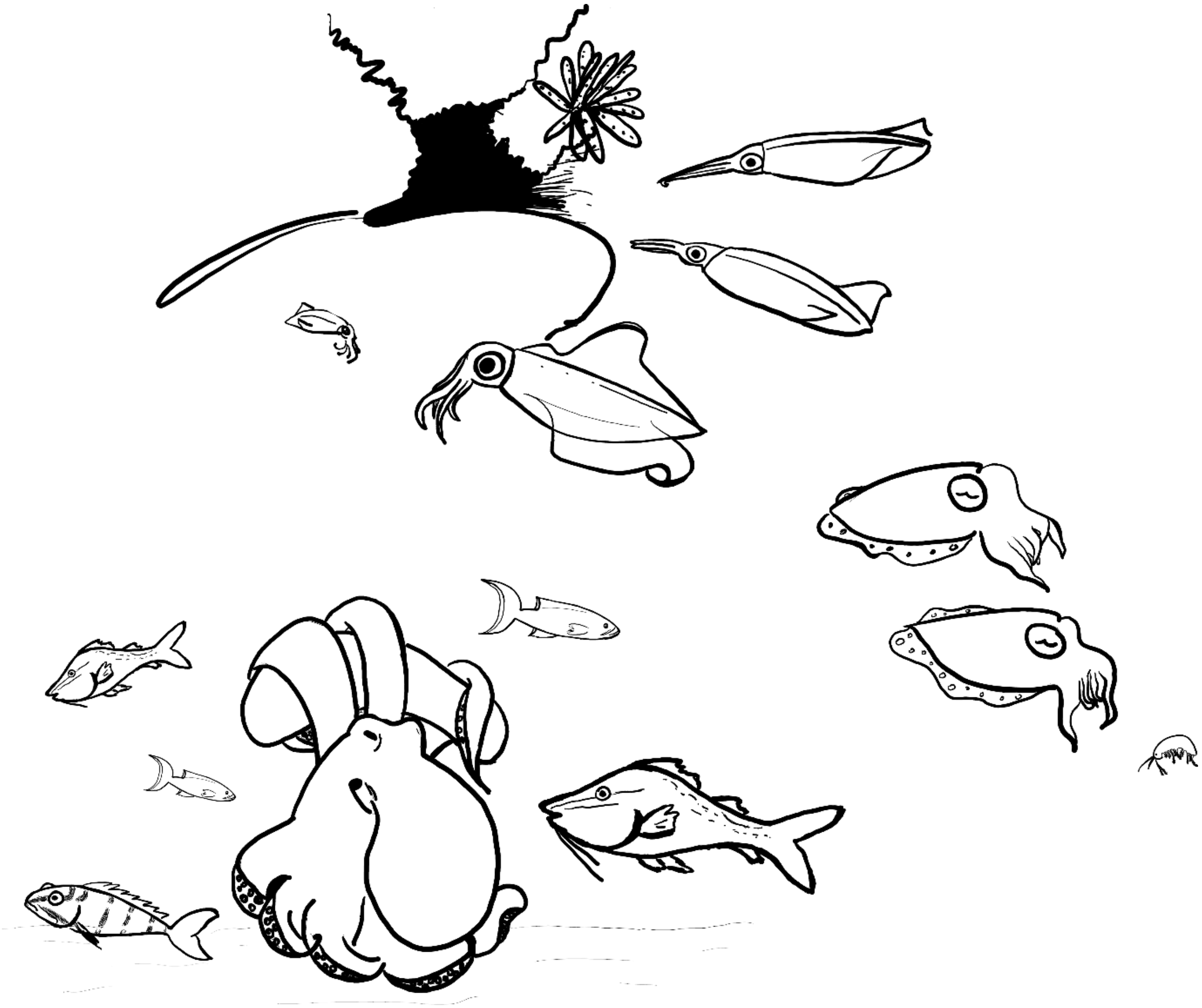

Figure 7.1. Graphic design representing the unveiled social aspects of the lives of cephalopods in this dissertation: squid location probing and complex sexual dynamics (Chapter 2), cuttlefish social learning of predatory behavior modulation (Chapter 3), and octopus social tool use (Chapter 4) and collective hunting with fish (Chapter 5 and 6). Credit: Maria Rita Pegado.

The short answer is: **Yes**. From the work performed within the framework of this dissertation, it can be concluded that complex interactions with other individuals can play a large role in the lives of all groups of the coleoid cephalopods (Figure 7.1).

First, from **Part I**, it can be concluded that aggregating cephalopods are social in the strictest sense of the word (interactions with conspecifics and partner-dependent behavior shaping decision-making of individuals), and that these social relationships can be more complex than previously thought by entailing multiple alternative tactics and complex social learning. Moreover, while no social learning process has been asserted in squid species, our observations during mating season combined with previous laboratory studies (Lin and Chiao 2017, Apostólico and Marian 2019) show that these animals consider the available social information in the environment and learn to choose between sexual tactics throughout their ontogeny based on conspecific feedback. The same seems to be true for cuttlefish (which we now know are capable of learning by observing others), with multiple studies reporting sexual partner- and rival-dependent behavior, as well as being influenced by recent mating history (Schnell et al. 2014, Mather 2016, Allen et al. 2017, Hanlon and Messenger 2018). Moreover, shoaling behavior in cuttlefish during subadult stages appears more frequent than previously thought (Yasumuro et al. 2015, Drerup and Cooke 2021), creating opportunities for social interactions and development at early ontogenetic stages.

Chapter 2 also may have provided the first evidence of paternal care in cephalopods, with male squid having an active (and potentially costly) role in selecting locations for females to lay their eggs. Thus, beyond extending behavioral options for each intervenient *per se*, this indicates that male sex roles potentially involve not only intrasexual competition, but also intersexual competition with females on the amount of parental care to provide. Generally, when anisogamy occurs, females have the largest sexual gamete and therefore invest more resources producing it

(Kodric-Brown and Brown 1987). Because of that, the operational sex ratio is shifted to males and these compete amongst themselves for females, while also in principle selecting for less parental care, as this would limit resources being used for intrasexual competition (Kokko and Jennions 2008). However, this type of sexual selection simultaneously prompts females to evolve mechanisms seeking to obtain or secure higher resources (Kodric-Brown and Brown 1987, Morse and Huffard 2019), be it a larger pool of high quality sperm to fertilize her eggs or by promoting the enforcement or 'extraction' of paternal care to ensure greater probabilities of reproductive success (Kirkpatrick 1982, Kokko and Jennions 2008). Such feedback mechanisms can represent yet another layer of complexity in the selection and evolution of squid decision-making processes hinging on partner-dependent behavior. We thus build on alternative hypotheses holding mating aggregations as potential grounds for behavioral flexibility and cognitive evolution (Amodio et al. 2018, Schnell et al. 2021), by expanding: a) the range of individual-specific behavioral strategies and sexual tactics existent, and b) potentially the underlying nature of sexual selection in cephalopods, with the inclusion of paternal investment and intersexual competition.

Part II delivers evidence of complex interspecific relationships, particularly between octopuses and fishes. As alluded throughout the introduction, despite varying the potential competitive or beneficial outputs depending on niche and life history overlap, there is little fundamental (or practical) difference in the nature of social relationships between different species or (unrelated) individuals of the same species. It is widely known that Darwinian selection does not occur at the species level, and that potential group selection (Nowak et al. 2010), can be explained by lower level individual selection and inclusive fitness (Abbot et al. 2011). Thus, when kin selection and related indirect benefits are non-existent, what is selected in interactions between unrelated individuals are the individuals themselves and the resulting direct benefits or costs, regardless of their species (a concept which, albeit useful in general, is a human construct). Recent publications have highlighted the same reasoning of expanding the concept of social behavior to heterospecific interactions ocurring at the same time and spatial scales (Sridhar and Guttal 2018, Oliveira and Bshary 2021), inciting more integrative approaches towards understanding social interactions between organisms.

Recent work has supported a larger role of the Ecological Intelligence hypothesis for the cognitive evolution of cephalopods (see Rosati 2017), which, given complex foraging niches and limited social interactions, seems to find particular support in solitary octopus species that have a generalist, but hunting skills-specific diet (Amodio et al. 2018). However, it is also known that comparatively neglected (Sridhar and Guttal 2018) heterospecific sociality can lead to cognitively complex processes as highlighted in cleaner-client systems (Grutter 1999, 2004, Bshary and Grutter 2006). If we now assess the studied octopus-fish hunting groups, these individuals hunt together in groups where decision-making is shared while displaying coordinated movement and having species-specific social preferences and hierarchies. Moreover, individuals can use interspecific referential gestures to point towards prey locations (active communication) and also use partner control mechanisms. Such complex interspecific feedback scenarios, in contrast to predator-prey dynamics, are more akin to the demands of intraspecific cooperation and intermittent group living.

Thus, I propose that the studied multispecific hunting groups, as well as other complex heterospecific interactions, can blur the lines between the Social and Ecological Intelligence hypotheses by promoting complex social relationships between co-ocurring heterospecific individuals, and may have played a role in the development of complex cognition of otherwise solitary octopuses. Moreover, given the complexities found within this dissertation relating to social aspects of squid and cuttlefish during their life histories, it is probable that complex interactions with other individuals (conspecific and heterospecific) played a role in shaping the evolution of decision-making, behavior, and cognition, of cephalopods in general, alongside the predator-prey interactions encompassed by the Ecological Intelligence hypothesis. One could tentatively name the merging of these two views as the **"*Socio-Ecological Intelligence Hypothesis*"**.

7.2. Future directions

As any scientific output, the answers provided along this dissertation end up raising even more questions. To ascertain that location probing is truly the first case of paternal investment in cephalopods, it must first be confirmed that the consort male's sperm is indeed passed on to the next generation, and is thus part of 'paternal behavior'. However, it seems very likely that location probing is included on the general consort male's behavioral repertoire and sustained across generations on *S. lessoniana* populations, as we reported this sexual behavior in populations separated by 10,000 km and 6 years apart. Concurrently, the matters of potential intersexual selection and social scenarios where simple and complex forms of social learning can occur provide more intriguing and exciting venues for future studies to explore.

In octopus-fish systems, consistency in hunting partnership is unknown, and it would be crucial to understand if specific individuals within the same fish species are recognized and preferentially followed. Such would indicate if, in hunting groups that can reach up to 10-12 individuals from different species, there is individual recognition between partners or individuals resort to more heuristic rules, such as species-level recognition. For that, more data collected on natural world scenarios and surveillance of identities for sequential days would enable time-extended comparisons. Moreover, experimental manipulations in the field could also help us better understand the social rules operating in these groups and the contexts leading up to the use of partner control mechanisms.

Multiple studies have been previously published across the world reporting fish 'scavenging' or 'following' octopuses (Diamant and Shpigel 1985, Mather 1992, Sazima et al. 2007, Machado and Barreiros 2008), including early descriptions with *Octopus cyanea* (Diamant and Shpigel 1985, Forsythe and Hanlon 1997). This of course begs the question: are these relationships truly nuclear-follower models as described or would a more quantitative analyses reveal hidden structures in these pairs or groups. Such would be important to understand how the ability to use social information is widespread across coastal octopuses, and would also allow us to better understand how collective hunting skills arose in these cephalopods. For example, one possibility is that it has evolved or was learned fairly recently, in response to the presence of following fish

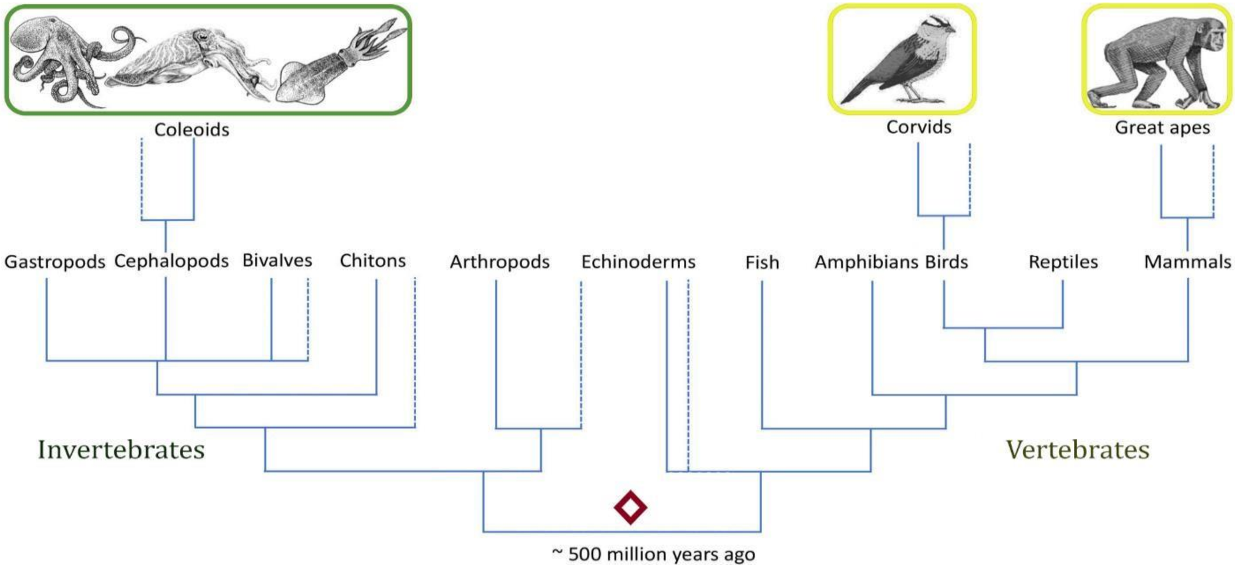

Figure 7.2. A simplified schematic of the phylogenetic tree of life. The most common ancestor between cephalopods (as well as other invertebrates) and vertebrates dates to ~500 million years ago (red diamond). Adapted from Schnell et al. 2021 and Vitti 2013.

while foraging in habitats with high visibility. As such, shared leadership of collective hunting would occur only sporadically in specific locations. Alternatively, if cephalopods worldwide use social information provided by fish to find other prey and maintain collective hunts for considerable amounts of time, that would suggest an earlier evolution of this form of social competence, and could have been already present in previous ancestors.

When inquiring on the convergent evolution of complex behavior and brain/cognitive development (Figure 7.2), cephalopods are excellent case studies as they do not fit the usual characteristics of complexly behaving organisms, such as apes and birds (Wirthlin et al. 2018, Schnell et al. 2021). On one hand, apes and birds are usually characterized by high longevity and long developmental periods, together with an omnivorous diet; cephalopods on the other hand, 'live fast and die young' with short developmental periods and longevity (at least in coastal species) and are highly carnivorous (Amodio et al. 2018, Schnell et al. 2021). Prolonged exposures to social stimuli in animal groups are hypothesized to help in establishing more complex social bonds which could stimulate cognition and partner-dependent behavioral flexibility, as part of the Social Intelligence Hypothesis (Barton and Dunbar 1997). The matter of prolonged bonds in cephalopods is still unanswered, and while it seems unlikely to occur in octopuses and in adult cuttlefish, it is possible that, after dispersing, squid aggregate in variable group sizes and remain together during the rest of their life. The potential existence of a consistency in social bonds could create the opportunity for more complex social relationships between individuals to occur, involving 'book-keeping' and/or other cognitive features requiring evaluations of iterated interactions (Dugatkin 2014), in for example intraspecific coordinated hunting (Benoit-Bird and Gilly 2012).

To understand and build hypotheses on the underlying evolutionary processes and selective pressures that living beings are subjected to, it is key that we are fully knowledgeable of the entirety of their life history and *umwelt*. Considering phylogenetic relationships and geographical characteristics can help us pinpoint environment-specific pressures modulating the reproductive success of individuals and leading to increases in the frequency of genes that provide higher fitness in populations. Together with the abiotic environment, socio-ecological landscapes, and the complexity of interactions therein, are crucial in shaping the evolution of decision-making processes, behavioral flexibility, and complex cognition. Finally, we must keep in mind that, even during our lifetime, populations are not static but unceasingly dynamic, and that organisms still "*…are being, evolved"*.

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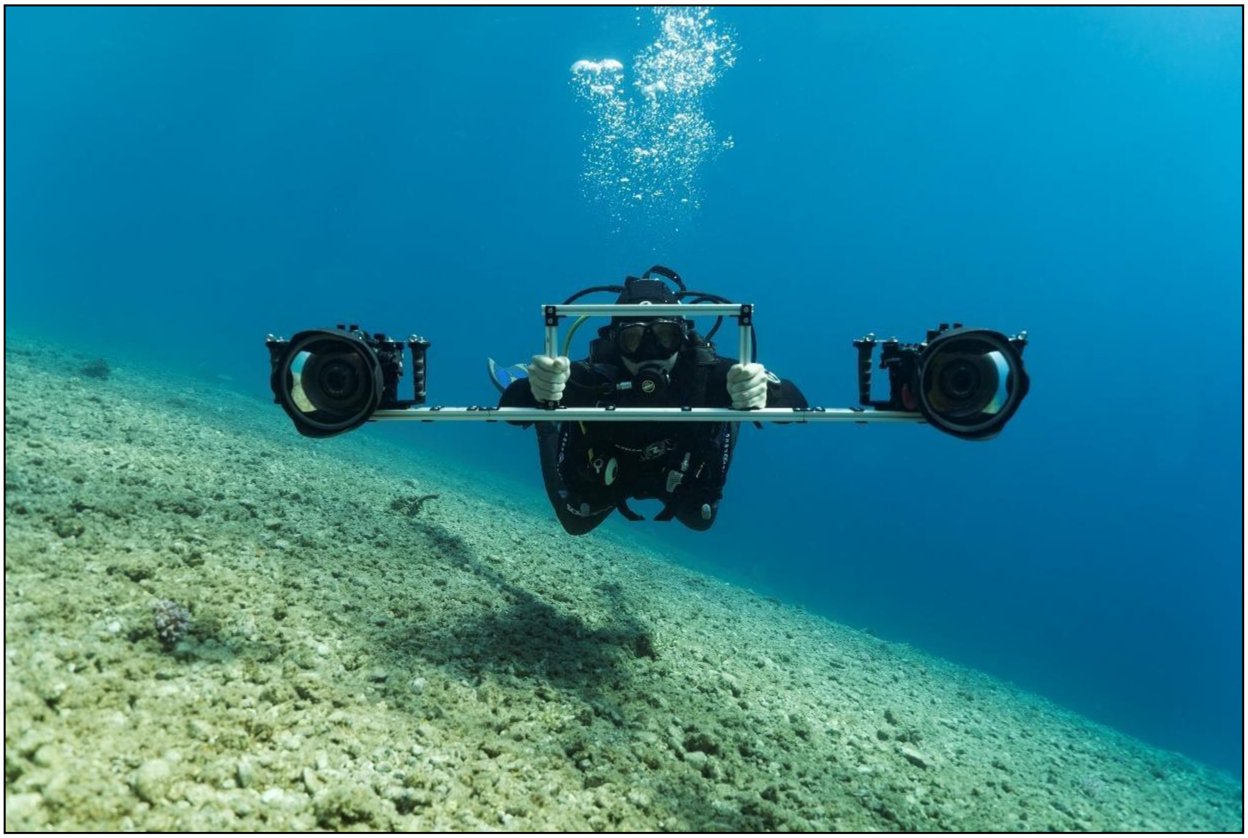
> “In conclusion, it appears that nothing can be more improving to a young naturalist, than a journey in distant countries.” *Charles Darwin, Voyage of the Beagle*

Annexes

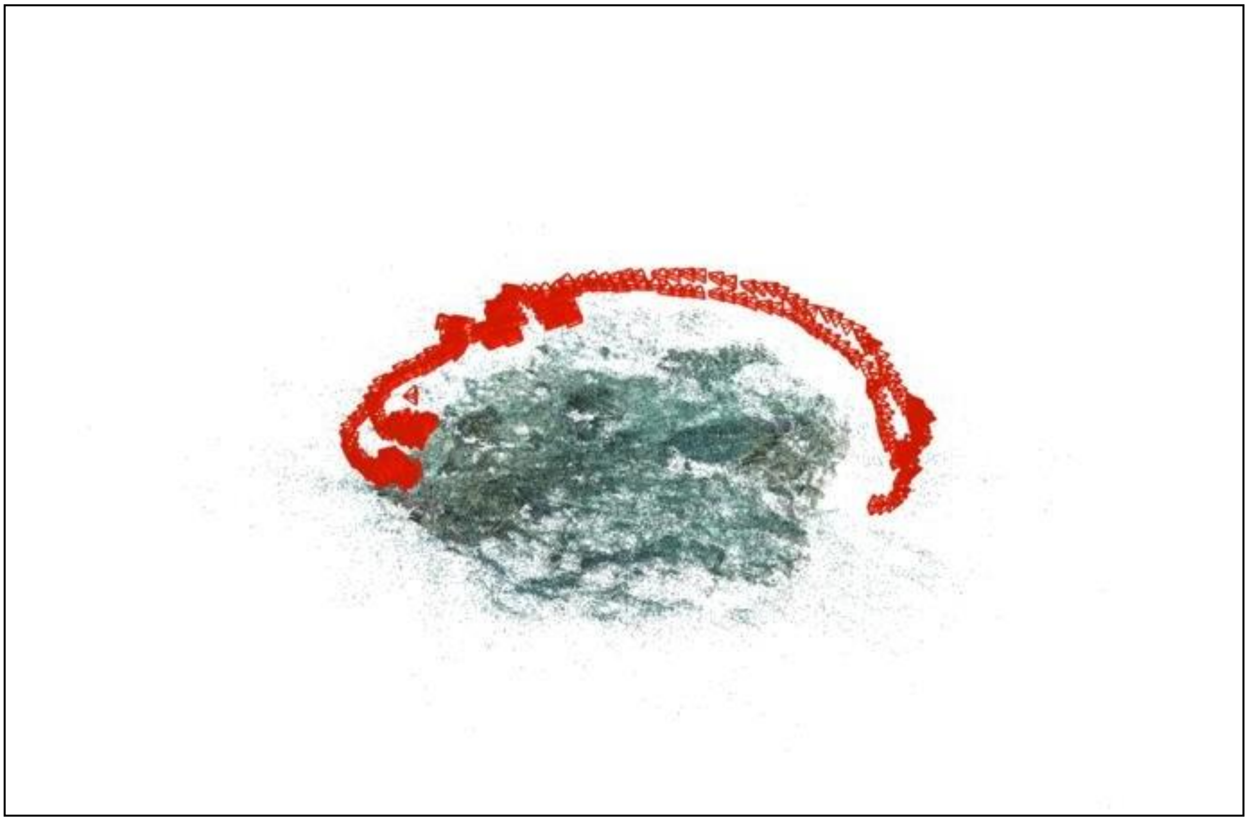
Supplemental Figures

Supplemental Figure 1. Snapshot from videos recorded during trial execution, demonstrating the experimental setup used to gauge social learning between cuttlefish.

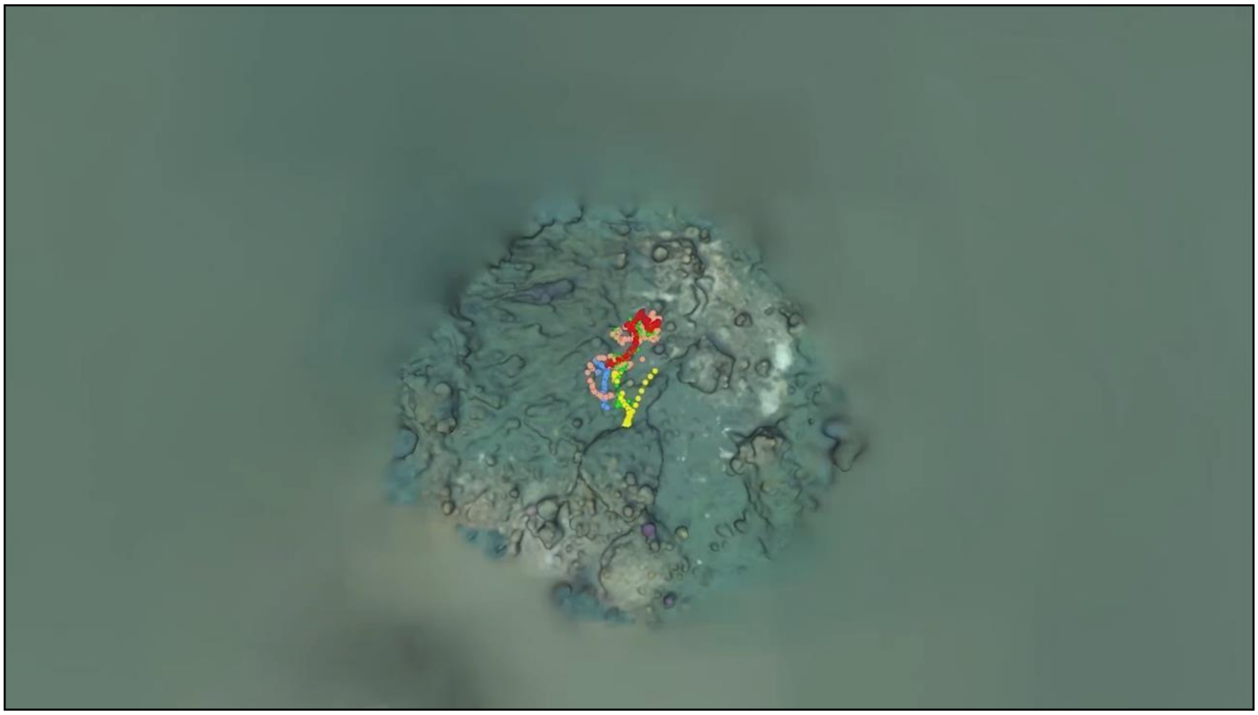
Supplemental Figure 2. Some of the most common species found in multi-specific hunting groups. **A.** blue octopus *Octopus cyanea*, **B.** lyretail grouper *Variola louti*, **C.** blacktip grouper *Epinephelus fasciatus*, **D.** Red sea goatfish *Parupeneus forsskali* (hereafter ‘greengoatfish’), and **E.** juvenile and **F.** adult yellowsaddle goatfish *Parupeneus cyclostomus*. Images from Wikimedia Commons.



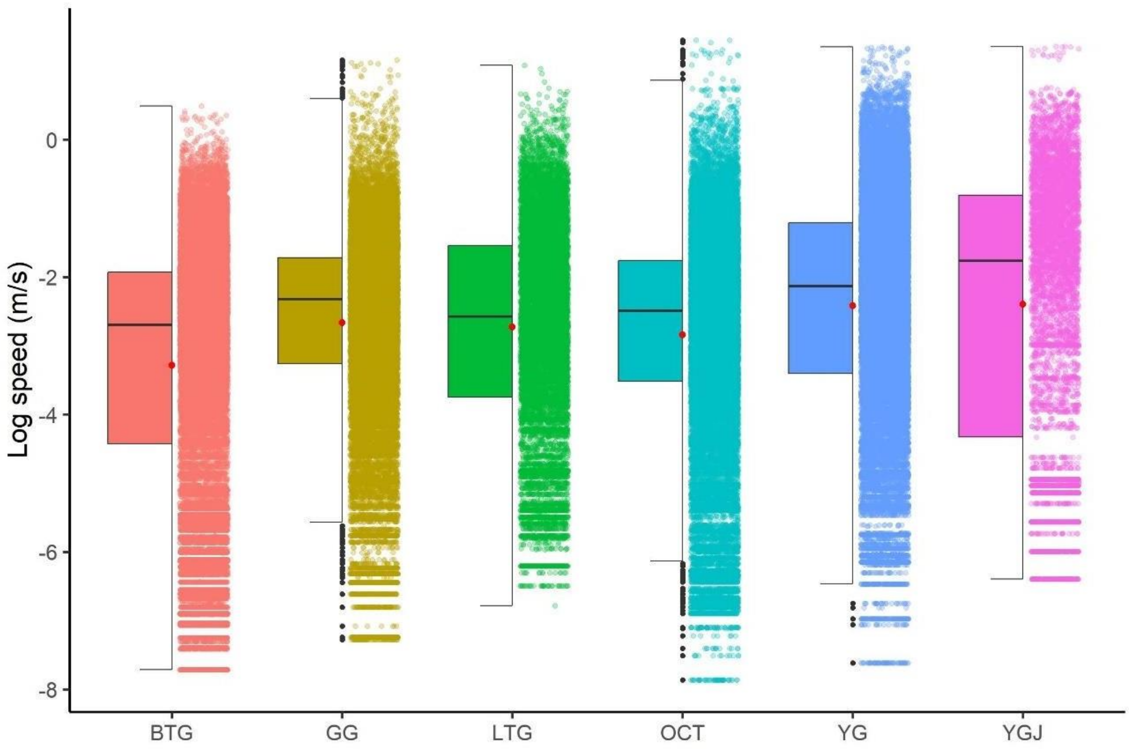
Supplemental Figure 3. The ‘Stereocamera Rig’, composed by two parallel cameras equiped with wide lenses, fixed on an aluminum structure with a length of 1.2 m.



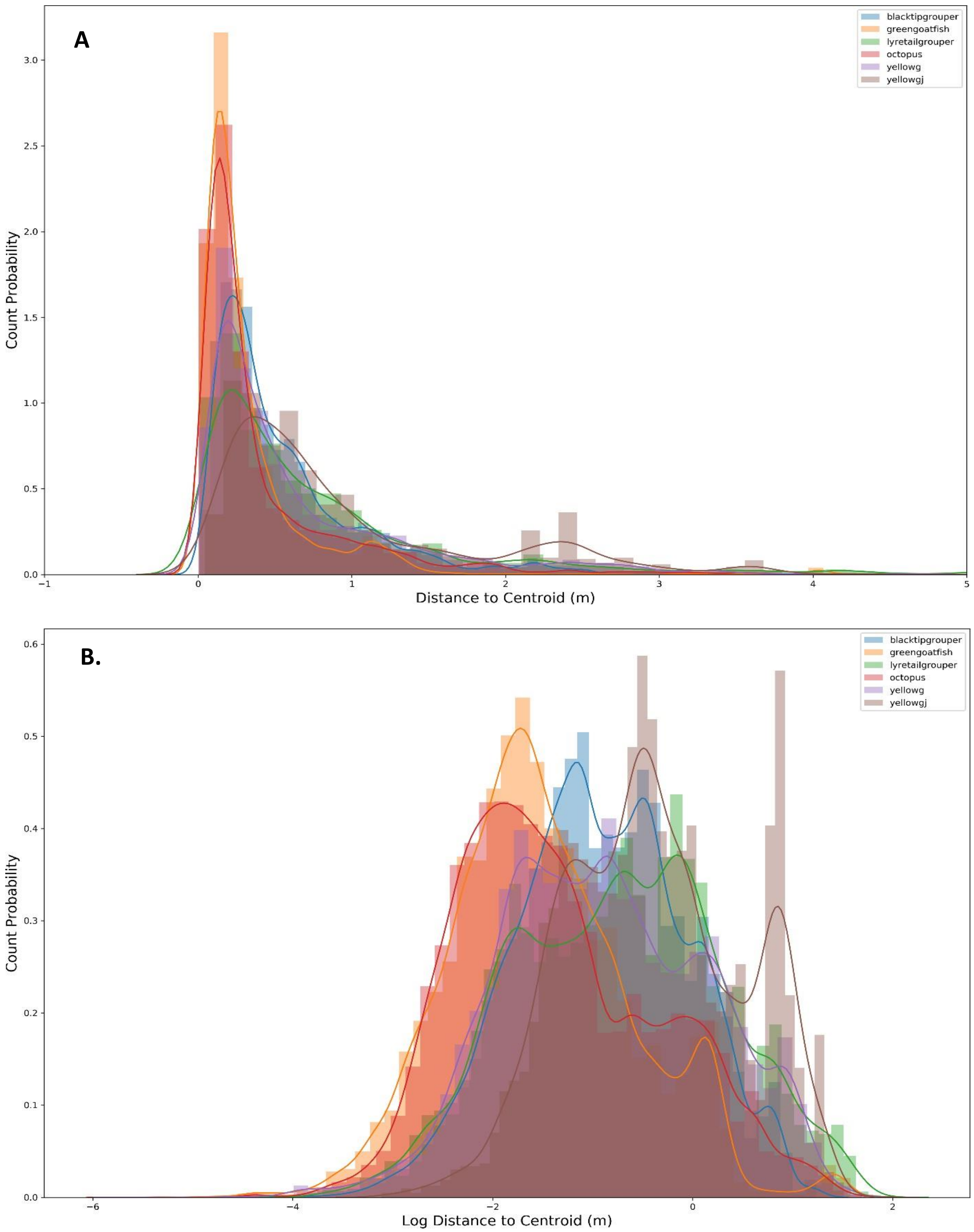
Supplemental Figure 4. Dense cloud point and camera positions outputed from *colmap* after image sampling and scene reconstructions (animation by ©Fritz Francisco).



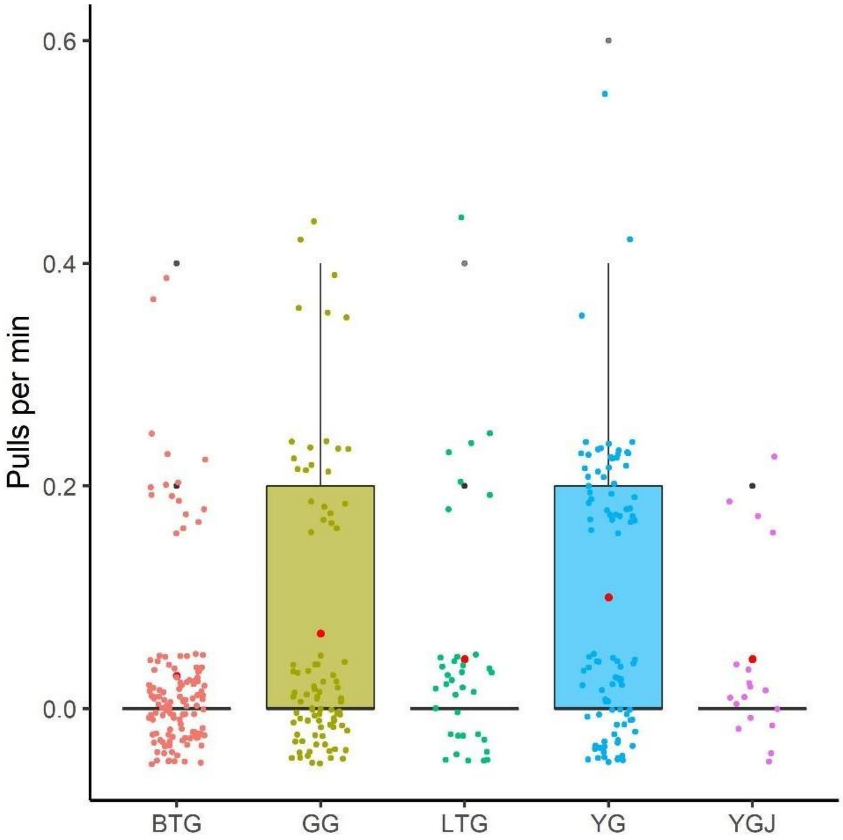
Supplemental Figure 5. Example of 3-dimensional reconstructed habitat and individual tracks after *mvt* pipeline.



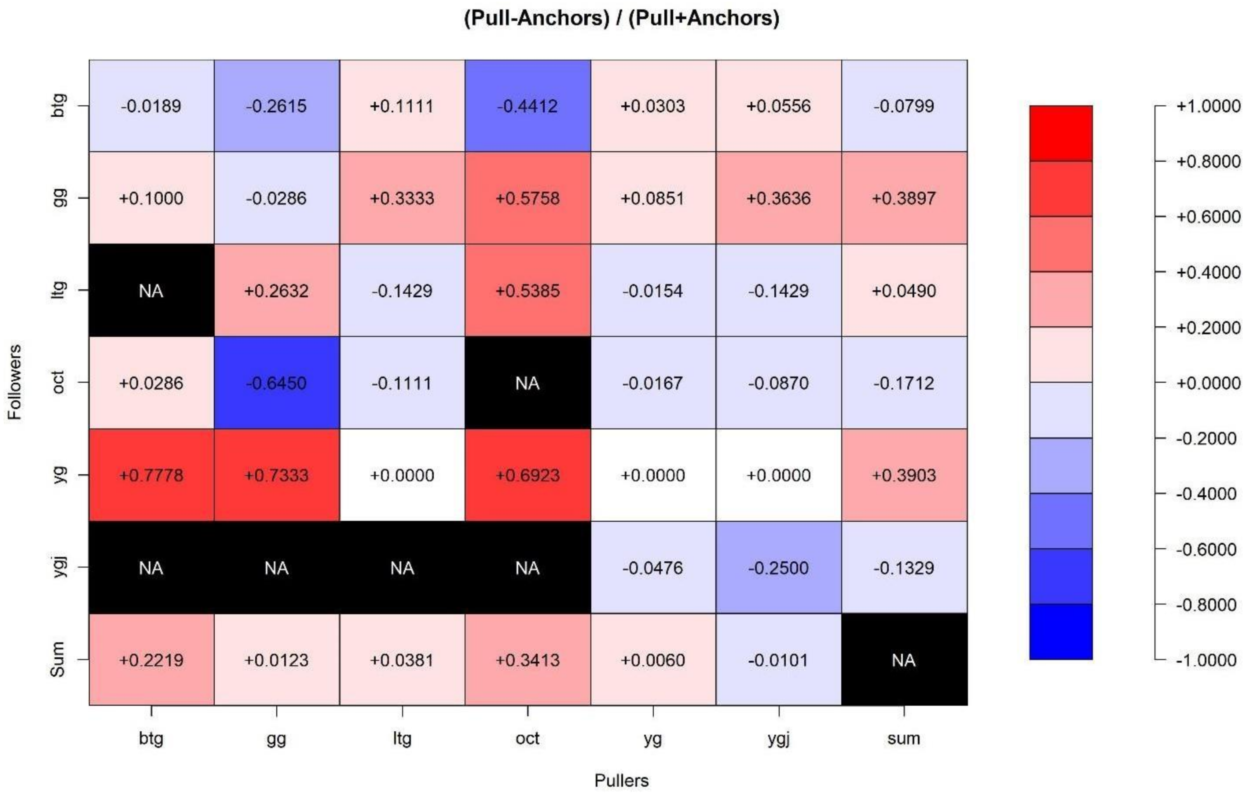
Supplemental Figure 6. The natural logarithm of speed (log speed) for the most prevalent species registered in collective interspecific hunts. BTG - Black tip grouper, GG - Green Goatfish, LGT - Lyretail grouper, OCT - Octopus, YG - yellowsaddle goatfish, YGJ - yellowsaddle goatfish juvenile.



Supplemental Figure 7. A. Distribution of individual distance to group centroid (calculated via the mean of all individual position in a given frame) and **B.** the natural logarithm of said distance, according to species. As described in the methods, the mode (i.e. highest probability value) of the kernel distribution estimate was used to rank species according to closeness to group centroid.

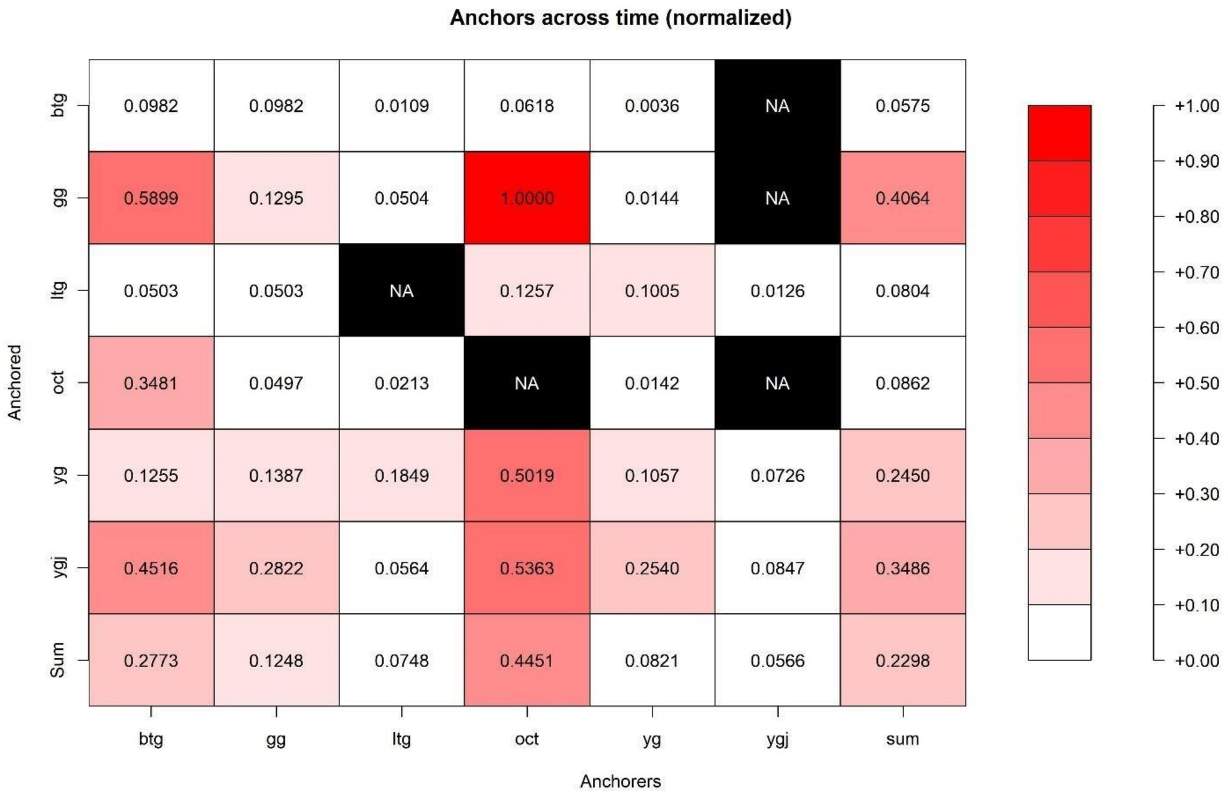


Supplemental Figure 8. Boxplots and datapoints showing the number of pulls per minute targeting the octopus as follower, according to species. Red dots indicate mean values for each species.



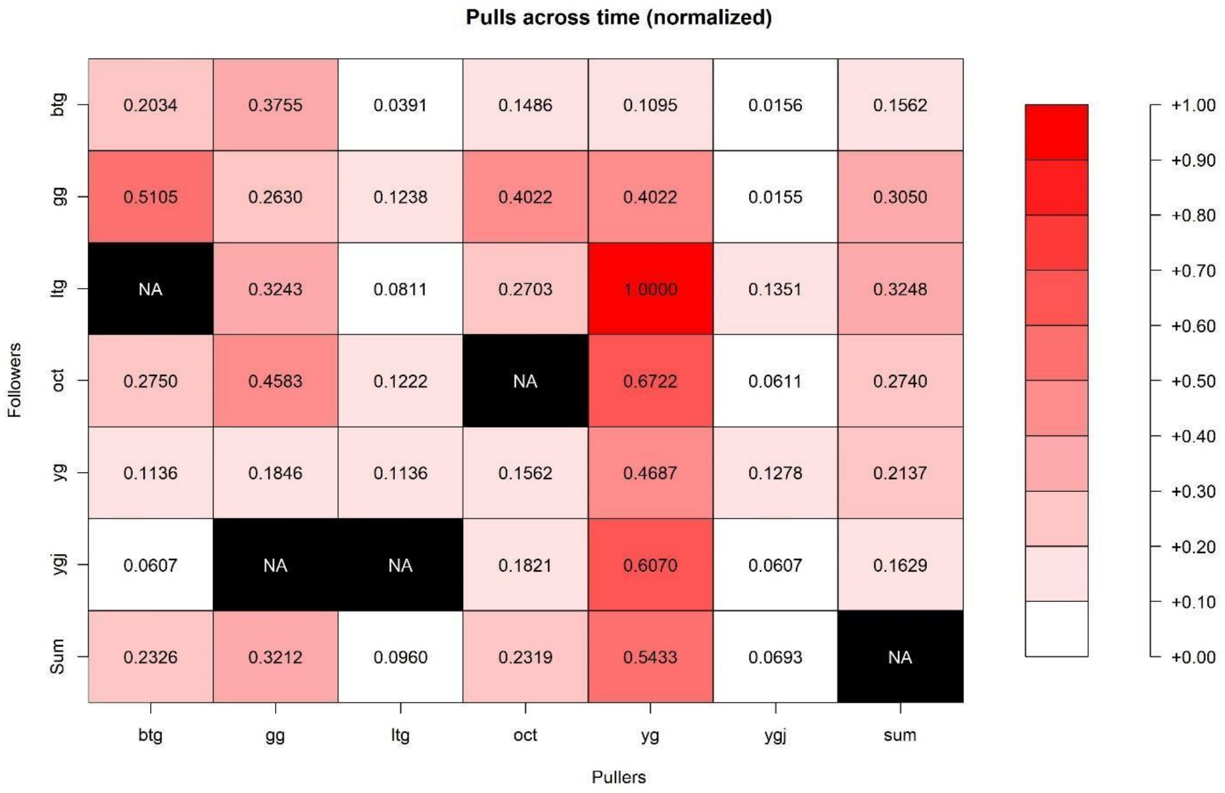
	btg	gg	ltg	oct	yg	ygj	sum
btg	-0.0189	-0.2615	+0.1111	-0.4412	+0.0303	+0.0556	-0.0799
gg	+0.1000	-0.0286	+0.3333	+0.5758	+0.0851	+0.3636	+0.3897
ltg	NA	+0.2632	-0.1429	+0.5385	-0.0154	-0.1429	+0.0490
oct	+0.0286	-0.6450	-0.1111	NA	-0.0167	-0.0870	-0.1712
yg	+0.7778	+0.7333	+0.0000	+0.6923	+0.0000	+0.0000	+0.3903
ygj	NA	NA	NA	NA	-0.0476	-0.2500	-0.1329
Sum	+0.2219	+0.0123	+0.0381	+0.3413	+0.0060	-0.0101	NA

Supplemental Figure 9. Species-specific breakdown of all pull-anchor interactions, measured as the difference between successful (pulls) and unsuccessful pulls (i.e. anchors), divided by the total number of attempts (pulls plus anchors). Rows depict detailed species performances in a pairwise fashion as followers, columns depict performance as leaders. Last row and column represent the sum of all interactions pertaining to that species as either follower or leader respectively, divided by the number of species interacted with (n > 3 pulls or anchors). Increasingly saturated shades of red indicate species performing more pulls than being anchored, whereas increasingly saturated shades of blue indicate species being more anchored than performing successful pulls. From the last column, a species ranking can be created assessing ratio leadership (see Figure 6). btg - black tip grouper, gg - green goatfish, ltg - lyretail grouper, oct - octopus, yg - yellowsaddle goatfish, ygj - yellowsaddle goatfish juvenile.



	btg	gg	ltg	oct	yg	ygj	sum
btg	0.0982	0.0982	0.0109	0.0618	0.0036	NA	0.0575
gg	0.5899	0.1295	0.0504	1.0000	0.0144	NA	0.4064
ltg	0.0503	0.0503	NA	0.1257	0.1005	0.0126	0.0804
oct	0.3481	0.0497	0.0213	NA	0.0142	NA	0.0862
yg	0.1255	0.1387	0.1849	0.5019	0.1057	0.0726	0.2450
ygj	0.4516	0.2822	0.0564	0.5363	0.2540	0.0847	0.3486
Sum	0.2773	0.1248	0.0748	0.4451	0.0821	0.0566	0.2298

Supplemental Figure 10. Species-specific breakdown of all anchor interactions over time, measured as the number of anchors performed while individuals of both species were present in a given hunt, afterwards normalized by dividing by the maximum number of anchors registered. Rows depict detailed species performances in a pairwise fashion as anchored, columns depict performance as anchorers. Last row and column represent the sum of all interactions pertaining to that species as either anchored or anchorer respectively, divided by the number of species interacted with (n > 3 anchors). Increasingly saturated shades of red indicate species interactions with more anchors per minute. From the last column, a species ranking can be created assessing effective anchoring (see Figure 7). btg - black tip grouper, gg - green goatfish, ltg - lyretail grouper, oct - octopus, yg - yellowsaddle goatfish, ygj - yellowsaddle goatfish juvenile.



Followers \ Pullers	btg	gg	ltg	oct	yg	ygj	sum
btg	0.2034	0.3755	0.0391	0.1486	0.1095	0.0156	0.1562
gg	0.5105	0.2630	0.1238	0.4022	0.4022	0.0155	0.3050
ltg	NA	0.3243	0.0811	0.2703	1.0000	0.1351	0.3248
oct	0.2750	0.4583	0.1222	NA	0.6722	0.0611	0.2740
yg	0.1136	0.1846	0.1136	0.1562	0.4687	0.1278	0.2137
ygj	0.0607	NA	NA	0.1821	0.6070	0.0607	0.1629
Sum	0.2326	0.3212	0.0960	0.2319	0.5433	0.0693	NA

Supplemental Figure 11. Species-specific breakdown of all pull interactions over time, measured as the number of pulls performed while individuals of both species were present in a given hunt, afterwards normalized by dividing by the maximum number of pulls registered. Rows depict detailed species performances in a pairwise fashion as followers (i.e. successfully being pulled), columns depict performance as pullers (i.e. successful leaders). Last row and column represent the sum of all interactions pertaining to that species as either follower or puller respectively, divided by the number of species interacted with (n > 3 anchors). Increasingly saturated shades of red indicate species interactions with more pulls per minute. From the last column, a species ranking can be created assessing effective leadership (see Figure 8). btg - black tip grouper, gg - green goatfish, ltg - lyretail grouper, oct - octopus, yg - yellowsaddle goatfish, ygj - yellowsaddle goatfish juvenile.

Table S1. GLMM analysis with first-order autoregressive variance structure concerning frame number for each scene, as well as scene and individual identity within each scene as nested random effects, measuring variation of speed (log transformed) according to species (6 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellow saddlegoatfish). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate $p < 0.05$.

Family: Gaussian (identity)

Formula: log_speed ~ species + (1 | dataset/id) + ar1(FRAME_IDX + 0 | dataset)

Random effects:

Groups	Name	Variance	Std.Dev.	Corr (ar1)
id.dataset	(Intercept)	0.367947	0.60659	
dataset	(Intercept)	0.000189	0.01376	
dataset.1	FRAME_IDX	1.070875	1.03483	0.99
Residual	1.5059202	1.22716		

Fixed Effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-3.3378	0.1518	-21.985	**< 0.0001**
speciesgreengoatfish	0.5001	0.2211	2.2620	**0.0237**
specieslyretailgrouper	0.8630	0.2347	3.6780	**0.0002**
speciesoctopus	0.4638	0.1993	2.3270	**0.0200**
speciesyellowg	1.0241	0.2205	4.6450	**< 0.0001**
speciesyellowgj	0.8837	0.3191	2.7700	**0.0056**

Tukey HSD

contrast	Estimate	df	t ratio	p value	p-value
blacktipgrouper - greengoatfish	-0.5001	0.2210	147881	-2.2620	0.2098
blacktipgrouper - lyretailgrouper	-0.8630	0.2350	147881	-3.6780	**0.0032**
blacktipgrouper - octopus	-0.4638	0.1990	147881	-2.3280	0.1829
blacktipgrouper - yellowg	-1.0241	0.2200	147881	-4.6460	**<.0001**
blacktipgrouper - yellowgj	-0.8837	0.3190	147881	-2.7700	0.0624
greengoatfish - lyretailgrouper	-0.3629	0.2680	147881	-1.3530	0.7551
greengoatfish - octopus	0.0363	0.2340	147881	0.1550	1.0000
greengoatfish - yellowg	-0.5240	0.2520	147881	-2.0830	0.2960
greengoatfish - yellowgj	-0.3836	0.3460	147881	-1.1080	0.8782
lyretailgrouper - octopus	0.3992	0.2340	147881	1.7050	0.5281
lyretailgrouper - yellowg	-0.1611	0.2450	147881	-0.6570	0.9865
lyretailgrouper - yellowgj	-0.0207	0.3440	147881	-0.0600	1.0000
octopus - yellowg	-0.5603	0.2210	147881	-2.5370	0.1134
octopus - yellowgj	-0.4199	0.3240	147881	-1.2960	0.7875
yellowg - yellowgj	0.1404	0.3220	147881	0.4360	0.9980

Table S2. GLMM analysis with first-order autoregressive variance structure concerning frame number for each scene, as well as scene and individual identity within each scene as nested random effects, measuring variation in distance to group centroid according to species (6 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellow saddlegoatfish). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate $p < 0.05$.

Family: Gamma (log)

Formula: dist_cent ~ species + (1 | dataset/id) + ar1(FRAME_IDX + 0 | dataset)

Random effects:

Groups	Name	Variance	Std.Dev.	Corr (ar1)
id.dataset	(Intercept)	0.05475	0.234	
dataset	(Intercept)	0.20127	0.4486	
dataset.1	FRAME_IDX9	0.70155	0.8376	1
Residual	1.50592	1.22716		

Fixed Effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.8068	0.1680	-4.8020	**< 0.0001**
speciesgreengoatfish	-0.4339	0.0873	-4.9680	**< 0.0001**
specieslyretailgrouper	-0.0454	0.0980	-0.4630	0.6432
speciesoctopus	-0.3631	0.0807	-4.5010	**< 0.0001**
speciesyellowg	-0.2696	0.0962	-2.8020	**0.0051**
speciesyellowgj	-0.0631	0.1309	-0.4820	0.6295

Tukey HSD:

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	0.1571	0.0397	148555	3.954	**0.0011**
blacktipgrouper - lyretailgrouper	0.0198	0.0426	148555	0.465	0.9973
blacktipgrouper - octopus	0.1359	0.0378	148555	3.594	**0.0044**
blacktipgrouper - yellowg	0.1054	0.0409	148555	2.579	0.1023
blacktipgrouper - yellowgj	0.0273	0.0557	148555	0.49	0.9965
greengoatfish - lyretailgrouper	-0.1373	0.0458	148555	-3.001	**0.0322**
greengoatfish - octopus	-0.0212	0.0278	148555	-0.763	0.9737
greengoatfish - yellowg	-0.0517	0.0344	148555	-1.501	0.6636
greengoatfish - yellowgj	-0.1298	0.058	148555	-2.236	0.2210
lyretailgrouper - octopus	0.1161	0.0407	148555	2.855	**0.0493**
lyretailgrouper - yellowg	0.0856	0.0405	148555	2.113	0.2803
lyretailgrouper - yellowgj	0.0075	0.0576	148555	0.13	1.0000
octopus - yellowg	-0.0304	0.0299	148555	-1.018	0.9121
octopus - yellowgj	-0.1086	0.0546	148555	-1.99	0.3481
yellowg - yellowgj	-0.0781	0.0526	148555	-1.487	0.6730

Table S3. GLMM analysis with scene and individual identity within each scene as nested random effects, measuring variation in number of unsuccessful pulls (i.e. times anchored) per minute (offset = 5 minutes) according to species (6 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellow saddlegoatfish). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate p < 0.05.

Family: poisson (log)
Formula: anchor ~ lead_spe + (1 | scene/individual) + (1 | block) + offset(time)

Random effects:

Groups	Name	Variance	Std.Dev.
individual:scene	(Intercept)	0.2714	0.5209
scene	(Intercept)	0.3095	0.5564
block	(Intercept)	0.3486	0.5904

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-6.4055	0.3032	-21.127	**< 0.0001**
lead_spegreengoatfish	1.5582	0.2677	5.8210	**< 0.0001**
lead_spelyretailgrouper	1.1569	0.4215	2.7450	**0.0061**
lead_speoctopus	-0.1622	0.3352	-0.4840	0.6285
lead_speyellowgoatfish	2.2294	0.3443	6.4740	**< 0.0001**
lead_speyellowgoatfishj	2.6602	0.4090	6.5040	**< 0.0001**

Tukey HSD:

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	-0.9197	0.2873	479	-3.2020	**0.0181**
blacktipgrouper - lyretailgrouper	-0.5346	0.2732	479	-1.9570	0.3688
blacktipgrouper - octopus	0.0367	0.0759	479	0.4840	0.9967
blacktipgrouper - yellowg	-2.0342	0.5585	479	-3.6420	**0.0040**
blacktipgrouper - yellowgj	-3.2616	1.2292	479	-2.6540	0.0868
greengoatfish - lyretailgrouper	0.3851	0.3732	479	1.0320	0.9071
greengoatfish - octopus	0.9565	0.3026	479	3.1610	**0.0206**
greengoatfish - yellowg	-1.1145	0.5669	479	-1.9660	0.3637
greengoatfish - yellowgj	-2.3419	1.2114	479	-1.9330	0.3831
lyretailgrouper - octopus	0.5714	0.2667	479	2.1420	0.2673
lyretailgrouper - yellowg	-1.4996	0.5130	479	-2.9230	**0.0420**
lyretailgrouper - yellowgj	-2.7270	1.2021	479	-2.2690	0.2091
octopus - yellowg	-2.0709	0.5524	479	-3.7490	**0.0027**
octopus - yellowgj	-3.2984	1.2293	479	-2.6830	0.0805
yellowg - yellowgj	-1.2274	1.1357	479	-1.0810	0.8889

Table S4. GLMM analysis with scene and individual identity within each scene as nested random effects, measuring variation in number of times anchoring (i.e. unsuccessfully pulled) per minute (offset = 5 minutes) according to species (6 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellow saddlegoatfish). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate p < 0.05.

Family: poisson (log)

Formula: fol ~ no_go + (1 | scene/individual) + (1 | block) + offset(time)

Random effects:

Groups	Name	Variance	Std.Dev.
individual:scene	(Intercept)	0.125	0.3536
scene	(Intercept)	0.1094	0.3307
block	(Intercept)	0.4069	0.6379

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-4.8952	0.1954	-25.0500	**< 0.0001**
fol_spegreengoatfish	-0.3914	0.1974	-1.9830	**0.0474**
fol_spelyretailgrouper	-0.3380	0.2771	-1.2200	0.2225
fol_speoctopus	0.5543	0.1869	2.9650	**0.0030**
fol_speyellowgoatfish	-1.0558	0.2353	-4.4870	**0.0000**
fol_speyellowgoatfishj	-0.9575	0.3451	-2.7750	**0.0055**

Tukey HSD:

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	0.3597	0.1880	476	1.9100	0.3972
blacktipgrouper - lyretailgrouper	0.3185	0.2520	476	1.2640	0.8043
blacktipgrouper - octopus	-0.8226	0.3010	476	-2.7310	0.0711
blacktipgrouper - yellowg	0.7241	0.2010	476	3.6000	**0.0047**
blacktipgrouper - yellowgj	0.6842	0.2230	476	3.0680	**0.0274**
greengoatfish - lyretailgrouper	-0.0412	0.2220	476	-0.1850	1.0000
greengoatfish - octopus	-1.1823	0.3080	476	-3.8450	**0.0019**
greengoatfish - yellowg	0.3644	0.1530	476	2.3870	0.1627
greengoatfish - yellowgj	0.3245	0.1890	476	1.7210	0.5186
lyretailgrouper - octopus	-1.1411	0.3350	476	-3.4070	**0.0093**
lyretailgrouper - yellowg	0.4056	0.1960	476	2.0650	0.3075
lyretailgrouper - yellowgj	0.3657	0.2280	476	1.6020	0.5977
octopus - yellowg	1.5467	0.3170	476	4.8790	**<.0001**
octopus - yellowgj	1.5068	0.3310	476	4.5510	**0.0001**
yellowg - yellowgj	-0.0399	0.1480	476	-0.2710	0.9998

Table S5. GLMM analysis with scene and individual identity within each scene as nested random effects, measuring variation in number of times followed (i.e. successfully pulled) per minute (offset = 5 minutes) according to species (6 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellow saddlegoatfish). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate p < 0.05.

Family: poisson (log)

Formula: fol ~ fol_spe + (1 | scene/individual) + (1 | block) + offset(time)

Random effects:

Groups	Name	Variance	Std.Dev.
individual:scene	(Intercept)	8.19E-10	2.86E-05
scene	(Intercept)	1.61E-01	4.02E-01
block	(Intercept)	1.75E-01	4.19E-01

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-5.1125	0.1667	-30.660	**< 0.0001**
fol_spegreengoatfish	0.1677	0.1349	1.2440	0.2140
fol_spelyretailgrouper	0.1742	0.1800	0.9670	0.3330
fol_speoctopus	0.0029	0.1431	0.0200	0.9840
fol_speyellowgoatfish	-0.1423	0.1619	-0.8790	0.3790
fol_speyellowgoatfishj	0.0574	0.2562	0.2240	0.8230

Tukey HSD:

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	-0.1632	0.1330	476	-1.2220	0.8258
blacktipgrouper - lyretailgrouper	-0.1700	0.1820	476	-0.9350	0.9374
blacktipgrouper - octopus	-0.0026	0.1280	476	-0.0200	1.0000
blacktipgrouper - yellowg	0.1186	0.1360	476	0.8720	0.9530
blacktipgrouper - yellowgj	-0.0528	0.2400	476	-0.2200	0.9999
greengoatfish - lyretailgrouper	-0.0068	0.1820	476	-0.0370	1.0000
greengoatfish - octopus	0.1606	0.1380	476	1.1610	0.8551
greengoatfish - yellowg	0.2817	0.1460	476	1.9270	0.3870
greengoatfish - yellowgj	0.1104	0.2470	476	0.4470	0.9977
lyretailgrouper - octopus	0.1674	0.1680	476	0.9950	0.9194
lyretailgrouper - yellowg	0.2885	0.1610	476	1.7970	0.4688
lyretailgrouper - yellowgj	0.1172	0.2550	476	0.4600	0.9974
octopus - yellowg	0.1211	0.1190	476	1.0200	0.9113
octopus - yellowgj	-0.0502	0.2300	476	-0.2190	0.9999
yellowg - yellowgj	-0.1713	0.2210	476	-0.7760	0.9715

Table S6. GLMM analysis with scene and individual identity within each scene as nested random effects, measuring variation in number of successful pulls per minute (offset = 5 minutes) according to species (6 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellowsaddle goatfish). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate $p < 0.05$.

Family: poisson (log)
Formula: pull ~ lead_spe + (1 | scene/individual) + (1 | block) + offset(time)

Random effects:

Groups	Name	Variance	Std.Dev.
individual:scene	(Intercept)	0.04265	0.2065
scene	(Intercept)	0.15527	0.394
block	(Intercept)	0.18309	0.4279

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-5.4998	0.1860	-29.564	**< 0.0001**
lead_spegreengoatfish	0.6271	0.1650	3.7990	**0.0001**
lead_spelyretailgrouper	0.1335	0.2395	0.5570	0.5774
lead_speoctopus	0.1749	0.1817	0.9630	0.3356
lead_speyellowgoatfish	1.0334	0.1805	5.7260	**0.0000**
lead_speyellowgoatfishj	0.8806	0.2624	3.3560	**0.0008**

Tukey HSD:

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	-0.5291	0.1610	479	-3.2860	**0.0139**
blacktipgrouper - lyretailgrouper	-0.0866	0.1600	479	-0.5410	0.9944
blacktipgrouper - octopus	-0.1160	0.1210	479	-0.9570	0.9310
blacktipgrouper - yellowg	-1.0985	0.2440	479	-4.4940	**0.0001**
blacktipgrouper - yellowgj	-0.8569	0.3500	479	-2.4450	0.1430
greengoatfish - lyretailgrouper	0.4425	0.1990	479	2.2210	0.2302
greengoatfish - octopus	0.4131	0.1690	479	2.4380	0.1455
greengoatfish - yellowg	-0.5694	0.2420	479	-2.3520	0.1755
greengoatfish - yellowgj	-0.3278	0.3590	479	-0.9130	0.9433
lyretailgrouper - octopus	-0.0294	0.1630	479	-0.1800	1.0000
lyretailgrouper - yellowg	-1.0118	0.2440	479	-4.1550	**0.0005**
lyretailgrouper - yellowgj	-0.7702	0.3590	479	-2.1430	0.2671
octopus - yellowg	-0.9825	0.2330	479	-4.2200	**0.0004**
octopus - yellowgj	-0.7409	0.3470	479	-2.1360	0.2706
yellowg - yellowgj	0.2416	0.3510	479	0.6890	0.9831

Table S7. GLMM analysis with scene and individual identity within each scene as nested random effects, measuring variation in number of successful pulls on the octopus per minute (offset = 5 minutes) according to species (5 levels: blacktip grouper, green goatfish, lyretail grouper, yellowsaddle goatfish, and juvenile yellowsaddle goatfish). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate $p < 0.05$.

Family: poisson (log)
Formula: pull ~ lead_spe + (1 | scene/individual) + (1 | block) + offset(time)

Random effects:

Groups	Name	Variance	Std.Dev.
individual:scene	(Intercept)	1.56E-02	1.25E-01
scene	(Intercept)	2.24E-09	4.73E-05
block	(Intercept)	2.23E-01	4.72E-01

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-7.0191	0.2614	-26.850	**< 0.0001**
lead_spegreengoatfish	0.7895	0.3085	2.5590	**0.0105**
lead_spelyretailgrouper	0.4295	0.4461	0.9630	0.3357
lead_speyellowgoatfish	1.2267	0.2955	4.1510	**< 0.0001**
lead_speyellowgoatfishj	0.5068	0.5771	0.8780	0.3799

Tukey HSD:

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	-0.7895	0.3090	351	-2.5590	0.0804
blacktipgrouper - lyretailgrouper	-0.4295	0.4460	351	-0.9630	0.8716
blacktipgrouper - yellowgoatfish	-1.2267	0.2950	351	-4.1510	**0.0004**
blacktipgrouper - yellowgoatfishj	-0.5068	0.5770	351	-0.8780	0.9048
greengoatfish - lyretailgrouper	0.3601	0.4180	351	0.8620	0.9107
greengoatfish - yellowgoatfish	-0.4372	0.2540	351	-1.7190	0.4238
greengoatfish - yellowgoatfishj	0.2828	0.5600	351	0.5050	0.9868
lyretailgrouper - yellowgoatfish	-0.7973	0.3940	351	-2.0220	0.2574
lyretailgrouper - yellowgoatfishj	-0.0773	0.6350	351	-0.1220	1.0000
yellowgoatfish - yellowgoatfishj	0.7200	0.5380	351	1.3370	0.6682

Table S8. GLMM analysis with scene and individual identity within each scene as nested random effects, measuring variation in the proportion of succesfuly leading when attempted, according to species (6 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellow saddlegoatfish). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate p <0.05.

Family: binomial (logit)
Formula: cbind(pull, attempts) ~ lead_spe + (1 | scene/individual) + (1 | block)

Random effects:

Groups	Name	Variance	Std.Dev.
individual:scene	(Intercept)	3.98E-09	6.31E-05
scene	(Intercept)	1.38E-01	3.71E-01
block	(Intercept)	5.98E-10	2.44E-05

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.3984	0.1814	-2.1970	**0.0280**
lead_spegreengoatfish	-0.4734	0.1625	-2.9130	**0.0036**
lead_spelyretailgrouper	-0.4942	0.2576	-1.9180	0.0551
lead_speoctopus	-0.0251	0.1906	-0.1320	0.8953
lead_speyellowgoatfish	-0.5087	0.1890	-2.6910	**0.0071**
lead_speyellowgoatfishj	-0.9055	0.2592	-3.4930	**0.0005**

Tukey HSD:

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	0.1068	0.0374	479	2.8540	0.0510
blacktipgrouper - lyretailgrouper	0.1111	0.0564	479	1.9720	0.3601
blacktipgrouper - octopus	0.0060	0.0457	479	0.1320	1.0000
blacktipgrouper - yellowg	0.1141	0.0433	479	2.6320	0.0915
blacktipgrouper - yellowgj	0.1882	0.0510	479	3.6910	**0.0034**
greengoatfish - lyretailgrouper	0.0043	0.0495	479	0.0870	1.0000
greengoatfish - octopus	-0.1008	0.0391	479	-2.5770	0.1050
greengoatfish - yellowg	0.0073	0.0346	479	0.2110	0.9999
greengoatfish - yellowgj	0.0814	0.0451	479	1.8040	0.4639
lyretailgrouper - octopus	-0.1051	0.0537	479	-1.9570	0.3690
lyretailgrouper - yellowg	0.0030	0.0435	479	0.0690	1.0000
lyretailgrouper - yellowgj	0.0771	0.0553	479	1.3950	0.7303
octopus - yellowg	0.1081	0.0401	479	2.6970	0.0777
octopus - yellowgj	0.1822	0.0499	479	3.6530	**0.0039**
yellowg - yellowgj	0.0741	0.0392	479	1.8890	0.4105

Table S9. GLMM analysis with scene and individual identity within each scene as nested random effects, measuring variation in the proportion of following when solicited, according to species (6 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellow saddlegoatfish). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate p <0.05.

Family: binomial (logit)

Formula: cbind(fol, fol_attempts) ~ fol_spe + (1 | scene/individual) + (1 | block)

Random effects:

Groups	Name	Variance	Std.Dev.
individual:scene	(Intercept)	6.81E-14	2.61E-07
scene	(Intercept)	4.39E-02	2.10E-01
block	(Intercept)	1.06E-08	1.03E-04

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.9935	0.1389	-7.1550	**< 0.0001**
fol_spegreengoatfish	0.3620	0.1603	2.2580	**0.0239**
fol_spelyretailgrouper	0.3246	0.2170	1.4960	0.1347
fol_speoctopus	-0.2630	0.1586	-1.6580	0.0973
fol_speyellowgoatfish	0.5091	0.1876	2.7130	**0.0067**
fol_speyellowgoatfishj	0.4577	0.3000	1.5260	0.1270

Tukey HSD:

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	-0.0770	0.0341	476	-2.2540	0.2154
blacktipgrouper - lyretailgrouper	-0.0685	0.0467	476	-1.4680	0.6846
blacktipgrouper - octopus	0.0486	0.0297	476	1.6390	0.5728
blacktipgrouper - yellowg	-0.1110	0.0411	476	-2.7020	0.0766
blacktipgrouper - yellowgj	-0.0990	0.0680	476	-1.4550	0.6933
greengoatfish - lyretailgrouper	0.0084	0.0475	476	0.1770	1.0000
greengoatfish - octopus	0.1256	0.0332	476	3.7870	**0.0024**
greengoatfish - yellowg	-0.0340	0.0426	476	-0.7990	0.9677
greengoatfish - yellowgj	-0.0220	0.0697	476	-0.3160	0.9996
lyretailgrouper - octopus	0.1172	0.0432	476	2.7120	0.0747
lyretailgrouper - yellowg	-0.0425	0.0461	476	-0.9220	0.9409
lyretailgrouper - yellowgj	-0.0304	0.0732	476	-0.4160	0.9984
octopus - yellowg	-0.1596	0.0370	476	-4.3090	**0.0003**
octopus - yellowgj	-0.1476	0.0656	476	-2.2500	0.2171
yellowg - yellowgj	0.0120	0.0687	476	0.1750	1.0000

Table S10. GLMM analysis with scene and individual identity within each scene as nested random effects, measuring variation in the proportion of leading attempts and following , according to species (6 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellow saddlegoatfish). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate $p < 0.05$.

Family: nbinom2 (log)
Formula: lead attempts ~ lead_spe + (1 | scene/individual) + (1 | block) + offset(fol)

Random effects:

Groups	Name	Variance	Std.Dev.
individual:scene	(Intercept)	5.36E-08	2.32E-04
scene	(Intercept)	1.88E-01	4.33E-01
block	(Intercept)	1.02E-09	3.20E-05

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.9404	0.2303	-4.0830	**< 0.0001**
lead_spegreengoatfish	1.2847	0.2072	6.2010	**< 0.0001**
lead_spelyretailgrouper	0.3697	0.3559	1.0390	0.2990
lead_speoctopus	0.2586	0.2268	1.1400	0.2540
lead_speyellowgoatfish	1.9362	0.2830	6.8400	**< 0.0001**
lead_speyellowgoatfishj	2.0759	0.3698	5.6130	**< 0.0001**

Tukey HSD:

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	-3.1200	0.8270	484	-3.7740	**0.0025**
blacktipgrouper - lyretailgrouper	-0.5340	0.5590	484	-0.9540	0.9318
blacktipgrouper - octopus	-0.3520	0.3120	484	-1.1270	0.8700
blacktipgrouper - yellowg	-7.0810	1.6680	484	-4.2440	**0.0004**
blacktipgrouper - yellowgj	-8.3210	3.2140	484	-2.5890	0.1018
greengoatfish - lyretailgrouper	2.5860	1.0100	484	2.5600	0.1092
greengoatfish - octopus	2.7670	0.8470	484	3.2660	**0.0147**
greengoatfish - yellowg	-3.9610	1.7740	484	-2.2330	0.2244
greengoatfish - yellowgj	-5.2020	3.2360	484	-1.6080	0.5940
lyretailgrouper - octopus	0.1820	0.5580	484	0.3250	0.9995
lyretailgrouper - yellowg	-6.5470	1.5730	484	-4.1630	**0.0005**
lyretailgrouper - yellowgj	-7.7880	3.2340	484	-2.4080	0.1555
octopus - yellowg	-6.7290	1.6270	484	-4.1360	**0.0006**
octopus - yellowgj	-7.9690	3.2120	484	-2.4810	0.1316
yellowg - yellowgj	-1.2410	3.2910	484	-0.3770	0.9990

Table S11. GLMM analysis with scene and individual identity within each scene as nested random effects, measuring variation in the proportion of succesfully leading and following , according to species (6 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellow saddlegoatfish). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate $p < 0.05$.

Family: nbinom2 (log)

Formula: pull ~ lead_spe + (1 | scene/individual) + (1 | block) + offset(fol)

Random effects:

Groups	Name	Variance	Std.Dev.
individual:scene	(Intercept)	1.32E-02	1.15E-01
scene	(Intercept)	1.61E-03	4.01E-02
block	(Intercept)	2.51E-09	5.01E-05

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-1.2521	0.1842	-6.7960	**< 0.0001**
lead_spegreengoatfish	0.6226	0.2501	2.4890	**0.0128**
lead_spelyretailgrouper	-0.3273	0.3453	-0.9480	0.3432
lead_speoctopus	0.0189	0.2593	0.0730	0.9419
lead_speyellowgoatfish	1.1518	0.2405	4.7890	**< 0.0001**
lead_speyellowgoatfishj	0.8423	0.4097	2.0560	**0.0398**

Tukey HSD:

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	-0.755	0.334	484	-2.259	0.2131
blacktipgrouper - lyretailgrouper	0.244	0.242	484	1.01	0.9147
blacktipgrouper - octopus	-0.0167	0.229	484	-0.073	1
blacktipgrouper - yellowg	-1.8912	0.465	484	-4.064	**0.0008**
blacktipgrouper - yellowgj	-1.1551	0.769	484	-1.502	0.6633
greengoatfish - lyretailgrouper	0.9989	0.348	484	2.872	**0.0485**
greengoatfish - octopus	0.7383	0.349	484	2.117	0.2803
greengoatfish - yellowg	-1.1362	0.523	484	-2.172	0.2529
greengoatfish - yellowgj	-0.4001	0.813	484	-0.492	0.9964
lyretailgrouper - octopus	-0.2606	0.254	484	-1.028	0.9085
lyretailgrouper - yellowg	-2.1352	0.472	484	-4.522	**0.0001**
lyretailgrouper - yellowgj	-1.399	0.779	484	-1.795	0.47
octopus - yellowg	-1.8745	0.47	484	-3.989	**0.0011**
octopus - yellowgj	-1.1384	0.775	484	-1.469	0.6842
yellowg - yellowgj	0.7361	0.878	484	0.839	0.9602

Table S12. GLMM analysis with scene and individual identity within each scene as nested random effects, measuring variation in the speed of leading attempts, according to species (6 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellow saddlegoatfish) and success/unscessful(2 levels: pull, or anchored). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate $p < 0.05$.

Family: Gamma (log)

Formula: lead_speed ~ lead_species + type + (1 | scene/leader)

Random effects:

Groups	Name	Variance	Std.Dev.
leader:scene	(Intercept)	0.1996	0.4468
scene	(Intercept)	0.1124	0.3353

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-4.2075	0.2032	-20.703	**< 0.0001**
lead_speciesgreengoatfish	-0.2884	0.2087	-1.3820	0.1671
lead_specieslyretailgrouper	0.2516	0.2992	0.8410	0.4004
lead_speciesoctopus	-0.7557	0.2445	-3.0910	**0.0020**
lead_speciesyellowgoatfish	0.2870	0.2455	1.1690	0.2424
lead_speciesyellowgoatfishj	0.0365	0.3092	0.1180	0.9061
typepull	0.1690	0.0602	2.8080	**0.0050**

Tukey HSD (factor = lead_species):

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	0.0041	0.0031	1358	1.3300	0.7682
blacktipgrouper - lyretailgrouper	-0.0046	0.0058	1358	-0.8020	0.9671
blacktipgrouper - octopus	0.0086	0.0032	1358	2.6560	0.0851
blacktipgrouper - yellowg	-0.0054	0.0046	1358	-1.1770	0.8478
blacktipgrouper - yellowgj	-0.0006	0.0051	1358	-0.1170	1.0000
greengoatfish - lyretailgrouper	-0.0087	0.0054	1358	-1.6210	0.5848
greengoatfish - octopus	0.0045	0.0025	1358	1.8120	0.4580
greengoatfish - yellowg	-0.0094	0.0040	1358	-2.3520	0.1742
greengoatfish - yellowgj	-0.0047	0.0049	1358	-0.9610	0.9301
lyretailgrouper - octopus	0.0132	0.0052	1358	2.5470	0.1116
lyretailgrouper - yellowg	-0.0008	0.0055	1358	-0.1360	1.0000
lyretailgrouper - yellowgj	0.0040	0.0065	1358	0.6210	0.9895
octopus - yellowg	-0.0140	0.0036	1358	-3.9330	**0.0012**
octopus - yellowgj	-0.0092	0.0046	1358	-2.0140	0.3348
yellowg - yellowgj	0.0048	0.0051	1358	0.9360	0.9372

Table S13. GLMM analysis with scene and individual identity within each scene as nested random effects, measuring variation in the speed of leading attempts targeting the octopus, according to species (5 levels: blacktip grouper, green goatfish, lyretail grouper, octopus, yellowsaddle goatfish, and juvenile yellow saddlegoatfish) and success/unscessful(2 levels: pull, or anchored). Tukey Honest Significant Differences (HSD) pairwise post hoc comparisons are displayed below. Bold values indicate $p < 0.05$.

Family: Gamma (log)

Formula: lead_speed ~ lead_species + type + (1 |scene/leader)

Random effects:

Groups	Name	Variance	Std.Dev.
leader:scene	(Intercept)	2.92E-09	5.41E-05
scene	(Intercept)	3.96E-01	6.29E-01

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-4.3821	0.2252	-19.460	**< 0.0001**
lead_speciesgreengoatfish	-0.1879	0.1357	-1.3850	0.1661
lead_specieslyretailgrouper	0.5253	0.2185	2.4050	**0.0162**
lead_speciesyellowgoatfish	0.7295	0.1615	4.5170	**< 0.0001**
lead_speciesyellowgoatfishj	0.8311	0.2037	4.0800	**< 0.0001**
typepull	0.2106	0.0848	2.4840	**0.0130**

Tukey HSD (factor = lead_species):

contrast	Estimate	Std. Error	df	t ratio	p value
blacktipgrouper - greengoatfish	0.0024	0.0019	376	1.2880	0.6989
blacktipgrouper - lyretailgrouper	-0.0096	0.0048	376	-2.0060	0.2651
blacktipgrouper - yellowgoatfish	-0.0149	0.0042	376	-3.5980	**0.0033**
blacktipgrouper - yellowgoatfishj	-0.0180	0.0063	376	-2.8800	**0.0340**
greengoatfish - lyretailgrouper	-0.0120	0.0048	376	-2.4950	0.0939
greengoatfish - yellowgoatfish	-0.0173	0.0043	376	-4.0480	**0.0006**
greengoatfish - yellowgoatfishj	-0.0204	0.0065	376	-3.1440	**0.0154**
lyretailgrouper - yellowgoatfish	-0.0053	0.0043	376	-1.2240	0.7374
lyretailgrouper - yellowgoatfishj	-0.0084	0.0065	376	-1.3020	0.6903
yellowgoatfish - yellowgoatfishj	-0.0031	0.0051	376	-0.6070	0.9739