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On potential cooperation in predator-prey interactions in fishes

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GENERAL SUMMARY

Predator – prey interaction provide the context for some of the best-studied cases of cooperation. Some predator species can hunt together and coordinate their moves within active and diverse roles to increase capture rates of their prey; this is known as cooperative hunting. Lionfish are common piscivores in the Indo-Pacific and invasive in the Caribbean. Since lionfishes hunt alone by nature, a study by a former researcher that demonstrated active recruitment, coordination, and alternated (perhaps reciprocal) striking in the dwarf lionfish *Dendrochirus zebra* has attracted much interest. Zebra lionfish have been seen to use a fin-flaring pattern that involves undulation of the caudal fin and successive flares of both pectoral fins to indicate the start of cooperative hunting. The findings suggested that the ability to hunt cooperatively may have contributed to the success of a sister lionfish species, *Pterois miles* and *P. volitans*, in invading the Caribbean.

Here, I investigated *Pterois miles* - one of the invasive species - in its natural range in the Red Sea. In the field, I found no signs of coordinated hunting. I supplemented field observations with a laboratory experiment, in which I exposed individuals to a possible hunting partner and inaccessible prey in a transparent housing. I observed the fin-flaring pattern, but it is vital to note that the partner was not the target of this fin-flaring signal. Also supporting the field findings that this species in the Red Sea does not rely on cooperative hunting to catch fish is the result that the two lionfish did not congregate at the prey patches. I further supported these findings by examining coordinated movement and strike alternation during hunting. I exposed subjects of *P. miles* pairings to inaccessible prey in three clear housings. In the presence of prey, the two lionfish did not congregate at the same prey house in space or time. In a second experiment, I put food pieces on a "feeding tree" stick to test for reciprocal alternation of strikes. I generally saw fewer alternations than expected by chance, and found that alternations can be increased by putting constaints on individual monopolization of food.

In conclusion, the fin flaring movement pattern seen in the Red Sea sister species *P. miles* that were previously thought to be a signal was now interpreted as a swimming mode. Additionally, pairs of *P. miles* in the Red Sea did not reciprocally alternate their strikes. I interpreted the result of the previous study of *D. zebra* that some alternation might be generated if prey items become alternately available at two corners in a confined space, with each lionfish preferring to monopolize one corner each.

Eventually, due to some challenges encountered in the original study, which prevented me from conducting more empirical research, I complimented the empirical chapters of the PhD thesis with a research review on the perspectives of predator inspection in fishes. The topic was chosen because predator inspection is another classic example of cooperation. This review looked at the possible goals and functions of prey fishes inspecting potential predators and how prey approach them based on the inspection goals. We discussed the assessment of prey motivations and indicators that moved from predator presence and state indicators that could show why an attack was made. Also, we discussed what types of games are involved in predator inspection behaviour. The review identified several important gaps in our knowledge that currently prevent a proper assessment of what cooperation games might apply.

In conclusion, the thesis demonstrates that it is an important scientific task to revisit apparently well-established examples and to challenge previous interpretations.

GENERAL INTRODUCTION

I.1. The evolution of helping

Helping is a behavior in which an individual improves the fitness of one or more other individuals. Helping is prevalent in nature and may be found at all levels of organization, from unicellular organisms to eusocial insects and sophisticated animal and human communities (1,2). As natural selection favors a certain behavior only if it boosts the individual's average lifetime fitness, the existence of helping creates challenges for evolutionary scientists.

Individuals who help others lower their own direct fitness in one type of helping, known as altruism (3). Meanwhile, (4) demonstrated that such altruistic behaviour can exist in nature when the act of helping is directed to kin. According to Hamilton’s rule ($b^* r > c$), the inclusive fitness of an actor (the helper) is increased if the benefits of the receiver (b) multiplied by the degree of relatedness (r) between actor and receiver outweigh the costs (c) for the actor. In the other form of helping; cooperation, all participants, actor and recipient enhance their direct fitness on average (3). The term "inclusive fitness" refers to both direct fitness, which is assessed by reproductive success, and indirect fitness, which is the influence of one's own behavior on the reproductive success of its kin (5).

Achieving stable cooperation can be challenging, as illustrated by the well-known prisoner's dilemma game PD (6,7). In the PD game, two players can either cooperate or defect, where the combination of actions affects individual payoffs. In the standard payoff matrix of the PD game, mutual cooperation yields three units to each player, while mutual defection yields only one. In the asymmetric circumstance in which one player defected and the other cooperated, the defecting player receives five units while the cooperating player receives zero. Therefore, mutual cooperation is better than mutual defection, but each player obtains more units upon defecting, independent of the other player's actions. The prisoner’s dilemma payoff matrix illustrates that to cooperate is often an investment, while to defect (to cheat) yields immediate benefits. This leads to defection being the only evolutionarily stable strategy (ESS) (8), resulting in a social dilemma (9), if there is only one interaction.

Stable cooperation may be achieved in an iterated version of the standard prisoner's dilemma, i.e. when the same participants interact repeatedly. In that case, cooperative individuals must ensure that their investments generate future benefits. In other words, investors must avoid being exploited by cheating partners. The solution is that players can learn about the behavioral tendencies of their partners and show conditionally cooperative strategies, i.e. Tit-For-Tat like (10–14).

Cooperation in N-player situations differs from the 2-players game in that it produces a public goods situation because the contribution made by an individual will not only benefit one specific member in the group, but the benefit can extend to the entire group members who did not participate in the public good. This can lead to the following problem explained in an example with three players (A, B, C). In the first round, players A and B cooperate by contributing to the public good, while player C cheats by not contributing, resulting in the highest payoff for C. In the second round, player B also cheats by not contributing, with the consequence that there will not only be a negative effect on the previous cheater C, but also on the cooperator A. Briefly said: not contributing in the public good affects all group members. The potentially resulting problem from the scenario described above can be illustrated with an N-player prisoner’s dilemma NPD, a specific type of public goods game (15). In the NPD, when only defecting is that evolutionary stable strategy ESS (16), resulting in the so-called “tragedy of the commons”.

Despite the conceptual issues raised by the prisoner’s dilemma, cooperation is frequently observed among unrelated individuals of the same species (17–22), and between individuals of different species (23–25). This latter form of mutual helping is termed mutualism (5). As it stands, few existing examples of mutual helping seem to fit the strict conditions of an iterated prisoner’s dilemma (6,11,14). Instead, conflicts can be intermediate or even absent. Different games have been developed with different payoff matrix, for example, the snow drift game SD (26). The payoff matrix of the SD is such that the best possible response to the partners’ defection is to cooperate with t> r> s> p (26). This game also exists in the N-player version, the volunteers dilemma (27). Recently scientists have argued that the payoff matrix of the volunteer's dilemma, which is either sigmoid or a step function of the individual contribution, might be more applicable to natural cooperating systems than the payoff distributions of the NPD, which is a linear function (15,28). A reasonable explanation is that just one or a few individuals can establish the public good by fulfilling a costly act (33), such as an alarm call in animals (29,30). Any conflicts over investments are entirely absent in the so-called prisoner's delight game (31,32). In this game, to cooperate yields the highest payoff, irrespective of what the partner is doing. The resulting cooperation is hence a by-product of self-serving actions (termed by-product mutualism by (33)).

I.2. Links between cooperation and cognition

Among species that lack a brain, examples of cooperation based on strategic decisions can emerge (e.g. mutualism between leguminous plants and rhizobia (34)). Furthermore, some cooperation challenges are independent of the issue of cheating, and successful cooperation may require the ability to coordinate actions in space and time**.** Coordination challenges may arise in interactions that fit a prisoner's dilemma, snowdrift**,** or prisoner's delight payoff matrix. Thus, many forms of cooperation are considered to require some forms of cognitive abilities. The important components that appear to be most relevant are individual recognition, book-keeping, planning and communication. For example, most species that perform cooperative interactions are mobile (35). Hence, the decision to conduct a gainful cooperative interactions is determined by the ability to recognize - and remember - past partners for being cooperative or defective (36). In the absence of previous experience of partner or interactions, it is important to prevent a potential defection through partner discipline in order to increase the net benefit, without lowering the total production of its investment (37,38). Such mechanisms, for example punishment (37,37,39), partner switching (40), or image scoring (41), were found to prevent partners from cheating. A more complex type of cooperation is for example collaborative hunting, where cooperators coordinate their actions in space and time and play diverse roles (22). Collaborative hunting may involve some planning, and individuals must learn to perform different behaviours while at the same time observing the actions of the other members in order to perform a successful hunt (35). Thus, many decisions an individual must make, like whether it is beneficial to recruit a partner in the first place, and if the partner is recruiting, whether the probability of success and per capita is increased with the presence of multiple partners, may demand cognitive abilities (21). Finally, cognition may play a key role in strategic decisions regarding the best behavioral options for a given situation (35). However, the precise cognitive processes underlying different components of cooperative hunting are currently unknown. Hence, providing a conclusive response to the question of whether or not collaboration involves ‘complex’ cognitive abilities would currently be difficult.

I.3. Predator-prey interactions to study cooperation

Predator-prey interactions can be seen as an arms race (42), however, as cooperation occurs in many different contexts, several classic examples involve interactions between predator and prey. For the predator side, the most common form of cooperation is cooperative/collaborative hunting. Carnivores like wolves, wild dogs, and lions frequently collaborate to capture and kill their prey (43,44). Other organisms, such as ants, spiders, and birds, also work together to find and attack their prey (43,45). On the other hand for the prey side; a prominent example is observed in shoal-living prey is the dilution effect, which entails that larger groups may have higher perception of safety and flee at shorter distances from the predator than if in small groups. In species that use dilution effect, larger groups respond with less flight distance and distance moved, because the risk is smaller per capita (46–48). Another antipredator behaviour is cooperative vigilance which implies shared monitoring, emitting alert calls or adopting alarm postures that trigger responses from peers of the group (49,50). As a consequence, prey tend to adopt and enhance anti-predator responses to reduce the capture success by predators, which in turn, lower the attack probabilities by predators.

I.3.1. Cooperative hunting

Cooperative hunting can be defined as two or more individuals working together to pursue a hunt, which on average, yields greater rewards per hunter than solitary hunting (51). For numerous mammals and birds, there is evidence of a positive link between hunting group size and group hunting success (52,53). While cooperative hunting may often represent cooperation based on by-product benefits (participation is better than standing aside as long as the benefits of participation outweigh the costs), behavioural biologists have emphasized cognitive challenges of more complex forms of cooperative hunting. Coordinated hunting, in which individual predators respond in time and space to each other's actions, is a complex and rare form of cooperative hunting (18,54). It has been observed and described in primates (22,55), carnivores (19,21,54), marine mammals (18,54,56), birds (57), and fishes (23). Coordination can range from simultaneous single hunts' to individuals organizing a hunt (intentional hunting) in which they play distinct roles (termed collaboration) (46, 42). For example, e.g. coordinated hunting between heterospecific (groupers *Plectropomus pessuliferus* and giant moray eels *Gymnothorax javanicus* (23)), conspecific (Yellow saddle goatfish *Parpenus cyclostomus* (58), and lions *Panthera leo* (19)). In its most complex form, collaborative hunting, individuals adopt specific hunting roles to herd and catch their prey (18,19,23,54,58). Complexity can be further increased if hunting partners actively communicate to initiate a hunt and during a hunting event (22,23).

I.3.2. Predator inspection

All animals can be targets of predation. The instinctive reaction for many prey encountering a predator is to flee. However, studies found that many prey animals approach predators with a sit-and-wait hunting mode (59–64). One predator assessment strategy that prey uses is *Predator inspection;* where one oer more prey fish separate from the safe shoal and approach a potential predator to identify, gain information and assess their risk, i.e. whether a predation attempt is likely to occur (62). Then inspecting individuals return to the group, exhibit changes in behavior, which imply that information had been gained and could be transferred among the shoal (59,60,63–65). Although inspecting predators appears risky there is no direct evidence that inspectors are at risk of being attacked (66–69); it is even claimed that they may even gain from pursuit deterrence (66,70,71). Since inspectors often have a companion nearby and make their further approach conditional (aka conditional approach depending on the contribution of the partner to the inspection approach) on the other one's presence (72–75), the companion potentially dilutes the risk (72). Thus, predator inspection is typically viewed as a cooperative behavior observed among vertebrates. It has been examined in most detail in fishes when encountering a stalking predator - real or model (63,67,69,76).

I.4. The Ph.D thesis

I.4.1. The study species

My main study species was the Pacific red lionfish *Pterois miles.* In the mid-1980s, this species and and *P. volitans* invaded the Western Atlantic via the aquarium trade (77). Genetic research suggests that the invasive fish were introduced off Florida's southeast coast via repeated aquarium releases (78). Lionfish have been recorded far from north Rhode Island and till south of Brazil (79–82). A combination of a high reproductive rate and the absence of native predators allowed lionfish populations in the western Atlantic to grow with density factors higher than in their native habitats (83,84). These high densities of lionfish imply that they have become invasive and can impact the richness and structure of reef fish communities, as well as cause substantial ecological change (85,86).

Lionfish are generalist predators with a diverse diet, consuming large amounts of small and juvenile fishes as well as small invertebrates (87–91). This opportunistic generalist feeding style has the potential to transform reef fish populations in the Bahamas, where the relative growth of lionfish from 23 to 40 percent of total predator biomass occurred concurrently with a 65 percent drop in prey fish biomass between 2008 and 2010. (92,93). While lionfish are typically opportunistic predators (87), contributions of various species to lionfish diets vary by location, depth, and season, suggesting that lionfish may consume specific prey species based on their availability or ease of capture (90,94,95). However, it is unknown if the distribution and availability of prey types may impact the abundance and dispersion of lionfish.

Two different hunting strategies are used by lionfish: a sit-and-wait ambush strategy and leisurely tracking of prey with the help of pectoral fins that resemble fans and herd prey (96). Blowing behaviour, lionfish's ability to "blow" a water stream at their prey when approaching them, increases the effectiveness of their hunting by confusing or distracting the prey. (97). Hunting takes place during crepuscular periods (96) when low light levels impede visually adapted prey. At invaded locations, lionfish consume a broad diversity of small fishes and crustaceans, including juveniles of herbivores (87), and even conspecifics (89). Behavioural comparisons between populations of both predator and prey in native vs invaded ranges might be highly informative because they may identify factors that foster invasion success (98–100) and help develop control measures (93,99,101). For invasive predators, such differences can mean access to more abundant or higher quality prey (100), enhanced by the substantial advantages novel invasive predators usually have over naïve native prey (102).

Of particular importance for my PhD thesis was a case study on zebra lionfish *Dendrochirus zebra*, proposing a particularly complex hunting pattern described as communicative and cooperative hunting (103). The authors mentioned field observations that lionfish species not only hunt alone (97) but also in the presence of conspecifics (104) and with moray eels (105). Based on such observations, Zebra lionfish were subjected to laboratory experiments (103), where subjects were initially exposed to inaccessible prey. The subjects were described to recruit conspecifics and heterospecific lionfish *Pterois antennata*, being held behind an opaque barrier, unaware of the presence of prey, to join the hunt. To do so, subjects approached the potential partner head-on, and pectoral fins flared, rapidly undulating their caudal fin, with flaring their pectorals (103). Then, subjects would swim back to the location of the prey ('leading' behavior) and then return to the partner to repeat the display

(103). Once the experimenter released the partner, the pair would then hunt on accessible shoaling prey in a cooperative and coordinated manner, alternating attacks in what looked to be a reciprocal manner. (103). These findings have been taken as proof that Zebra lionfish exhibit very sophisticated social behavior in order to achieve high hunting success rates (92). Furthermore, the result showed similar behaviour by *P. antennata*, and the authors also hypothesized that sophisticated cooperative hunting might explain Pterois lionfish species' strong feeding rates throughout their invasive area in the Caribbean. After I started my PhD project, concerns were raised about the credibility of the published data as sample sizes were smaller than declared (106). Nevertheless, knowing the hunting strategies of Pterois species may contribute to understanding their invasion success in the Caribbean, and recently the Mediterranean (80,107,108).

I.4.2. Aim of the thesis

The original aim of the study was to conduct a comparative study of the cooperative hunting lionfish *Pterois miles* between the native range in the Red Sea and the invaded range in the Caribbean. In addition, I intended to investigate the predator-prey interaction and the efficiency of the anti-predator responses of lionfish common prey in both the the native range in the Red Sea and the invaded range in the Caribbean. However, after the comparative plan has been finalised, I faced several obstacles. First, the primary key study by Lönnstedt et al., 2014 (103) received a note of concern (106), and was reported as unreliable research due to fraud and misconduct (109). Second, I faced major challenges in the data collection in the Caribbean zone, including the rarity of the study species due to frequent culling on Barbados, and a lack of quality facilities in the hosting research station on Jamaica to keep lionfish healthy in captivity. In addition, the Covid-19 pandemic has limited the time dedicated to field data collection. As a result, I dedicated our research to continue investigating and provide reliable result on the cooperative hunting behavior in the Red Sea by lionfish *P. miles,* and on providing a review on the predator inspection behaviour in fishes.

The specific objectives for this thesis are:

1) Investigate the recruitment of lionfish *P. miles* conspecifics and interspecific signaling.

2) Explore the tendency of the coordinated hunt and reciprocal attacks performed by the *P. miles* within paired individuals.

3) Focus on a theoretical review on the perspectives of predator inspection behaviour, and investigate the types of cooperation games played by inspectors.

I.4.3. Thesis chapters

The aim of *Chapter 1* was to investigate the cooperative hunting behavior of the Red Sea lionfish *P. miles,* and focus on the lionfish conspecifics recruitment and signaling occurring between the conspecifics. First, I conducted field observation to investigate the duration and frequency of any *P. miles* interspecific hunting associations. Second, using both field observations and a laboratory experiment by Lönnstedt *et al.* (103), which tested whether *P. miles* actively recruit naïve partners to hunt in pairs. I investigated whether the fin flaring pattern described for the Zebra lionfish *D. zebra* is also shown by *P. miles* in the Red Sea. I adjusted the availability of prey (present or absent) and partners (available initially in a separate compartment, or absent) and recorded the prevalence of fin flaring patterns. If the patterns of fin flaring function as a recruitment signal, I anticipated a strong interaction in which fin flaring would be frequent near the separate compartment if both prey and a partner were present.

Consequently, the aim of *Chapter 2* was to investigate the coordinated hunt and alternated attacks aspect in conspecific hunting of wild lionfish *P. miles*. I conducted a laboratory experiment in controlled conditions and different experimental setups to investigate whether lionfish tend to join a partner when confronted with multiple prey locations. Moreover, if they do, do lionfish individuals alternate their attacks in a reciprocal manner, or are there potentially alternative explanations for alternating strikes?

As complementary, the aim of *Chapter 3* was to focus on the predator inspection strategy in fishes as a means to include research on prey behavior in the thesis. First, I analyzed the potential goals and functions underlying predator inspection behaviour by prey fishes, and assessing the evidence for the functional hypotheses: reputation gains, information gains, and prey communication to the predator, with examples covering laboratory experiments and wild observations. In addition, I shed light on the rules of the predator approach by prey, and how predators inspection can affect the attack probabilities by the potential predators, with discussion of why prey tend to perform repeated visits. Furthermore, given the previous functions, I discuss the kind of cooperation games that prey inspectors may play during predator inspection.

I.6. References

1. Clutton-Brock T. Cooperation between non-kin in animal societies. Nature. 2009 Nov;462(7269):51–7.

2. Dugatkin LA. The Evolution of Cooperation. BioScience. 1997;47(6):355–62.

3. Lehmann L, Keller L. The evolution of cooperation and altruism–a general framework and a classification of models. Journal of evolutionary biology. 2006;19(5):1365–76.

4. Wd H. The genetical theory of social behaviour. I, II. J Thour Biol. 1964;7:1–52.

5. West SA, Griffin AS, Gardner A. Evolutionary explanations for cooperation. Current biology. 2007;17(16):R661–72.

6. Scodel A, Minas JS. The Behavior of Prisoners in a “Prisoner’s Dilemma” Game. The Journal of Psychology. 1960;50(1):133–8.

7. Tucker AW. A two-person dilemma. Prisoner’s Dilemma. 1950;

8. Smith JM, Maynard SJ. The theory of evolution. Cambridge University Press; 1993.

9. Dawes RM. Social dilemmas, economic self-interest, and evolutionary theory. In: Frontiers of mathematical psychology. Springer; 1991. p. 53–79.

10. Press WH, Dyson FJ. Iterated Prisoner’s Dilemma contains strategies that dominate any evolutionary opponent. Proceedings of the National Academy of Sciences. 2012;109(26):10409–13.

11. Fogel DB. Evolving behaviors in the iterated prisoner’s dilemma. Evolutionary Computation. 1993;1(1):77–97.

12. Wedekind C, Milinski M. Human cooperation in the simultaneous and the alternating Prisoner’s Dilemma: Pavlov versus Generous Tit-for-Tat. Proceedings of the National Academy of Sciences. 1996;93(7):2686–9.

13. Hauert Ch, Schuster HG. Extending the Iterated Prisoner’s Dilemma without Synchrony. Journal of Theoretical Biology. 1998 May 21;192(2):155–66.

14. Raihani NJ, Bshary R. Resolving the iterated prisoner’s dilemma: theory and reality: Resolvings the IPD. Journal of Evolutionary Biology. 2011 Aug;24(8):1628–39.

15. Archetti M, Scheuring I. Coexistence of cooperation and defection in public goods games. Evolution: International Journal of Organic Evolution. 2011;65(4):1140–8.

16. Smith JM. Evolution and the Theory of Games. Cambridge university press; 1982.

17. Yamamoto C, Kashiwagi N, Otsuka M, Sakai M, Tomonaga M. Cooperation in bottlenose dolphins: bidirectional coordination in a rope-pulling task. PeerJ. 2019 Oct 2;7:e7826.

18. Gazda SK, Connor RC, Edgar RK, Cox F. A division of labour with role specialization in group–hunting bottlenose dolphins (Tursiops truncatus) off Cedar Key, Florida. Proceedings of the Royal Society B: Biological Sciences. 2005;272(1559):135–40.

19. Stander PE. Cooperative hunting in lions: the role of the individual. Behavioral ecology and sociobiology. 1992;29(6):445–54.

20. Pitman RL, Durban JW. Cooperative hunting behavior, prey selectivity and prey handling by pack ice killer whales (Orcinus orca), type B, in Antarctic Peninsula waters. Marine Mammal Science. 2012;28(1):16–36.

21. Creel S, Creel NM. Communal hunting and pack size in African wild dogs, Lycaon pictus. Animal Behaviour. 1995;50(5):1325–39.

22. Boesch C, Boesch H. Hunting behavior of wild chimpanzees in the Tai National Park. American journal of physical anthropology. 1989;78(4):547–73.

23. Bshary R, Hohner A, Ait-el-Djoudi K, Fricke H. Interspecific communicative and coordinated hunting between groupers and giant moray eels in the Red Sea. PLoS Biol. 2006;4(12):e431.

24. Sampaio E, Seco MC, Rosa R, Gingins S. Octopuses punch fishes during collaborative interspecific hunting events. Ecology. n/a(n/a):e03266.

25. Ruusila V, Pesonen M. Interspecific cooperation in human (Homo sapiens) hunting: the benefits of a barking dog (Canis familiaris). In JSTOR; 2004. p. 545–9.

26. Tanimoto J. Promotion of cooperation by payoff noise in a $2\ifmmode\times\else\texttimes\fi{}2$ game. Phys Rev E. 2007 Oct 24;76(4):041130.

27. Diekmann A. Volunteer's Dilemma. Journal of Conflict Resolution. 1985 Dec 1;29(4):605–10.

28. Archetti M, Scheuring I, Hoffman M, Frederickson ME, Pierce NE, Yu DW. Economic game theory for mutualism and cooperation. Ecology letters. 2011;14(12):1300–12.

29. Townsend SW, Rasmussen M, Clutton-Brock T, Manser MB. Flexible alarm calling in meerkats: the role of the social environment and predation urgency. Behavioral Ecology. 2012 Nov 1;23(6):1360–4.

30. Clutton-Brock TH, O'Riain MJ, Brotherton PNM, Gaynor D, Kansky R, Griffin AS, et al. Selfish Sentinels in Cooperative Mammals. Science. 1999 Jun 4;284(5420):1640–4.

31. Allen B, Nowak MA. Games among relatives revisited. Journal of theoretical biology. 2015;378:103–16.

32. Cooney D, Allen B, Veller C. Assortment and the evolution of cooperation in a Moran process with exponential fitness. Journal of Theoretical Biology. 2016 Nov 21;409:38–46.

33. Brown JL. Cooperation—a biologist's dilemma. In: Advances in the Study of Behavior. Elsevier; 1983. p. 1–37.

34. Kiers ET, Rousseau RA, West SA, Denison RF. Host sanctions and the legume–rhizobium mutualism. Nature. 2003;425(6953):78–81.

35. Brosnan SF, Salwiczek L, Bshary R. The interplay of cognition and cooperation. Philosophical Transactions of the Royal Society B: Biological Sciences. 2010;365(1553):2699–710.

36. Brosnan SF, de Waal F. A proximate perspective on reciprocal altruism. Human Nature. 2002;13(1):129–52.

37. Schino G, Aureli F. Reciprocity in group-living animals: Partner control versus partner choice. Biological Reviews. 2017;92(2):665–72.

38. Leimar O. Reciprocity and communication of partner quality. Proceedings of the Royal Society of London Series B: Biological Sciences. 1997 Aug 22;264(1385):1209–15.

39. Grutter AS, Bshary R. Cleaner wrasse prefer client mucus: support for partner control mechanisms in cleaning interactions. Proceedings of the Royal Society of London Series B: Biological Sciences. 2003;270(suppl_2):S242–4.

40. Bshary R, Grutter AS. Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. Biology Letters. 2005;1(4):396–9.

41. Wedekind C, Milinski M. Cooperation through image scoring in humans. Science. 2000;288(5467):850–2.

42. Endler JA. Interactions between predator and prey. Behavioural ecology. 1991;

43. Alves MT, Hilker FM. Hunting cooperation and Allee effects in predators. Journal of theoretical biology. 2017;419:13–22.

44. Scheel D, Packer C. Group hunting behaviour of lions: a search for cooperation. Animal behaviour. 1991;41(4):697–709.

45. Uetz GW. Foraging strategies of spiders. Trends in Ecology & Evolution. 1992;7(5):155–9.

46. Lima SL. Back to the basics of anti-predatory vigilance: the group-size effect. Animal Behaviour. 1995;49(1):11–20.

47. Stankowich T. Ungulate flight responses to human disturbance: a review and meta-analysis. Biological conservation. 2008;141(9):2159–73.

48. Fairbanks B, Dobson FS. Mechanisms of the group-size effect on vigilance in Columbian ground squirrels: dilution versus detection. Animal Behaviour. 2007;73(1):115–23.

49. Hoogland JL. The evolution of coloniality in white-tailed and black-tailed prairie dogs (Sciuridae: Cynomys leucurus and C. ludovicianus). Ecology. 1981;62(1):252–72.

50. Cassini M. Foraging under predation risk in the wild guinea pig Cavia aperea. Oikos. 1991;20–4.

51. Packer C, Ruttan L. The evolution of cooperative hunting. The American Naturalist. 1988;132(2):159–98.

52. Lang SDJ, Farine DR. A multidimensional framework for studying social predation strategies. Nat Ecol Evol. 2017 Sep;1(9):1230–9.

53. Steinegger M, Sarhan H, Bshary R. Laboratory experiments reveal effects of group size on hunting performance in yellow saddle goatfish, Parupeneus cyclostomus. Animal Behaviour. 2020;168:159–67.

54. Bailey I, Myatt JP, Wilson AM. Group hunting within the Carnivora: physiological, cognitive and environmental influences on strategy and cooperation. Behav Ecol Sociobiol. 2013 Jan 1;67(1):1–17.

55. Stiner MC, Barkai R, Gopher A. Cooperative hunting and meat sharing 400–200 kya at Qesem Cave, Israel. Proceedings of the National Academy of Sciences. 2009;106(32):13207–12.

56. Chivers DP, Smith RJF. Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. Ecoscience. 1998;5(3):338–52.

57. Griffith SC. Cooperation and Coordination in Socially Monogamous Birds: Moving Away From a Focus on Sexual Conflict. Frontiers in Ecology and Evolution [Internet]. 2019 [cited 2022 Jun 22];7. Available from: https://www.frontiersin.org/article/10.3389/fevo.2019.00455

58. Strübin C, Steinegger M, Bshary R. On group living and collaborative hunting in the yellow saddle goatfish (Parupeneus cyclostomus) 1. Ethology. 2011;117(11):961–9.

59. Brönmark C, Hansson LA. Chemical Ecology in Aquatic Systems. OUP Oxford; 2012. 318 p.

60. Brown GE, Paige JA, Godin JGJ. Chemically mediated predator inspection behaviour in the absence of predator visual cues by a characin fish. Animal Behaviour. 2000 Sep 1;60(3):315–21.

61. Curio E, Ernst U, Vieth W. The adaptive significance of avian mobbing: II. Cultural transmission of enemy recognition in blackbirds: Effectiveness and some constraints. Zeitschrift für Tierpsychologie. 1978;48(2):184–202.

62. Fishman MA. Predator Inspection: Closer Approach as a Way to Improve Assessment of Potential Threats. Journal of Theoretical Biology. 1999 Jan 21;196(2):225–35.

63. Pitcher TJ, Green DA, Magurran AE. Dicing with death: predator inspection behaviour in minnow shoals. Journal of Fish Biology. 1986;28(4):439–48.

64. Saavedra I, Amo L. The importance of chemical, visual and behavioral cues of predators on the antipredatory behavior of birds. Journal of Avian Biology [Internet]. 2020 [cited 2021 Jul 12];51(7). Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/jav.02431

65. Brown GE, Magnavacca G. Predator Inspection Behaviour in a Characin Fish: an Interaction between Chemical and Visual Information? Ethology. 2003;109(9):739–50.

66. Magurran AE. The adaptive significance of schooling as an anti-predator defence in fish. In JSTOR; 1990. p. 51–66.

67. Magurran AE, Seghers BH. Population differences in predator recognition and attack cone avoidance in the guppy Poecilia reticulata. Animal Behaviour. 1990;40(3):443–52.

68. Dugatkin LA, Godin JGJ. Prey approaching predators: a cost-benefit perspective. Annales Zoologici Fennici. 1992;29(4):233–52.

69. Pitcher T. Who Dares, Wins: the Function and Evolution of Predator Inspection Behaviour in Shoaling Fish. Netherlands Journal of Zoology. 1991 Jan 1;42(2–3):371–91.

70. hasson O. Pursuit-deterrent signals: communication between prey and predator. Trends in Ecology & Evolution. 1991 Oct;6(10):325–9.

71. Godin J guy J, Davis SA. Who dares, benefits: predator approach behaviour in the guppy (Poecilia reticulata) deters predator pursuit. Proceedings of the Royal Society of London Series B: Biological Sciences. 1995 Feb 22;259(1355):193–200.

72. Milinski M. Tit for tat in sticklebacks and the evolution of cooperation. nature. 1987;325(6103):433–5.

73. Dugatkin LA. Do guppies play TIT FOR TAT during predator inspection visits? Behav Ecol Sociobiol. 1988 Dec 1;23(6):395–9.

74. Milinski M, K¨lling D, Kettler R. Tit for Tat: sticklebacks (*Gasterosteus aculeatus*) ‘trusting’ a cooperating partner. Behavioral Ecology. 1990;1(1):7–11.

75. Dugatkin LA, Alfieri M. Tit-For-Tat in guppies (Poecilia reticulata): the relative nature of cooperation and defection during predator inspection. Evol Ecol. 1991 Jul 1;5(3):300–9.

76. Dugatkin LA. N-person games and the evolution of co-operation: A model based on predator inspection in fish. Journal of Theoretical Biology. 1990 Jan;142(1):123–35.

77. Semmens BX, Buhle ER, Salomon AK, Pattengill-Semmens CV. A hotspot of non-native marine fishes: evidence for the aquarium trade as an invasion pathway. Marine Ecology Progress Series. 2004;266:239–44.

78. Betancur-R. R, Hines A, Acero P. A, Ortí G, Wilbur AE, Freshwater DW. Reconstructing the lionfish invasion: insights into Greater Caribbean biogeography. Journal of Biogeography. 2011;38(7):1281–93.

79. Schofield PJ. Update on geographic spread of invasive lionfishes (Pterois volitans [Linnaeus, 1758] and P. miles [Bennett, 1828]) in the Western North Atlantic Ocean, Caribbean Sea and Gulf of Mexico. Aquatic Invasions. 2010;5(Supplement 1):S117–22.

80. Johnston MW, Purkis SJ. Are lionfish set for a Mediterranean invasion? Modelling explains why this is unlikely to occur. Marine Pollution Bulletin. 2014 Nov 15;88(1):138–47.

81. Frazer TK, Jacoby CA, Edwards MA, Barry SC, Manfrino CM. Coping with the Lionfish Invasion: Can Targeted Removals Yield Beneficial Effects? Reviews in Fisheries Science. 2012 Oct;20(4):185–91.

82. Ferreira CE, Luiz OJ, Floeter SR, Lucena MB, Barbosa MC, Rocha CR, et al. First record of invasive lionfish (Pterois volitans) for the Brazilian coast. PloS one. 2015;10(4):e0123002.

83. Darling ES, Green SJ, O’Leary JK, Côté IM. Indo-Pacific lionfish are larger and more abundant on invaded reefs: a comparison of Kenyan and Bahamian lionfish populations. Biol Invasions. 2011 Sep;13(9):2045–51.

84. Kulbicki M, Beets J, Chabanet P, Cure K, Darling E, Floeter SR, et al. Distributions of Indo-Pacific lionfishes Pterois spp. in their native ranges: implications for the Atlantic invasion. Marine Ecology Progress Series. 2012;446:189–205.

85. Lesser MP, Slattery M. Phase shift to algal dominated communities at mesophotic depths associated with lionfish (Pterois volitans) invasion on a Bahamian coral reef. Biological Invasions. 2011;13(8):1855–68.

86. Albins MA. Effects of invasive Pacific red lionfish Pterois volitans versus a native predator on Bahamian coral-reef fish communities. Biological Invasions. 2013;15(1):29–43.

87. Morris JA, Akins JL. Feeding ecology of invasive lionfish (Pterois volitans) in the Bahamian archipelago. Environmental Biology of Fishes. 2009;86(3):389–98.

88. Layman CA, Allgeier JE. Characterizing trophic ecology of generalist consumers: a case study of the invasive lionfish in The Bahamas. Marine Ecology Progress Series. 2012 Feb 23;448:131–41.

89. Valdez-Moreno M, Quintal-Lizama C, Gómez-Lozano R, García-Rivas M del C. Monitoring an Alien Invasion: DNA Barcoding and the Identification of Lionfish and Their Prey on Coral Reefs of the Mexican Caribbean. Gratwicke B, editor. PLoS ONE. 2012 Jun 1;7(6):e36636.

90. Dahl KA, Patterson WF. Habitat-Specific Density and Diet of Rapidly Expanding Invasive Red Lionfish, Pterois volitans, Populations in the Northern Gulf of Mexico. Layman CA, editor. PLoS ONE. 2014 Aug 29;9(8):e105852.

91. Dahl KA, Patterson WF. Movement, home range, and depredation of invasive lionfish revealed by fine-scale acoustic telemetry in the northern Gulf of Mexico. Marine Biology. 2020;167(8):1–22.

92. Green SJ, Dulvy NK, Brooks AM, Akins JL, Cooper AB, Miller S, et al. Linking removal targets to the ecological effects of invaders: a predictive model and field test. Ecological Applications. 2014;24(6):1311–22.

93. Green S, Underwood E, Atkins L. Fishing derbies for invasive lionfish: a tool for public engagement and population control. Proceedings of the 66th Gulf and Caribbean Fisheries Institute Corpus Christi, Texas, USA, http://www gcfi org/proceedings/sites/de fault/files/procs/GCFI_66-55_5 pdf(accessed 01 February 2017). 2013;

94. Green SJ, Côté IM. Trait-based diet selection: prey behaviour and morphology predict vulnerability to predation in reef fish communities. Journal of Animal Ecology. 2014;83(6):1451–60.

95. Eddy C, Pitt J, Jr JAM, Smith S, Goodbody-Gringley G, Bernal D. Diet of invasive lionfish (Pterois volitans and P. miles) in Bermuda. Marine Ecology Progress Series. 2016 Oct 25;558:193–206.

96. Randall JE. Reef and shore fishes of the South Pacific. University of Hawai’i Press, Honolulu. 2005;

97. Albins MA, Lyons PJ. Invasive red lionfish Pterois volitans blow directed jets of water at prey fish. Marine Ecology Progress Series. 2012;448:1–5.

98. Holway DA, Suarez AV. Animal behavior: an essential component of invasion biology. Trends in ecology & evolution. 1999;14(8):328–30.

99. Morris Jr JA, Whitfield PE. Biology, ecology, control and management of the invasive Indo-Pacific lionfish: an updated integrated assessment. 2009;

100. Meyer AL, Dierking J. Elevated size and body condition and altered feeding ecology of the grouper Cephalopholis argus in non-native habitats. Marine Ecology Progress Series. 2011;439:203–12.

101. Rittermann A. A Review of Present and Alternative Lionfish Controls in the Western Atlantic. :53.

102. Cox JG, Lima SL. Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. Trends in Ecology & Evolution. 2006;21(12):674–80.

103. Lönnstedt OM, Ferrari MCO, Chivers DP. Lionfish predators use flared fin displays to initiate cooperative hunting. Biol Lett. 2014 Jun;10(6).

104. Rizzari JR, Lönnstedt OM. Cooperative hunting and gregarious behaviour in the zebra lionfish, Dendrochirus zebra. Marine Biodiversity. 2014;44(4):467–8.

105. Naumann MS, Wild C. Foraging association of lionfish and moray eels in a Red Sea seagrass meadow. Coral Reefs. 2013;32(4):1111–1111.

106. The Biology Letters Editorial Team. Expression of concern: Lionfish predators use flared fin displays to initiate cooperative hunting. Biology Letters. 2018 Feb 28;14(2):20180032.

107. Kletou D, Hall-Spencer JM, Kleitou P. A lionfish (Pterois miles) invasion has begun in the Mediterranean Sea. Marine Biodiversity Records. 2016 Jun 30;9(1):46.

108. They are here to stay: the biology and ecology of lionfish (Pterois miles) in the Mediterranean Sea - Savva - 2020 - Journal of Fish Biology - Wiley

Online Library [Internet]. [cited 2022 Jun 16]. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/jfb.14340?casa_token=v69ipxLTNt4AAAAA%3A2ZgYKhQEQh8b9abR7ity-Yyw0Y-cIVHLAXTlpDE13NitiOZZOrVEBLApjokRo2zpxmFkonffprz3E2M

109. Biology Letters Editorial Team. Correction to ‘Lionfish predators use flared fin displays to initiate cooperative hunting’. Biology letters. 2019;15(11):20190766.

CHAPTER 1 | NO EVIDENCE FOR CONSPECIFIC FOR RECRUITMENT FOR COOPERATIVE HUNTING IN LIONFISH *PTEROIS MILES*

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Abstract

Lionfish are common piscivores in the Indo-Pacific and invasive in the Caribbean. A fin flaring pattern, involving a rapid undulation of the caudal fin and sequential turning of both pectoral fins, was described in zebra lionfish as a signal to initiate cooperative hunting, and it was hypothesized that such hunting tactics may also exist in other lionfish species and contribute to their successful invasion in the Caribbean. Here, we investigated one of those invasive species, Pterois miles, in its natural range in the Red Sea. We did not observe evidence for cooperative hunting in the field. We complemented field observations with a laboratory experiment aimed at inducing subjects to recruit partners for cooperative hunts, exposing subjects to inaccessible prey in a transparent housing as well as to a potential partner. We regularly observed the fin flaring pattern, but importantly it was not directed at the partner. Thus, rather than being a signal, the fin flaring movement pattern seems to be a swimming mode in a confined environment. Furthermore, the two lionfish did not aggregate at the prey housing, reinforcing the field results that this species in the Red Sea does not depend on cooperation to hunt fish.

Keywords: cooperative hunting, signaling, recruitment, lionfish.

1.1. Introduction

One way in which animals may benefit from grouping is because of the transfer of information regarding the distribution of patchy food (1). The transfer of information may be passive, i.e., based on cues, or active due to communication. Active communication warrants shared interest between signaler and receiver. One possible cause is kin selection, like in the waggle dance in honey bees (2) or pioneer ants leaving trails of pheromones that lead foraging ants between food-rich areas and their nests (3). Alternatively, recruiting partners yields direct benefits to the signaler. For example, ravens recruit conspecifics to overcome defense of food by dominant individuals and competitors (4). Recruitment may also occur between individuals belonging to different species. Groupers may perform a head stand signal to attract various partner species (moray eels, Napoleon wrasse or octopus) to a location where prey is hiding in a crevice, eliciting a cooperative hunting attempt on that prey (5). Cooperative hunting occurs when two or more individuals interact in pursuing a hunt that eventually produces on average greater payoffs per individual than solitary hunting (6). Correlative evidence for a positive relationship between hunting group size(s) exists for a variety of mammals and birds (7).

Much research on cooperative hunting has focused on the complexity of the coordination between hunters. Coordination can range from spontaneous ‘simultaneous single hunts’ to individuals planning a hunt (intentional hunting) in which they play different roles (termed collaboration) (9,7). Intentional and collaborative hunting was initially reported in few endotherm vertebrate species (Harris hawks (10); chimpanzees (9); lions (11); dolphins (12)) and hence assumed to rely on advanced cognitive abilities that require at least an endotherm vertebrate brain (9). However, the more recent description of intentional communication and collaborative hunting in various fish species (5, 13, 14) changed this view. A case study yielding seemingly particularly complex hunting patterns in a fish described communicative and cooperative hunting in zebra lionfish (*Dendrochirus zebra*) (13). Building on field observations that lionfish species not only hunt alone (15) but also in the presence of conspecifics (16) and with moray eels (17), Zebra lionfish were subjected to laboratory experiments (13). In these experiments, subjects were initially exposed to inaccessible prey. The subjects were described to recruit naïve conspecifics and heterospecific lionfish (*Pterois antennata*), confined behind an opaque barrier, to join the hunt. To do so, subjects approached the potential partner head on and pectoral fins flared, rapidly undulated their caudal fin with a slow and separate waving of the pectorals ((13), also see Figure 1.C). Subjects would then swim back to the prey location ('leading' behavior) and return to a non-following partner to repeat the display (13). Once the partner was released by the experimenter, the resulting pair would then hunt in highly coordinated ways on now accessible shoaling prey, alternating attacks in what appeared to be a reciprocal manner (13). The findings were interpreted as evidence that Zebra lionfish display highly sophisticated social behavior to achieve high hunting success rates (18). Furthermore, as recruited *P. antennata* behaved very similarly the authors also hypothesized that sophisticated cooperative hunting may explain the high feeding rates of Pterois lionfish species in their invasive range in the Caribbean.

Pterois miles and *P. volitans* were introduced in Florida water in the 1980s, apparently from home aquaria (19). The following invasion across the Caribbean has attracted great attention by conservationists as the negative effects are very strong (20). Some parameters have already diverged from the native populations in the Indo-Pacific: lionfish in the native range exhibit slower life histories and/or narrower resource use (15–17). One proposed reason for lionfish being successful hunters in their invasive range is that they display a rather unique combination of adaptations to hunting that Caribbean prey had not encountered before. Lionfish show striped coloration for crypsis (21), slow movement to avoid prey flight responses (22–25), and they blow water towards prey to make it orient against the resulting current and hence towards its predator (21). The hypothesized cooperative hunting with active recruitment of partners based on the study of Zebra lionfish (13,16) would hence add to an already fearsome list of features.

Given that *P. miles* is one of the two lionfish species of Indo-pacific origin that is found in the Caribbean, we decided to investigate whether this species exhibits communicative cooperative hunting in its native range that is similar to what has been described for zebra lionfish. Only after our plans had been made, a note of concern was published indicating that the data by Lönnstedt et al. (13) may be at least in part manipulated and/or fabricated, as the number of subjects is higher than the records for captured fish (26,27) **.** We conducted a series of field observations and laboratory experiments. First, we investigated the frequency and duration of any *P. miles* interspecific hunting associations in the field, by use of snorkeling and diving. Second, we explored whether the fin flaring pattern described for the Australian Zebra lionfish *D. zebra* is also shown by *P. miles* in the Red Sea, using both field observations and a laboratory experiment (see S1 videos in supplements on *P. miles* and video by Lönnstedt et al. 2014 for *D. zebra*). The experiment was based on the design by Lönnstedt *et al.* (13), aimed at testing whether *P. miles* actively recruit naïve partners to hunt in pairs. We manipulated prey availability (present or absent) and partner availability (present initially in a separate compartment, or absent) and recorded the occurrence of fin flaring patterns. If the fin flaring patterns serve as a recruitment signal, we expected a significant interaction where fin flaring should be frequent near the separate compartment if both prey and a partner are present.

1.2. Material and methods

1.2.1. *Field Observations*

Lionfish *Pterois miles* (Figure 1.A) were observed on four reefs near Dahab city located in the Northern Red Sea (Figure 1.B), from August to November 2017. The four sites for data collection were (1) Assalah coast with estimated area of 0.09 km², (2) Light house (0.06 km²), (3) Soliman reef (0.09 km²), (4) Three pinnacles (0.06 km²), all located in Dahab city, South Sinai, Red Sea, Egypt (Figure 1.A). Field observations were conducted via snorkeling and diving during the crepuscular times of the day (4:30 - 6:00 and 18:00 - 19:30), i.e. when the species is most active (25, 26). Only active individuals were picked in an opportunistic way, i.e. the first individual found was followed for a maximum of 30 min, and then the next encountered active individual was followed. A distance of 3 m was respected to avoid disturbance. The length of focal individuals as well as associated individuals was estimated in centimeter with the help of a reference scale fixed on the observer's fin. During observations, lionfish swam in depth ranging from 1-15 meter.

Regarding the maximum of four follows during a single observation session, we are confident that different individuals were sampled. In contrast, the same individual may have been resampled during repeated visits to the same locations, as we did not recognize lionfish individually. The lionfish density in the Dahab area is very high, with estimated 87.3 ± 117.3 individuals per 0.01 km² (29). Therefore, the estimated number of lionfish at each site was 785, 524, 785 and 524, respectively, and our 302 individual follows comprised 72, 74, 78 and 78 follows at the four sites, respectively. Based on these numbers, we estimated that repeated sampling of individuals was probably rare. We are therefore confident that our results represent what lionfish typically do at our study sites, despite the small amount of uncontrolled pseudoreplication.

The most basic measure was to quantify how much time lionfish spent in association with conspecifics and/or record any occasions of associations with moray eels as these had been described by Naumann and Wild (17). We used 1 m maximal distance as association criterion (30,31) and asked whether lionfish spend more time in such distance to conspecifics than expected by chance as an indicator whether or not they seek each other. We looked for the fin flaring pattern described by Lönnstedt *et al.* (13) during the entire observation period. Similarly, we recorded any attempt to herd prey by spreading the pectoral fins like a shield (17), as this spreading almost always preceded any foraging attempt. Such attempts sometimes included orienting the head towards a nearby prey (typically close or on a substrate, i.e. reef or the sandy bottom), mostly accompanied with a rapid mouth opening to suck prey in. Note, however, that it was impossible to ascertain whether or not a fish was caught in these events.

1.2.2. *Laboratory experiment and fish collection*

The laboratory experiment was conducted at Open Ocean Science Center (OOSC) at Dahab city in September to November 2019. Fish capturing, handling, and acclimatizing process was performed carefully to minimize stress responses and to provide a neutral environment for the captured individuals. 48 Lionfish individuals were captured using handmade plastic net and gloves from depths from 0 to 10 meters. Individuals were brought slowly to the surface to avoid air bladder inflation. Fish were directly transferred into holding tanks for three days without being fed, then assigned into the experimental tanks. The round experimental tanks with 153 cm diameter and 60 cm high, with the water level at 40 cm (Figure 2.A). A semi-closed system was used to maintain continuous seawater change with a flow rate of 2 L/min, and an air pump as well as a water pump to provide oxygen and some current.

In each tank, a pair of lionfish was placed for ten days to acclimate before the experimental trails. Starting from the third night, individuals were fed twice during the crepuscular time 2 pieces (0.5 x 0.5 cm) of dead shrimps for each individual, while during experimental days individuals were fed only after trails to ensure that they were motivated to hunt during trials. Aquaria were cleaned once a day to prevent biotic accumulation. All fish were released at sites of capture after completing the experiment.

In parallel with collecting lionfish individuals, we caught 25 Blue-green chromis (*Chromis viridis*), using a barrier net 1.5 x 1.5 m with a 0.5 x 0.5 cm mesh size to present them as potential prey to lionfish. The chromis were kept in a single aquarium (50 x 30 x 30 centimeter), and left to acclimatize for 7-10 days. The aquarium was provided with sea water open system flow (2L per min), and an air pump to provide appropriate aeration. An artificial coral block and small PVC tubes were added to serve as shelters and blue background were placed all around the aquarium. Fish were fed twice per day with commercial prawn flakes, and food residuals were removed daily, and tank surfaces were cleaned at least every third day.

1.2.2.1. Experimental design

The design of the experiment and the setup of the round experimental tanks were inspired by the study of Lönnstedt *et al.* (13), the main difference being that we had round tanks. Inside each tank, we placed two holding compartments separated by a channel (Figure 2. A and B). Pairs of individuals were locked separately inside the compartments with the ability to see each other through two removable transparent barriers. In contrast, the barrier facing the rest of the tank was opaque, preventing locked individuals to see the location where a tube containing either 4 prey fish (experimental treatment) or nothing (control treatment) would be presented. Consequently, a released focal individual trying to catch a prey would not be able to see the potential partner locked away behind the barrier through the opaque barriers installed facing the prey housing. The tube was placed close to the opposite side of the holding compartments, such that lionfish could still swim all around it (Figure 2.B).

We run experiments in a full 3 x 2 design, yielding six conditions. The focal individual could be in three association states regarding its partner, referred to as ‘social state’ from now on: (1) Singleton: the partner had been removed to another tank (100 x 60 centimeter) before the trial. (2) Partner locked: the partner was contained in a holding compartment. (3) Partner released: the partner could swim around freely in the tank. The second and third conditions were tested within the same trial, invariably starting with the partner locked. Regarding our second factor prey availability, the Plexiglas tube either contained four prey fish (prey present) or no prey fish at all (prey absent). In order to be able to define a visit, as well as to define hunting activity by either the focal or its partner, we divided the tank into three zones (Figure 2.B): The ‘partner visit zone’ was the corridor between the two holding compartments. The ‘hunting zone’ was a rectangle 60 x 100 cm around the lying Plexiglas tube. The ‘neutral zone’ as a general activity zone contained all the other areas of the tank.

1.2.2.2. Experiment protocol and data collection

All trials took place at night as lionfish were more active during that time compared to during the day. Nevertheless, white light wrapped with towels was put on to allow direct observation as well as filming. A Gopro hero +3 camera was installed 130 cm above the rim of the tank to film the trials. Before each trial, the focal individual and its partner (if present) were guided into the holding compartments, the compartments being randomly assigned. This was achieved by gently pushing objects (a plexiglass sheet or stick) behind them. Lionfish were then left to settle for ten minutes. Meanwhile, we placed a Plexiglas tube (40 x 15 cm) in the tank at its predetermined location. The tube had a removable cap to allow the insertion of prey for trials in which prey was presented to the lionfish. The four prey fish were always randomly caught from the holding tank; individual identity was not known. A trial started by removing the transparent barrier of the focal's holding compartment. Focal individuals left the compartment within the next 60 sec. In the singleton condition, a trial lasted 15 min. With a partner, the focal invariably started to hunt alone. If it revisited its partner, scored as entering the channel between the two holding compartments, the partner would be released five minutes later. This delay in the release gave us opportunity to record any repeated signaling if it occurred. If the focal individual did not visit the partner, the partner would be released after 15 min. The total length of a trial with partner was 20 min, so the two conditions 'partner locked' and 'partner released' occurred with variable durations. The focal and partner compartments were considered as part of the neutral zone once the partner had been released. A trial ended with the removal of the Plexiglas tube; and any prey were returned to their holding tank.

The following information was extracted from the videos, using Boris 7.9.7 software. (1) Any fin movement sequence as described as fin flaring signal by Lönnstedt *et al.* (13) (Figure.1.B) and its context (which zone and location of partner), and (2) The time from releasing the focal to its returning to the 'partner visit zone'. See supplementary material for statistical analysis.

1.3. Statistical analysis

1.3.1. *Field observations*

We used Waser's gas model (WGM) (30–32) as the simplest option to calculate a null hypothesis regarding the mean duration of associations between lionfish. Using the model to calculate the predicted mean duration needs rather minimal information, namely the average swimming speed (v) of individuals, and a set maximal distance criterion (r) between two individuals that they are still considered to be associated. Application of the model warrants to consider movements only in a two-dimensional space, which works well for an observer watching from above. Under these assumptions, the mean duration of associations (t) predicted if individuals move independently of each other is given by equation 1:

$$(1) \qquad t = \frac{1.745\ r}{v}$$

We used a mean speed of 3 cm/sec as reported by Dahl and Patterson (33), and 100 cm as the distance criterion (30,31).

1.3.2. Laboratory experiments

In general, all our data represent independent measures as each fish was tested only once. As we had several data points per fish in the test, individual ID was added as a random factor. Data distributions were negatively binomial and the normality was always violated. As our data contained zeros, we applied generalized linear mixed-effect models using Template Model Builder with the beta zero-inflation properties to calculate the frequencies, time and proportions (GLMM: “glmmTMB”). The data and the R codes as well as a videos of fin flaring and recruitment tendency are available to public on dryad at https://doi.org/10.5061/dryad.w3r2280q8

The frequency of the fin flaring was analyzed as a function of prey presence/absence, social state (singleton, partner locked, and partner released) and the focal individual’s location (hunting zone, neutral zone and partner zone). The focal and partner ID as well as observation time were random factors. If the fin flaring movement pattern serves as a signal to initiate cooperative hunting, we expected a 3-way interaction. If lionfish try to recruit the partner to guide it towards prey, the pattern should occur most often when prey is present, in the partner zone with a partner locked up. Alternatively, if lionfish try to make partners join at the prey location, the pattern should occur most often when prey is present, in the hunting zone with a partner locked up. For the analyses, we quantified how much time the focal spent in each zone in each condition. We then used the occurrences of fin flaring in each zone and time spent in each zone to calculate fin flaring frequencies.

As an additional test whether lionfish are likely to seek a partner when confronted with inaccessible prey, we analyzed whether the time from release to returning for the first time to the ‘partner zone’ varied as a function of prey presence/absence and partner absent/locked. We obtained a single value in seconds for each trial. If lionfish seek the presence of a partner for joint hunting, we expected that subjects will revisit the partner zone soonest if there is prey and a partner locked up. Subject and partner IDs were added as random variable.

1.4. Results

1.4.1. Field observations

We collected a total of 302 follows, yielding 172 hours of observations. In general, lionfish spend 14.3 % time in association with conspecifics. The mean duration of the associations was 4.2± 1.4 min (Figure 3). Based on the gas model, assuming an average speed of 3 cm per second, the expected mean duration of an association was 59 s. There is thus some indication that associations last approximately four times longer than predicted by chance. However, the more critical question was whether lionfish would hunt together when in association. We observed a total of 103 events of lionfish spreading their fins like a shield. In 12 of these 103 cases, a conspecific was within 1 m, i.e. slightly less than the 14.7 cases expected by chance if associations do not serve to hunt cooperatively. No fin flaring sequences were recorded during any association, and the sequence was only observed once in a singleton.

1.4.2. Laboratory experiments

Results were based on a total of 48 individuals that formed 24 pairs. We observed a total of 1512 fin flaring, yielding 48 hours of observations. We regularly observed the fin flaring pattern during trials, if it serves as a recruitment signal, we expected it to occur most frequently in the partner zone but only if there is prey and the partner locked up. However, we did not find such a 3-way interaction but instead two 2-way interactions (Figure 4.A). As expected if fin flaring was a recruitment signal, it occurred most frequently in the corridor between the two holding compartments when prey was present (2-way interaction, Chi2 = 6.49, p = 0.039). However, opposite to the recruitment hypothesis, fin flaring occurred most frequently in the corridor after the partner had been released and hence was not in the zone anymore (2-way interaction, Chi2=12.2, p = 0.016).

Regarding the time focals spent in the three zones we found a significant 3-way interaction opposite to what would be expected if lionfish hunt cooperatively: focals spent more time in the hunting zone (and less time in the neutral zone) if there was prey and the partner was still locked up (Chi2 = 10.08, p = 0.039). Focals spent little time in the hunting zone when the partner was released, irrespective of prey presence/absence (Figure 2.B).

The time it took focals to return to the partner zone was explained by an interaction between prey condition and social state (Chi2 = 10.06, p= 0.0015). Focals took longest to return if there was a partner locked up and prey absent (Figure 2.C). In the presence of prey, return times were similar between partner locked and the singleton condition.

1.5. Discussion

We had asked whether lionfish *P. miles* in the Red Sea show any evidence for cooperative hunting based on active recruitment, as such hunting techniques might have helped them to become such a highly successful invasive species in the Caribbean. While the fin flaring pattern described in *D. zebra* was virtually absent in *P. miles* the field, it occurred frequently during our laboratory experiments. The field data contrast with Lönnstedt *et al.* (13) who apparently observed the pattern regularly in nature (though no data were presented). The two key differences between the two studies and hence the two species were that we found no evidence that the fin flaring is used as a signal and that *P. miles* individuals would coordinate to hunt cooperatively. A hypothesis for the frequent occurrence of the fin flaring pattern in *P. miles* that needs further investigation is that its occurrence is linked to maneuvering (34–37). The main current argument in favor of this hypothesis is that it occurred mostly in the narrow partner zone in our experiment. Hence the question arises whether the fin flaring pattern could also be used for maneuvering in *D. zebra*. Speaking against this hypothesis are the observations that partners apparently responded to a visit and fin flaring pattern with their own one (see Fig. 2b in (13)). On the other hand, the interaction site between the test fish and its contained partner had to happen in a narrow place, like in our experiment. Furthermore, we are uncertain how to interpret the information provided by Lönnstedt et al. (13) in the supplementary video that shows the fin flaring pattern. We invite colleagues to watch it for themselves (Supplementary video S1. A): in our view it looks like the focal individual used the pattern to orient towards a prey that is subsequently eaten, an interpretation that favours the maneuvering hypothesis.

The space use of *P. miles* subjects during experiments conforms with the interpretation that this species does not hunt cooperatively, at least not at our study site in the Red Sea. Our *Chromis* prey can be considered attractive, just as *Apogon sp.* is for *D. zebra* (38–40), and both were presented in small groups. Prey was behind a transparent barrier in the study by Lönnstedt et al. (2014), while inside a transparent tube that could be approached from all sides in our study. Though the details of the presentation hence differed, subjects in both studies experienced that prey is inaccessible when hunting alone. Thus, despite the fact that various variables were slightly different (prey species, aquarium size, transparent housing structure), we think that the situation should have triggered partner recruitment in our study as well. However, in our study inaccessible prey and a locked-away partner did not result in a quick return to the partner (Supplementary video S1.B), which contradicts the hypothesis that *P. miles* recruits' partners for joint hunting proposed in Lönnstedt study.

In line with the lack of evidence for recruitment, we did not find any evidence that *P. miles* hunts cooperatively in nature. While associations in the field lasted on average longer than expected by chance, they were much shorter than for example between collaboratively hunting fish, like groupers and moray eels (41) or yellow goatfish (42). Also, during experiments, the lionfish did not aggregate in the hunting zone, which suggests a rather chance character of lionfish associations (15,43). Taken together, our results suggest that the success of invasive *P. miles* in the Caribbean is not based on cooperative hunting but on other features. Clearly, the reported variation in cooperative hunting between lionfish species and/or study sites warrants further investigation**.**

1.6. Acknowledgments

We thank Radu Slobodeanu for statistic support, the Open Ocean science station in Dahab for logistic support, and Ahmed Hassan for fish catching support. RB is supported by the Swiss Science Foundation grant 310030_192673 / 1.

1.7. Author contributions

Both authors designed the study, HS collected all data and wrote a first draft, which was finalized by RB.

1.8. Ethics

Ethics and research permit was granted by Ras Mohamed National Park office before starting the study.

1.9. Funding

Research was funded by a Swiss Government Excellence Scholarship to HS and a Swiss National Science foundation grant to RB.

1.10. References

1. Ward P, Zahavi A. The importance of certain assemblages of birds as “information-centres” for food-finding. Ibis. 1973;115(4):517–34.
2. Von Frisch K. The dance language and orientation of bees: Harvard University Press. Cambridge, Mass. 1967;
3. Heinrich B. Winter foraging at carcasses by three sympatric corvids, with emphasis on recruitment by the raven, Corvus corax. Behavioral Ecology and Sociobiology. 1988;23(3):141–56.
4. Marzlufi JM, Heinrich B. Foraging by common ravens in the presence and absence of territory holders: an experimental analysis of social foraging. Animal Behaviour. 1991;42(5):755–70.
5. Vail AL, Manica A, Bshary R. Referential gestures in fish collaborative hunting. Nat Commun. 2013 Jun;4(1):1765.
6. Packer C, Ruttan L. The evolution of cooperative hunting. The American Naturalist. 1988;132(2):159–98.
7. Lang SDJ, Farine DR. A multidimensional framework for studying social predation strategies. Nat Ecol Evol. 2017 Sep;1(9):1230–9.
8. Steinegger M, Sarhan H, Bshary R. Laboratory experiments reveal effects of group size on hunting performance in yellow saddle goatfish, Parupeneus cyclostomus. Animal Behaviour. 2020;168:159–67.
9. Boesch C, Boesch H. Hunting behavior of wild chimpanzees in the Tai National Park. American journal of physical anthropology. 1989;78(4):547–73.
10. Bednarz JC. Cooperative hunting Harris’ hawks (Parabuteo unicinctus). Science. 1988;239(4847):1525–7.
11. Stander PE. Cooperative hunting in lions: the role of the individual. Behavioral ecology and sociobiology. 1992;29(6):445–54.
12. Gazda SK, Connor RC, Edgar RK, Cox F. A division of labour with role specialization in group–hunting bottlenose dolphins (Tursiops truncatus)

off Cedar Key, Florida. Proceedings of the Royal Society B: Biological Sciences. 2005;272(1559):135–40.

13. Lönnstedt OM, Ferrari MCO, Chivers DP. Lionfish predators use flared fin displays to initiate cooperative hunting. Biol Lett. 2014 Jun;10(6).
14. Steinegger M, Roche DG, Bshary R. Simple decision rules underlie collaborative hunting in yellow saddle goatfish. Proc R Soc B. 2018 Jan 31;285(1871):20172488.
15. Albins MA, Lyons PJ. Invasive red lionfish Pterois volitans blow directed jets of water at prey fish. Marine Ecology Progress Series. 2012;448:1–5.
16. Rizzari JR, Lönnstedt OM. Cooperative hunting and gregarious behaviour in the zebra lionfish, Dendrochirus zebra. Marine Biodiversity. 2014;44(4):467–8.
17. Naumann MS, Wild C. Foraging association of lionfish and moray eels in a Red Sea seagrass meadow. Coral Reefs. 2013;32(4):1111–1111.
18. Green SJ, Dulvy NK, Brooks AM, Akins JL, Cooper AB, Miller S, et al. Linking removal targets to the ecological effects of invaders: a predictive model and field test. Ecological Applications. 2014;24(6):1311–22.
19. Courtenay W. Marine fish introductions in southeastern Florida. Am Fish Soc Introd Fish Newsl 14: 2–3. 1995;
20. Côté IM, Green SJ, Hixon MA. Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. Biological Conservation. 2013 Aug;164:50–61.
21. Albins MA, Hixon MA. Worst case scenario: potential long-term effects of invasive predatory lionfish (Pterois volitans) on Atlantic and Caribbean coral-reef communities. Environ Biol Fish. 2013 Nov;96(10–11):1151–7.
22. Cure K, Benkwitt C, Kindinger T, Pickering E, Pusack T, McIlwain J, et al. Comparative behavior of red lionfish Pterois volitans on native Pacific

versus invaded Atlantic coral reefs. Mar Ecol Prog Ser. 2012 Oct 25;467:181–92.

23. Randall JE. Reef and shore fishes of the South Pacific. University of Hawai'i Press, Honolulu. 2005;

24. Green S, Akins J, Côté I. Foraging behaviour and prey consumption in the Indo-Pacific lionfish on Bahamian coral reefs. Mar Ecol Prog Ser. 2011 Jul 18;433:159–67.

25. Green S, Underwood E, Atkins L. Fishing derbies for invasive lionfish: a tool for public engagement and population control. Proceedings of the 66th Gulf and Caribbean Fisheries Institute Corpus Christi, Texas, USA, http://www gcfi org/proceedings/sites/de fault/files/procs/GCFI_66-55_5 pdf(accessed 01 February 2017). 2013;

26. The Biology Letters Editorial Team. Expression of concern: Lionfish predators use flared fin displays to initiate cooperative hunting. Biology Letters. 2018 Feb 28;14(2):20180032.

27. Enserink M. Can you spot the duplicates? Critics say these photos of lionfish point to fraud [Internet]. Science | AAAS. 2019 [cited 2021 Aug 17]. Available from: https://www.sciencemag.org/news/2019/09/can-you-spot-duplicates-critics-say-these-photos-lionfish-point-fraud

28. Fishelson L. Experiments and observations on food consumption, growth and starvation in Dendrochirus brachypterus and Pterois volitans (Pteroinae, Scorpaenidae). Environmental Biology of Fishes. 1997;50(4):391–403.

29. McTee SA, Grubich JR. Native densities, distribution, and diurnal activity of Red Sea lionfishes (Scorpaenidae). Marine Ecology Progress Series. 2014;508:223–32.

30. Holenweg AK, Noë R, Schabel M. Waser's gas model applied to associations between red colobus and Diana monkeys in the Taï National Park, Ivory Coast. Folia Primatologica. 1996;67(3):125–36.

31. Waser PM. Cerococebus albigena: site attachment, avoidance, and intergroup spacing. The American Naturalist. 1976;110(976):911–35.

32. Waser PM. “Chance” and mixed-species associations. Behavioral Ecology and Sociobiology. 1984;15(3):197–202.

33. Dahl KA, Patterson WF. Movement, home range, and depredation of invasive lionfish revealed by fine-scale acoustic telemetry in the northern Gulf of Mexico. Marine Biology. 2020;167(8):1–22.

34. Larouche O, Benton B, Corn KA, Friedman ST, Gross D, Iwan M, et al. Reef-associated fishes have more maneuverable body shapes at a macroevolutionary scale. Coral Reefs [Internet]. 2020 Jul 29 [cited 2020 Aug 26]; Available from: http://link.springer.com/10.1007/s00338-020-01976-w

35. Weihs D. Stability Versus Maneuverability in Aquatic Locomotion. Integr Comp Biol. 2002 Feb 1;42(1):127–34.

36. Does body and fin form affect the maneuverability of fish traversing vertical and horizontal slits? | SpringerLink [Internet]. [cited 2020 Sep 4]. Available from: https://link.springer.com/article/10.1007/BF00001692

37. Webb PW. Control of Posture, Depth, and Swimming Trajectories of Fishes. Integrative and Comparative Biology. 2002 Feb 1;42(1):94–101.

38. Côté I, Green S, Morris J, Akins J, Steinke D. Diet richness of invasive Indo-Pacific lionfish revealed by DNA barcoding. Mar Ecol Prog Ser. 2013 Jan 9;472:249–56.

39. Ritger AL, Fountain CT, Bourne K, Martín-Fernández JA, Pierotti ME. Diet choice in a generalist predator, the invasive lionfish (Pterois volitans/miles). Journal of Experimental Marine Biology and Ecology. 2020;524:151311.

40. Dahl KA, Patterson WF. Habitat-Specific Density and Diet of Rapidly Expanding Invasive Red Lionfish, Pterois volitans, Populations in the Northern Gulf of Mexico. Layman CA, editor. PLoS ONE. 2014 Aug 29;9(8):e105852.

41. Bshary R, Hohner A, Ait-el-Djoudi K, Fricke H. Interspecific communicative and coordinated hunting between groupers and giant moray eels in the Red Sea. PLoS Biol. 2006;4(12):e431.

42. Strübin C, Steinegger M, Bshary R. On Group Living and Collaborative Hunting in the Yellow Saddle Goatfish (Parupeneus cyclostomus)1: On Group Living and Collaborative Hunting. Ethology. 2011 Nov;117(11):961–9.

43. Morris JA, Akins JL. Feeding ecology of invasive lionfish (Pterois volitans) in the Bahamian archipelago. Environmental Biology of Fishes. 2009;86(3):389–98.

1.11. Figures

A.

B.

C.

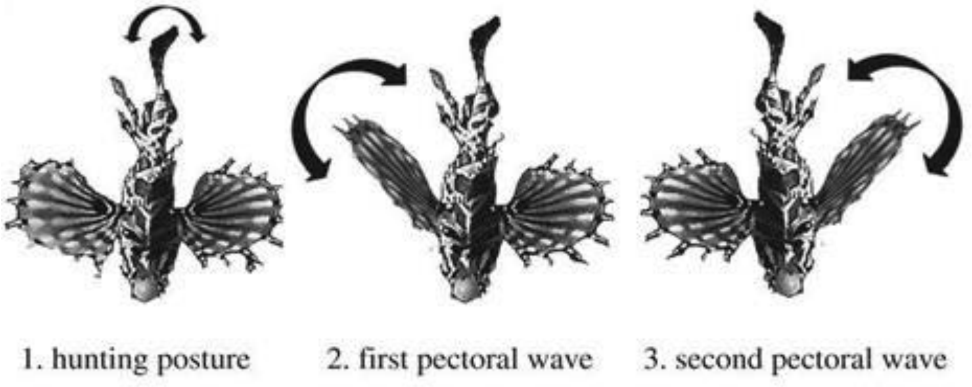


Figure 1: (A) The study species P. miles. (Photo credit: Hanaa Sarhan. Three pinnacles, Dahab, Egypt). (B) Location of 4 reef sites surveyed for lionfish species fin flaring behavior in the norther Red Sea region. Image modified from Google earth v.6.2 (2021). (C) The flared fin display sequence by initiator predators: where one individual approach partner head on, it rapidly undulates its caudal fin for 3–9 s. then followed by a slow and separate waving of the flared pectorals (Photo credit: Oona Lönnstedt,[13]).

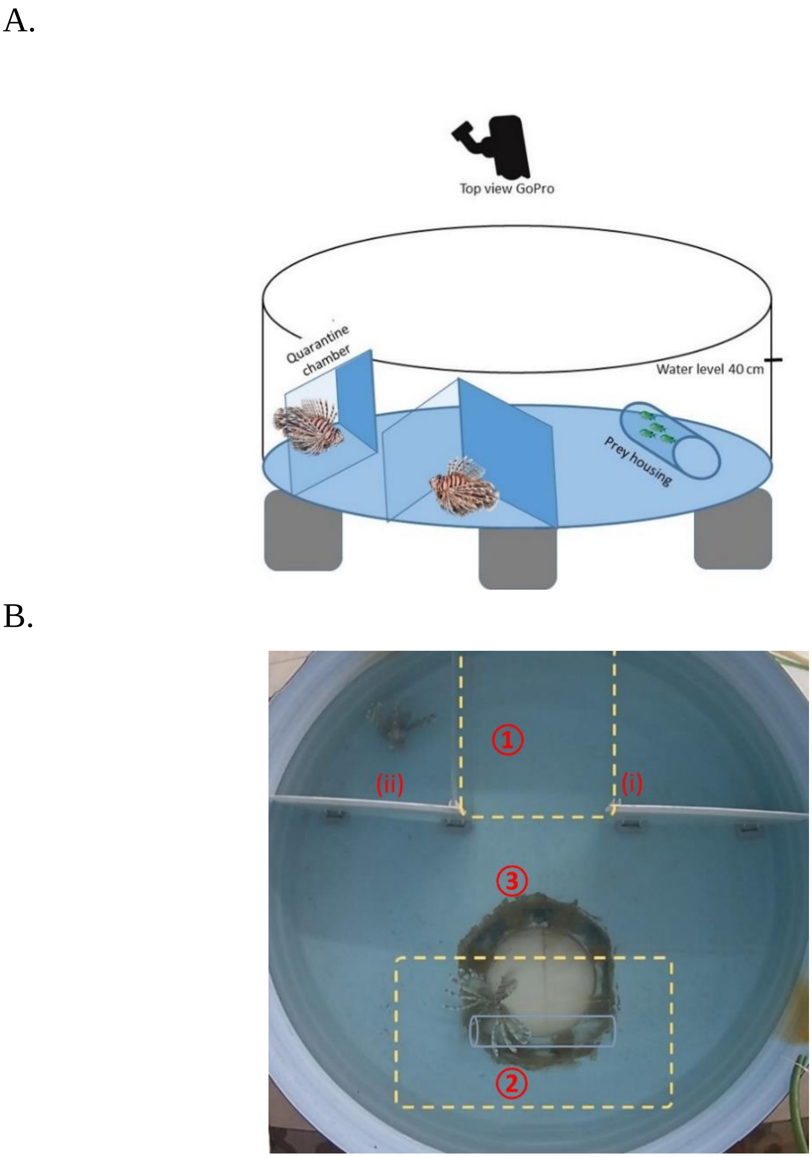


Figure 2: Tank setup to test recruitment tendency of lionfish. A. an illustration of the initial tank structure prior the experiment. B. A life shot of the zone references after focal fish were released, and the quarantined partner has no information about the existence of prey, where (i) focal chamber, and (ii) partner chamber, and zones (1) partner zone, (2) hunting zone -the transparent prey housing is edited with grey border for reader’s visibility, and (3) neutral zone. The round circle located in the hunting zone is dedicated to further studies.

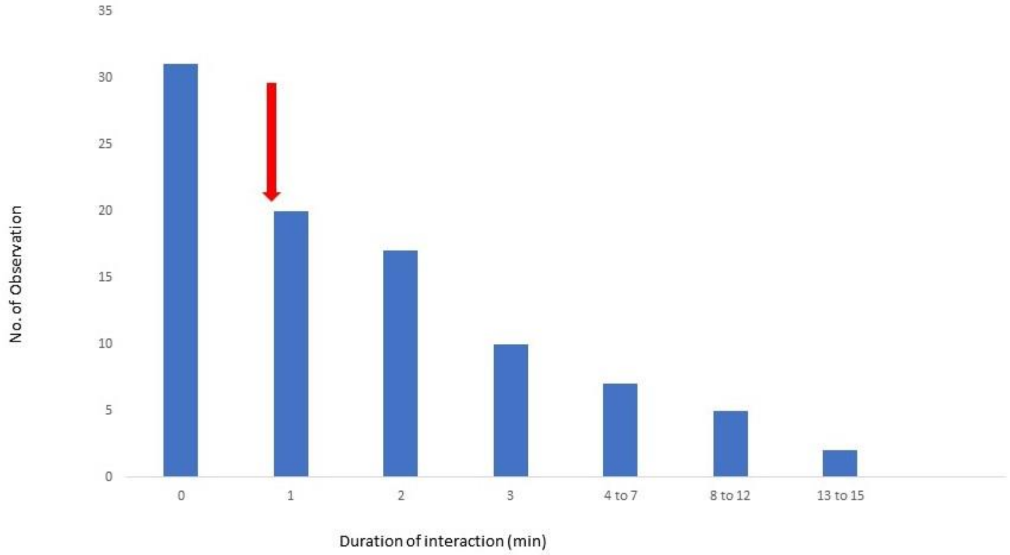
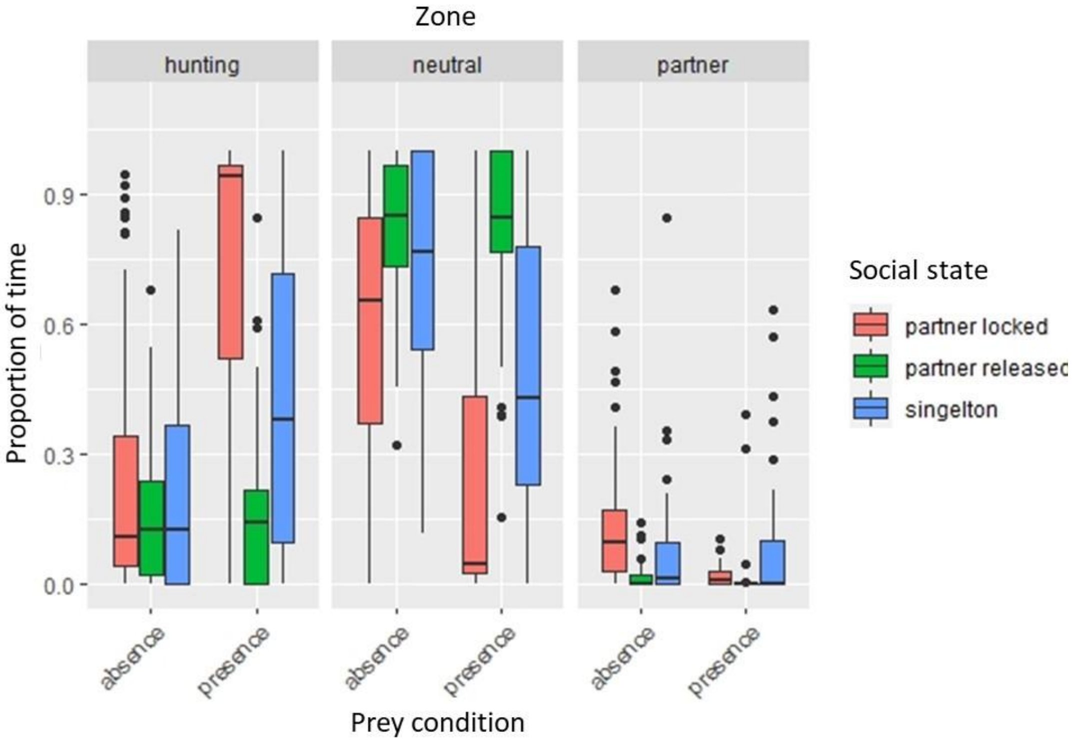
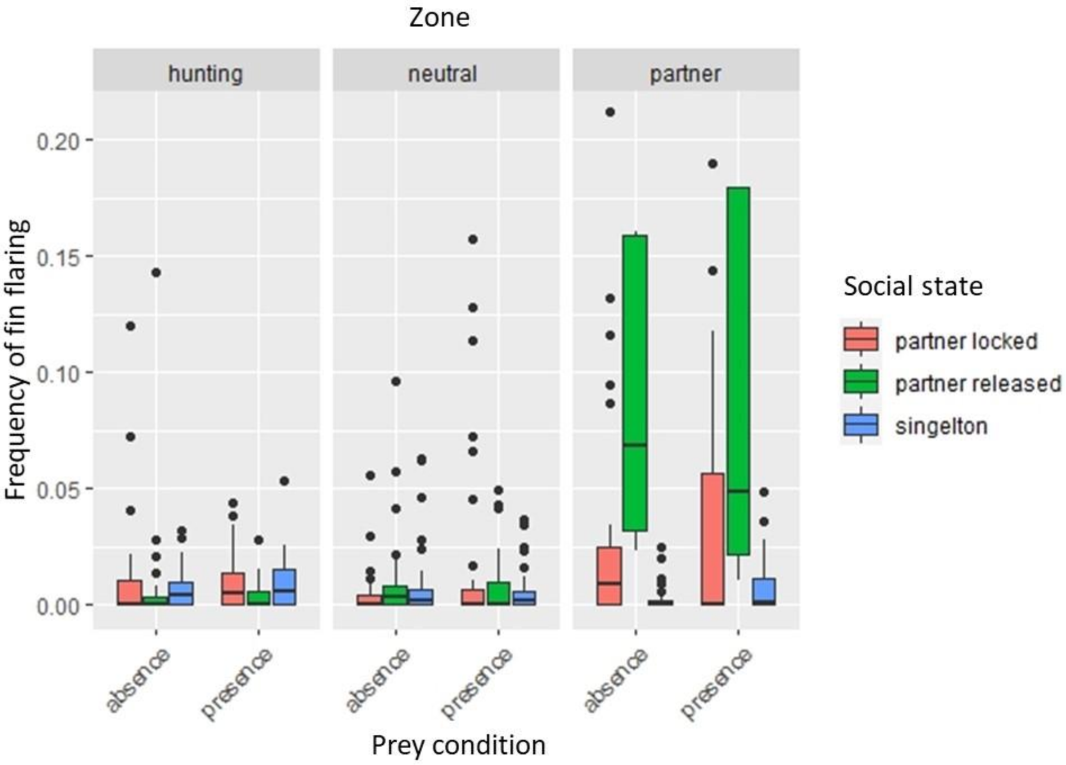


Figure 3. Observed frequency distributions of durations (min) of associations between lionfish conspecifics. The x-axis shows different time categories that were grouped in a non-linear fashion. The arrow almost above the 1 min time category indicates the average duration of associations (59 s) predicted by a null model, assuming independent movements of individuals observed.

A.


Zone
hunting
neutral
partner
Proportion of time
0.0
0.3
0.6
0.9
absence
presence
absence
presence
absence
presence
Prey condition
Social state
partner locked
partner released
singelton

B.


Zone
hunting
neutral
partner
Frequency of fin flaring
0.20
0.15
0.10
0.05
0.00
absence
presence
absence
presence
absence
presence
Prey condition
Social state
partner locked
partner released
singelton

C.

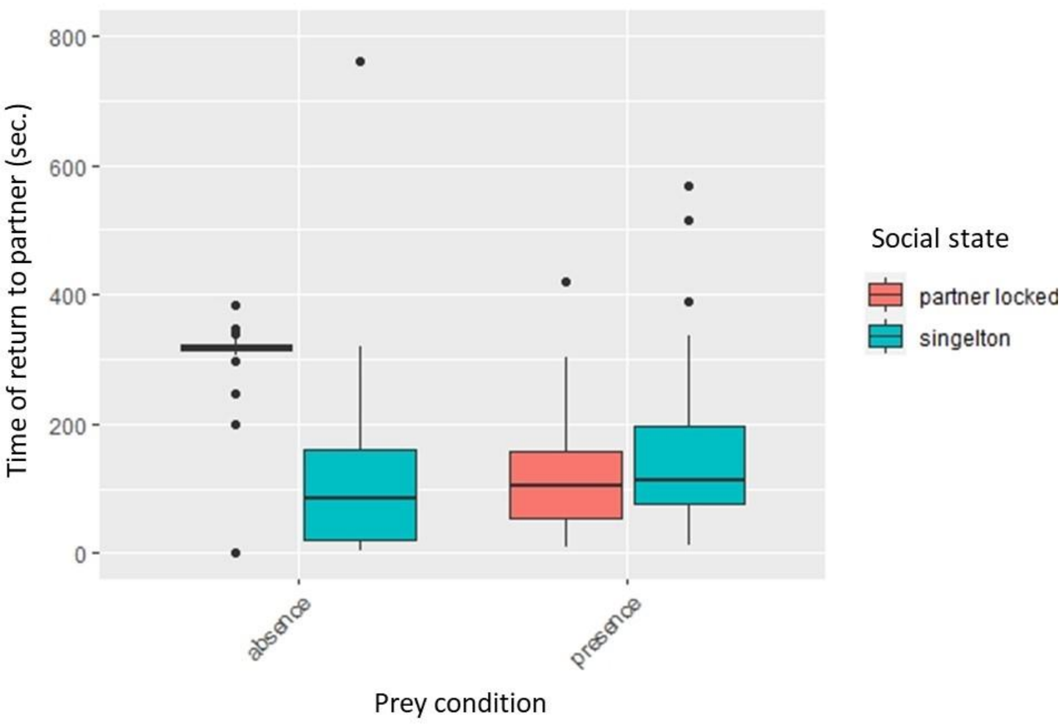


Figure 4. Experimental results. (A) the frequency of fin flaring patterns and (B) proportional space use; both variables as a function of location (hunting, neutral and partner zones) and absence/presence of prey. (C) The time in seconds) it took subjects from being released to return to the partner zone, depending on whether prey was

CHAPTER 2 | NO EVIDENCE THAT LIONFISH *PTEROIS MILES* COORDINATE AND RECIPROCATE DURING HUNTING

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Status: Submitted 2021.

Abstract

Decision rules underlying cooperative hunting can range from very simple to very complex. As lionfishes are naturally solitary hunters; an experimental study documenting active recruitment, coordination and alternating (potentially reciprocal) striking in dwarf lionfish *Dendrochirus zebra* received major attention. A hypothesis was that sophisticated coordinated hunting may also contribute to the successful invasion of another lionfish species, *Pterois miles,* in the Caribbean. However, we did not find recruitment signaling in *P. miles* in parts of its native range, the Red Sea. Here, we expand on these results, testing for coordinated movements and for alternation in strikes**.** We exposed subject pairs to inaccessible prey in three transparent housings. The two lionfish did not aggregate at the same prey housing or even share larger space units in the presence of prey. In a second experiment, we found that some alternation can be induced if prey items become alternatingly accessible at two corners, with each lionfish tending to monopolize one corner each. When the movement of prey is slow or even absent, we observed less alternation than expected by chance. In conclusion*, P. miles* in the Red Sea does not use any coordination to hunt prey.

Keywords: cooperative hunting, coordination , alternation, lionfish.

2.1. Introduction

Cooperative hunting occurs when two or more individuals interact in a hunt that produces higher payoffs per individual than solitary hunting (1). Coordinated cooperative hunting, in which individual predators respond in time and space with each other's actions, is considered to be a complex form of cooperative hunting (2,3). It has been observed and described in primates (4,5), carnivores (3,6,7), marine mammals (2,3,8), birds (9), and fishes (10). In its most complex form, a collaborative hunting, individuals adopt specific hunting roles to herd and catch their prey (2,3,6). Complexity can be further increased if hunting partners actively communicate with each other, for example to initiate a hunting event (4,10).

Given that complex forms of cooperative hunting are rare and proposed to be linked to specific ecological challenges, one would not expect evidence for complex cooperative hunting in predators like lionfish, which typically hunt alone (11–13), though they have been rarely observed to hunt in the presence of conspecifics lionfishes (14) or moray eels (15). However, the hunting behavior of lionfish *Dendrochirus zebra* (Scorpaenidae: Pteroinae) described in laboratory experiments (16) was very sophisticated. More precisely, *D. zebra* subjects swam away from an inaccessible shoal of prey fish (behind a glass barrier) to join a partner initially out of sight. There, subjects repeatedly showed a stereotyped fin flaring pattern that was interpreted as a recruitment signal to elicit joint hunting with both conspecifics and another lionfish species *Pterois antennata*. The signal recipient would join the signaler and return to the prey that was made accessible by the experimenter. Both lionfish would then simultaneously herd prey by spreading own pectoral fins, and alternate feeding strikes at the shoal (16). The alternation of strikes was interpreted as reciprocal acts to ensure sharing of prey in a cooperative task. While *D. zebra* only occurs in the Indo-Pacific, *P. antennata* is one of the two Pterois species invasive to the Caribbean (17,18). It was therefore hypothesized that cooperative hunting may be an important part of lionfish invasion success (16).

While concerns were raised on the credibility of the published data as sample sizes were lower than declared (19) and attempts to hide this fact with a collage of pictures (20), knowing the hunting strategies of Pterois species may potentially contribute to understand their invasion success in the Caribbean, and more recently the Mediterranean (21–23). In a first study on *Pterois miles* in the Red Sea, no evidence was found that individuals seek each other's' presence in the field (13). Furthermore, the stereotyped fin flaring movement pattern was also observed in *P. miles* but it was not associated with signaling situations. The authors concluded that fin flaring is linked to maneuvering in tight places (13), which would also have applied to the area in front of the partner in the study by Lönnstedt et al. (16).

Here, we expand on (13) and investigate two main questions using laboratory experiments. First, we asked to what degree two *P. miles* individuals coordinate in space and time when exposed to three alternative hunting locations, i.e. glass aquaria of identical sizes spread out inside the larger lionfish holding tank, each containing four prey fish. If *P. miles* individuals seek cooperative hunting, we predicted that pairs would aggregate at the same prey aquarium more than expected by chance. Second, we investigated to what extent and under which conditions two *P. miles* individuals alternate striking at prey. Lönnstedt (16) had interpreted the alternation as reciprocity, i.e. individuals deciding to alternate as a cooperative solution to the competition over resources. We tested this hypothesis of reciprocity against an alternative hypothesis that alternations might be linked to the laboratory setting imposing constraints on monopolisation. More specifically, in a rectangular tank as used by (16) it is conceivable that prey might aggregate in one corner when facing two lionfish, where the nearest lionfish conducts the first strike. The strike might make the surviving prey flee into the adjacent corner, where now the other individual is nearest and hence will strike, which will make surviving prey return to the first corner, and the circle restarts. Thus, alternation could be due to prey behavior rather than based on reciprocity between lionfish. In order to test our hypothesis versus the reciprocity interpretation indicated by the alternation of feeding strikes and equality of food acquisition outcome, we created a 'feeding tree', a hand-held stick with several branches and a piece of food items (fresh tuna or prawn) on each end. The experimenter moved the stick through the aquarium, making single food items at a time accessible at specific locations in various pre-defined patterns that either hindered or facilitated monopolization by one fish. According to the reciprocity interpretation introduced by (16) , our manipulations should not affect the alternation pattern of strikes (e.g. the equality and turn taking between individuals to take food items). Alternatively, some movement patterns should yield asymmetries in foraging success while other promote alternating strikes due to imposed constraints on individual monopolization.

2.2. Material and methods

2.2.1. Spatial coordination

Fish handling and experiment setup: the coordination experiments were conducted in the Open Ocean Research Center (OOSC) located in Dahab, South Sinai, Red Sea, Egypt (28.5091° N, 34.5136° E), from May to June 2021 (Figure 1. A). Fish capturing, handling and acclimatizing process was performed carefully to minimize stress responses and to provide a neutral environment for the captured individuals. For lionfish capture, we built a plastic net fixed to a hand net frame (40cm diameter) and gloves and captured 46 individuals varying in size from 20 cm to 29 cm total lengths via scuba diving with a slow approach from depths of 0 to 10 m. Individuals were brought slowly to the surface to avoid air bladder inflation. We assigned individuals directly into single pairs in each round tank and left to acclimatize for 10 days. The round experiment tank was 153 cm diameter x 60 cm high with a water level of 40 cm. Starting from the third night, individuals were fed twice during the crepuscular time 2 pieces of dead shrimps/individual, while during experimental days, individuals were fed only after trials to ensure that they were motivated to hunt during trials. Blue-green Chromis (*Chromis viridis*, Figure 1. C) were captured in parallel to present them as potential prey to lionfish. For Blue-green Chromis, we caught 25 individuals with average size of 4 cm using a barrier net of 1.5 x 1.5 m and 0.5 x 0.5 cm mesh size. Chromis were kept in a single aquarium (70 x 40 x 40 cm) and left to acclimatize for 7-10 days. An artificial coral block and small PVC tubes were added to serve as shelters and blue background was placed all around the aquarium. Fish were fed twice per day with commercial flakes, food residuals were removed daily. We kept each species in a separate seawater system where aquaria were filled two days before fish accommodation, provided with seawater open system flow (2L per minute), an air pump to provide appropriate aeration, and water submersible pumps to provide current. All aquaria were cleaned once a day to prevent biotic accumulation. All fish were released at sites of capture after completing the experiment.

Experimental protocol: The lionfish partners were first pushed slowly into an opaque compartment and isolated there for 15 min, while setting up the experiment. Three prey housing glass aquaria (30x 15x 25 cm) were placed in the tank with 45 cm space in between to allow subjects to move freely around them (Figure 2. A, B). After the prey housings were installed and prey individuals were assigned randomly to the prey housings (Figure 2. A), we let the fish settle for 10 minutes. Then, the partners were released simultaneously by lifting the opaque compartment, and filmed for 10 min from above. Each lionfish pair was tested three times in each of the two conditions: 1) general prey absence and 2) four prey individuals per housing aquarium. For data collection and analysis purposes, we divided the tank into four *zones:* three separate hunting zones around each of the three prey housing aquaria, and a fourth 'neutral zone' away from prey (Figure 2B).

2.2.2. Alternated strikes

Fish handling and experiment setup: the alternation experiments were conducted in Hurghada grand aquarium, Hurghada (27.1337°N, 33.8217°E) in March 2022. Lionfish sample individuals were collected in 2020-2022 and kept in the exhibition and in the hold tanks. Fishermen used cages to capture lionfish individuals to avoid harmful contact, and transferred to the aquarium hospital tanks to be treated from potential injuries or infections, then let to be acclimatized. After recovery, individuals were fed pieces of fresh tuna (1cmx 1cm) twice a day by throwing food items randomly in the tank. 24 Lionfish individuals assigned to be tested were transferred in pairs to the round experimental tanks (153 cm diameter x 60 cm high, with a water level of 40 cm) one week prior to the experimental trials, matching individuals in size as much as possible. Training consisted of moving our ‘feeding tree’, a hand-hold main stick with three or four branches to attach food items (Figure 3), randomly in the tank, so that the two lionfish could simultaneously access it and hence compete over food. Within 3-4 days, all individuals followed the stick and ate food items off it. In this way, by moving the food tree selectively, individuals were fed twice during the crepuscular time periods 2 pieces of fresh tuna/individual. On the fifth day, experimental trials started.

Experimental protocol: Like in the previous experiment, lionfish pairs were first confined to one side of the tank, behind an opaque barrier. We then placed a transparent barrier (100x 60 cm) on the opposite side, creating an inaccessible compartment. That barrier had three vertical cuts 20 cm long and 3 cm wide, 30 cm apart from each other (Figure 3). The experimenter could slide side arms of the feeding tree holding uni-sized pieces of tuna at pre-determined schedules (time and location) through these cuts such that they became accessible to lionfish during trials. We conducted a single final training round in which three items were offered successively through each of the three slits, with the order counterbalanced across pairs. We did not score how many items each individual ate during that training. Then, the actual experiments began, consisting of three different treatments. As we were interested in the subjects’ spontaneous behavior, each pair was tested only once in each treatment. The order of treatments was counterbalanced across pairs to avoid that potential sequence effects could influence overall results. During the first two treatments, four items became successively accessible through the two side cuts. Crucially, accessibility alternated between the two cuts: as soon as one item was eaten on one side, the feeding tree was retracted and moved to the other cut, and moved back once a second item had been eaten, and finally again moved for the fourth food item to become accessible. The difference between the two treatments was the time the experimenter took to move the tree from one cut to the other: 5s versus 15s. The third treatment involved a feeding tree with only three branches and hence three food items. The branches were invariably introduced to the lionfish side through the middle cut, with all three branches being simultaneously accessible. In all three treatments, a trial started by having the feeding tree in its first accessible position and then lifting the opaque partition to release the partners simultaneously. A trial ended in principal when all pieces had been eaten. Only once, individuals had not eaten all items after 180s exposure, and the feeding tree was removed. This data point was removed from the analysis. We recorded the order of attacks by individuals with a Gopro Hero+3 camera attached to the tank side.

2.3. Statistical analysis

All statistical analyses were conducted with RStudio Inc., Version 1.2.5033, and Videos were analyzed using BORIS software v7.9.7-2021. All the data are available as supplementary material on Dryad: https://doi.org/10.5061/dryad.g79cnp5s8.

We aim in the first experiment to investigate the level of coordination of lionfish in space and time to hunt their prey. For the analyses on spatial coordination, we extracted the following information separately for trials in which prey was present and trials in which prey was absent. A) Time in seconds from release until the two fish were in the same hunting zone for the first time. B) The time in seconds they would spent together in that zone before separating. C) The overall time in seconds spent together in the same hunting zone, and overall time spent together in the neutral zone. The first two data sets were directly compared between trials in which prey was present and trials in which prey was absent. For the third data set, we first quantified the time each individual spent in each of the four zones. The data allowed us to calculate an expected value for the two fish being simultaneously in each of the four zones based on the null hypothesis that the two fish move independently of each other (Equation 1). We then added up the three observed values for simultaneous presence in each of the three hunting zones, and we added up the three expected values, for a comparison. We also compared observed and expected values of co-occurrence in the neutral zone. Due to the repeated measures, pair IDs were added as a random variable. In general, we applied a linear mixed-effect model to investigate how treatment affected our variables of interest (nlme: “lme”).

Equation (1)

$$Expected\ duration\ to\ overlap\ in\ zone\ in\ seconds = \left(\frac{observed\ in\ zone\ .ind\ 1\ in\ sec.}{trial\ duration\ in\ sec.}\right) \times \left(\frac{observed\ in\ zone\ .ind\ 2\ in\ sec.}{trial\ duration\ in\ sec.}\right) \times trial\ duration\ in\ sec.$$

To test whether lionfish alternate strikes in the second experiment, we scored the number of switches in each trial (0-3 possible during the first two treatments, 0-2 possible during the third treatment). Then we calculated the difference between the observed alternation count and expected alternation value based on the null hypothesis that individuals feed at random. To calculate the expected value of the null hypothesis that individual success is random for each item presentation, we divided the maximal possible number of switches for each trial by 2. As the first two treatments involved four food items, there were three occasions on which the successful individual could change or remain the same, each event with 50% probability. Hence, for the first two treatments (5 sec. and 15 sec intervals), the expected value of the null hypothesis was 1.5 alternations, for the third treatment (steady) it was 1 alternation. As we faced many ties between observed values and the null hypothesis, we used a simple Sign Test. We calculated the positive and negative ranks, which were used to extract the test statistics and *p* value.

2.4. Results

2.4.1. Spatial coordination experiment

We had 230 min video material for 23 pairs. In the presence of prey, it took individuals longer to join each other for the first time in one prey zone (i.e. focusing on the same prey holding aquarium) compared to the treatment in which prey was absent (Presence: mean= 55.68± 10.76 sec., and Absence: mean = 17.57± 5.42 sec., Chi^2=4.354, *df*= 1, *p*= 0.037, Figure 4.A). Once together, pairs tended to stay for longer in the same hunting zone when prey was present (Presence: mean= 14.33±6.42, and Absence: mean= 4.50± 2.33, *Chi²*= 3.63, *df*= 1, *p*= 0.056, Figure 4.B).

Overall, however, lionfish either ignored or tended to avoid each other during the trials. On a purely descriptive level, expected mean values of being together were higher than observed mean values in all four conditions (hunting zones/neutral zone x prey present/prey absent). The full model revealed a significant effect of location (Chi²= 24,6, df= 1, $p < 0.0001$) but not of prey presence/absence (Chi²= 0.01, df= 1, p= 0.91), and no interaction between the two factors (Chi²= 0.942, df= 1, p= 0.331, Figure 4.C). Post hoc tests revealed the lionfish pair generally spent significantly less time together than expected in the neutral zone (prey present: t ratio= -5.27, $p < 0.0001$; prey absent: t ratio= -4.11, p = 0.0001). In the presence of prey, expected and observed values did not differ significantly (prey present: t ratio= -1.22, p = 023; prey absent: t ratio= -1.97, p = 0.054).

2.4.2. Alternated strikes experiment

Due to the deliberately short training period, we did not observe individuals focusing on any food insertion location. We did record that lionfish would distribute or separate themselves such that each individual is waiting at each slit in any of the 12 pairs. Between the insertion's duration, both partners followed the movement of the feeding tree on the other side of the transparent barrier. As a result, both individuals where typically near the feeding tree when a food item was made accessible through a slit. Keeping up with the food tree movement was simply more challenging when the interval was 5s rather than 15s.

Using 12 pairs, we generally found that individuals did not alternate their strikes above chance levels. The amount of alternations apparently depended on the time interval between successive presentations during the first two treatments. The 5s interval treatment yielded a random pattern (Sign-Test, N=12, X=6, p = 1). In contrast, the 15s treatment yielded significantly less alternations than expected by chance (Sign-Test, N= 12, X=2, p=0.038, Figure

5.). Finally, in the steady treatment, observed alternations occurred less than expected by chance but not significantly so (Sign-Test, N=12, 4 ties, remaining N=8, X=2, p = 0.29, Figure 5).

2.5. Discussion

We had aimed to test whether lionfish *P. miles* inhabiting the Red Sea expressed any evidence of hunting in a coordinated way as a first step towards assessing the hypothesis that such coordination may contribute to their successful expansion in their invasive range in the Caribbean Sea. We did not find any evidence for coordination in time and space, nor for alternation in hunting strikes. Thus, we failed to replicate results reported on another lionfish species *D. zebra* in Australia. As a consequence, the literature on cooperative hunting and potentially underlying cognitive mechanisms, including signaling, coordination, or reciprocity, does not apply to P. miles in the Red Sea.

Regarding coordination in space and time (first experiment), the presence of prey delayed lionfish pairing up in the same prey zone. While they then stayed together for longer than in the control, this result was apparently driven by both being independently attracted to the prey. If lionfish coordinated in space and time, we would have expected that they spent overall more time together in the same zones than expected by chance. If anything, the data suggest that lionfish tend to avoid each other and hence prefer to hunt alone. This result aligns with pre-existing field observation reporting that lionfish *P. miles* in nature are mostly solitary hunters (12,17,25). It contrasts with the described coordination of movements in *D. zebra* (16). Note, however, that the data are not directly comparable as only we offered alternative prey locations and quantified each individual's location over the entire trial.

Given that our lionfish did not coordinate in time and space, it may not be surprising that they did not alternate their strikes at prey as reported for *D. zebra* (16). Importantly, one experimental design caused more alternating strikes, i.e. when fast movement of the feeding tree (aka prey shoal) from one end of the separation to the other allowed each individual to focus on a different gap. Thus, prey movement patterns could in principle cause alternating strikes in confined aquarium space, especially as prey would move faster between locations than our feeding tree. Unfortunately, the experimental videos of Lönnstedt et al. (16) are not available. Otherwise, re-analysing the videos would reveal whether prey indeed moved back and forth between corners, whether they thereby alternated their proximity to the two lionfish, and hence whether prey movement caused the reported alternations in strikes.

In summary, our previous (13) and the current study on *P. miles* fail to reproduce results on *D. zebra* (16), finding no evidence for active recruitment, coordination in time and space, or reciprocity-like alternation in strikes. Thus, lionfish are not in general sophisticated cooperative hunters. We therefore consider it parsimonious to assume that the success of the genus *Pterois* in the Caribbean (16, 17) is linked to other features already documented.

2.6. Acknowledgments

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2.7. Author contributions

Both authors designed the study, HS collected all data and wrote the first draft, which was finalized by RB.

2.8. Ethics

The ethics and research permit were granted by Ras Mohamed National Park office (RMNP), and Hurghada Environmental and Protection and Conservation Association (HEPCA).

2.9. Funding

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2.10. Reference

1. Packer C, Ruttan L. The evolution of cooperative hunting. Am Nat. 1988;132(2):159–98.

2. Gazda SK, Connor RC, Edgar RK, Cox F. A division of labour with role specialization in group–hunting bottlenose dolphins (Tursiops truncatus) off Cedar Key, Florida. Proc R Soc B Biol Sci. 2005;272(1559):135–40.

3. Bailey I, Myatt JP, Wilson AM. Group hunting within the Carnivora: physiological, cognitive and environmental influences on strategy and cooperation. Behav Ecol Sociobiol. 2013 Jan 1;67(1):1–17.

4. Boesch C, Boesch H. Hunting behavior of wild chimpanzees in the Tai National Park. Am J Phys Anthropol. 1989;78(4):547–73.

5. Stiner MC, Barkai R, Gopher A. Cooperative hunting and meat sharing 400–200 kya at Qesem Cave, Israel. Proc Natl Acad Sci. 2009;106(32):13207–12.

6. Stander PE. Cooperative hunting in lions: the role of the individual. Behav Ecol Sociobiol. 1992;29(6):445–54.

7. Creel S, Creel NM. Communal hunting and pack size in African wild dogs, Lycaon pictus. Anim Behav. 1995;50(5):1325–39.

8. Chivers DP, Smith RJF. Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. Ecoscience. 1998;5(3):338–52.

9. Griffith SC. Cooperation and Coordination in Socially Monogamous Birds: Moving Away From a Focus on Sexual Conflict. Front Ecol Evol [Internet]. 2019 [cited 2022 Jun 22];7. Available from: https://www.frontiersin.org/article/10.3389/fevo.2019.00455

10. Bshary R, Hohner A, Ait-el-Djoudi K, Fricke H. Interspecific communicative and coordinated hunting between groupers and giant moray eels in the Red Sea. PLoS Biol. 2006;4(12):e431.

11. Albins MA, Hixon MA. Worst case scenario: potential long-term effects of invasive predatory lionfish (Pterois volitans) on Atlantic and Caribbean

coral-reef communities. Environ Biol Fishes. 2013 Nov;96(10–11):1151–7.

12. Albins MA, Lyons PJ. Invasive red lionfish Pterois volitans blow directed jets of water at prey fish. Mar Ecol Prog Ser. 2012;448:1–5.

13. Sarhan H, Bshary R. No evidence for conspecific recruitment for cooperative hunting in lionfish Pterois miles. R Soc Open Sci. 2021;8(9):210828.

14. Rizzari JR, Lönnstedt OM. Cooperative hunting and gregarious behaviour in the zebra lionfish, Dendrochirus zebra. Mar Biodivers. 2014;44(4):467–8.

15. Naumann MS, Wild C. Foraging association of lionfish and moray eels in a Red Sea seagrass meadow. Coral Reefs. 2013;32(4):1111–1111.

16. Lönnstedt OM, Ferrari MCO, Chivers DP. Lionfish predators use flared fin displays to initiate cooperative hunting. Biol Lett. 2014 Jun;10(6).

17. Côté IM, Smith NS. The lionfish *Pterois* sp. invasion: Has the worst-case scenario come to pass?: RE-EVALUATING THE LIONFISH INVASION. J Fish Biol. 2018 Mar;92(3):660–89.

18. Darling ES, Green SJ, O'Leary JK, Côté IM. Indo-Pacific lionfish are larger and more abundant on invaded reefs: a comparison of Kenyan and Bahamian lionfish populations. Biol Invasions. 2011 Sep;13(9):2045–51.

19. The Biology Letters Editorial Team. Expression of concern: Lionfish predators use flared fin displays to initiate cooperative hunting. Biol Lett. 2018 Feb 28;14(2):20180032.

20. Journal stands by paper facing misconduct allegations, but expresses concern about related images [Internet]. [cited 2022 Dec 8]. Available from: https://www.science.org/content/article/journal-stands-paper-facing-misconduct-allegations-expresses-concern-about-related

21. Kletou D, Hall-Spencer JM, Kleitou P. A lionfish (Pterois miles) invasion has begun in the Mediterranean Sea. Mar Biodivers Rec. 2016 Jun 30;9(1):46.

22. Johnston MW, Purkis SJ. Are lionfish set for a Mediterranean invasion? Modelling explains why this is unlikely to occur. Mar Pollut Bull. 2014 Nov 15;88(1):138–47.

23. They are here to stay: the biology and ecology of lionfish (Pterois miles) in the Mediterranean Sea - Savva - 2020 - Journal of Fish Biology - Wiley Online Library [Internet]. [cited 2022 Jun 16]. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/jfb.14340?casa_token=v69ipxLTNt4AAAAA%3A2ZgYKhQEQh8b9abR7ity-Yyw0Y-cIVHLAXTlpDE13NitiOZZOrVEBLApjokRo2zpxmFkonffprz3E2M

24. Pritschet L, Powell D, Horne Z. Marginally Significant Effects as Evidence for Hypotheses: Changing Attitudes Over Four Decades. Psychol Sci. 2016 Jul 1;27(7):1036–42.

25. Dahl KA, Patterson WF. Habitat-Specific Density and Diet of Rapidly Expanding Invasive Red Lionfish, Pterois volitans, Populations in the Northern Gulf of Mexico. Layman CA, editor. PLoS ONE. 2014 Aug 29;9(8):e105852.

2.11. Figures

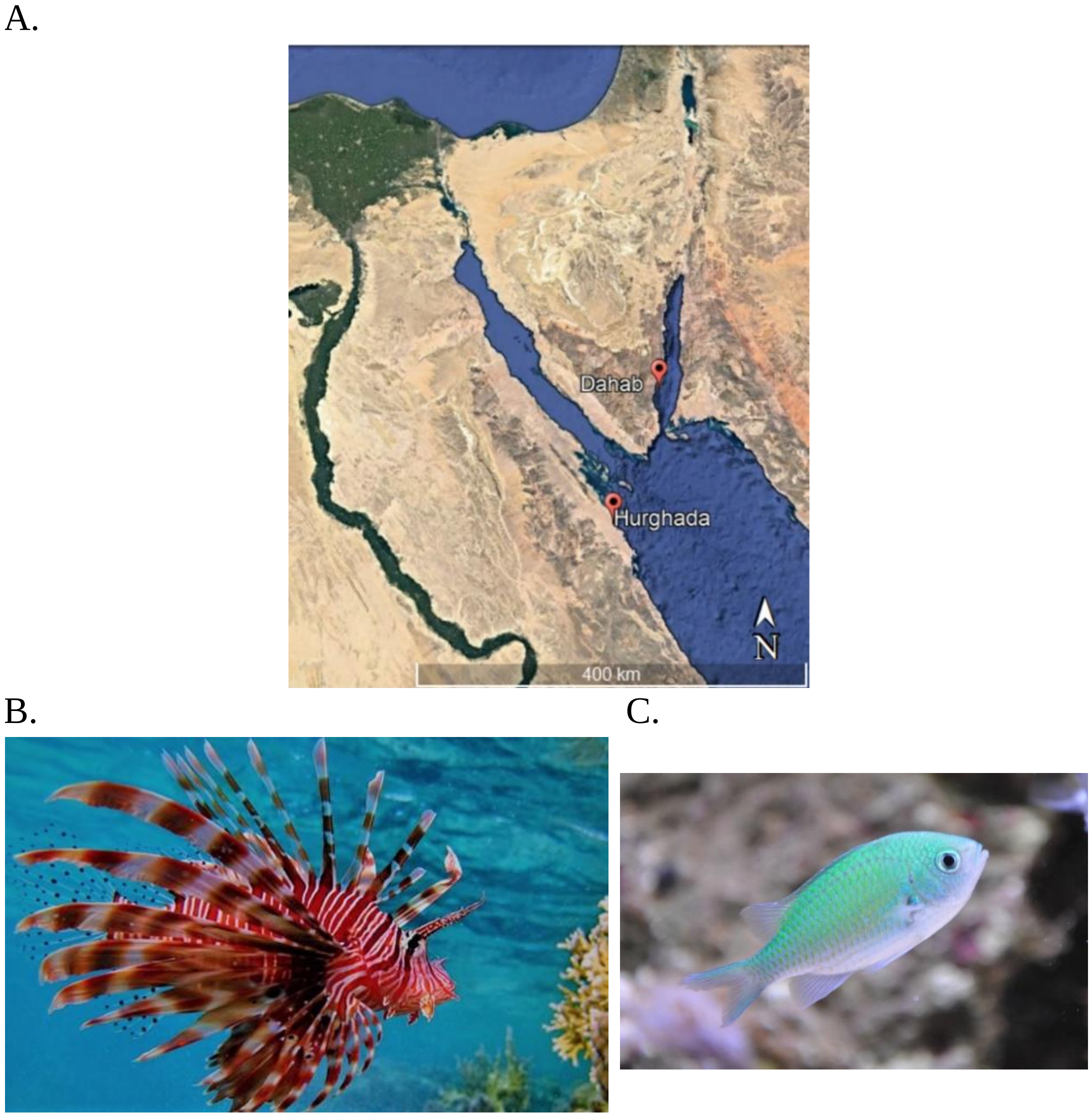


Figure 1. (A) Location of the conducted study in the northern Red Sea region. Image modified from Google earth v.6.2 (2021). (B) The study species *P. miles*. (Photo credit: Hanaa Sarhan). (C) The prey species *Chromis viridis* provided as bait during experiment trails. (Photo credit: Lubomír Klátil)

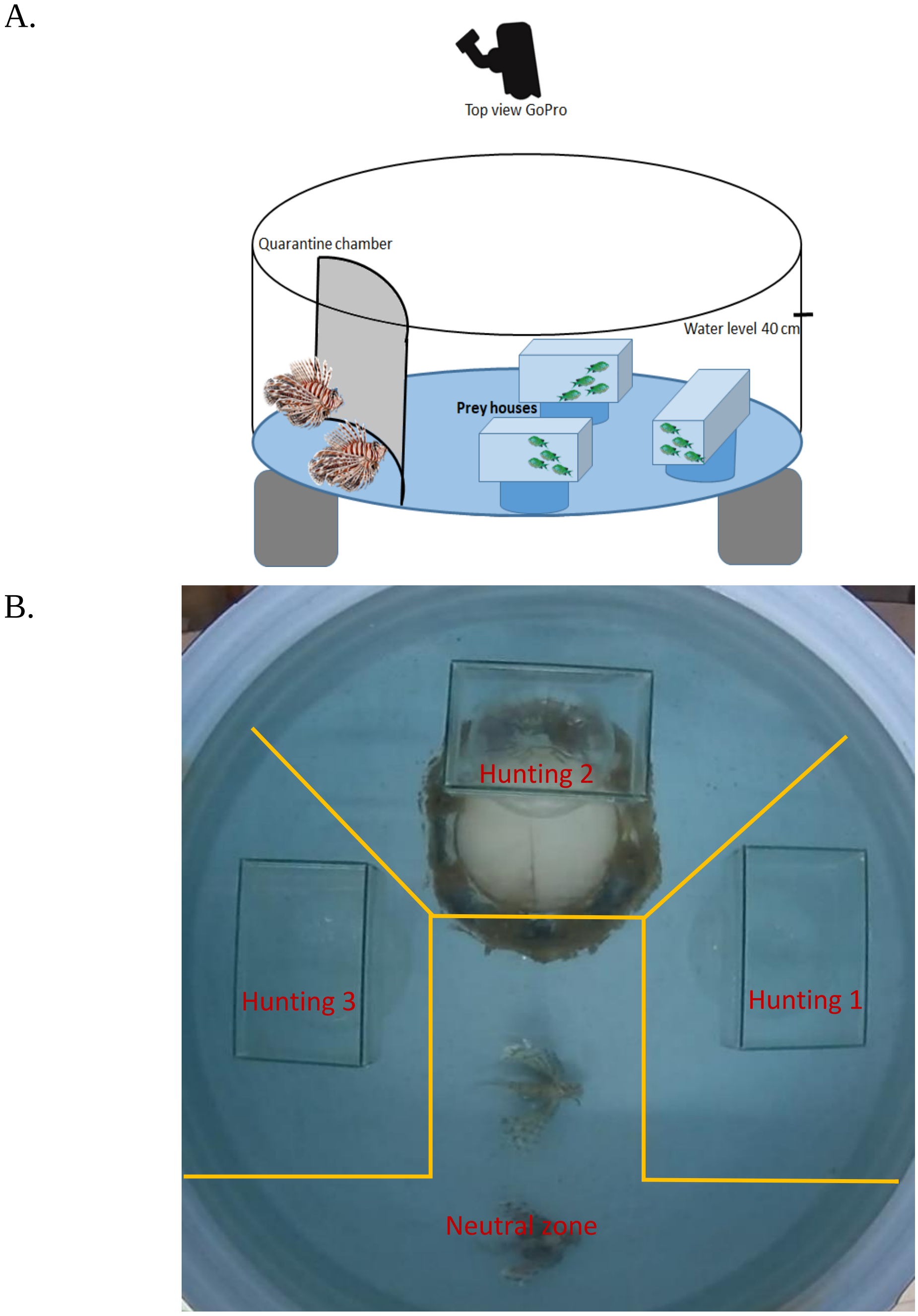


Figure 2: Tank setup to test the coordination of lionfish partners. (A) Illustrating the tank structure and locations of installments before starting the trial. (B) Live photo of experiment zones in control condition after partners were released from the quarantine.

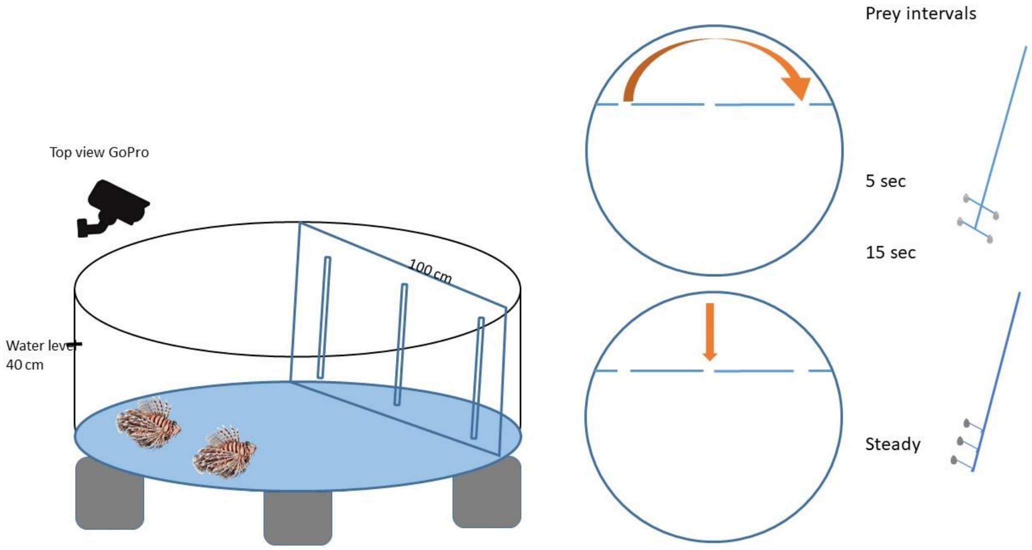
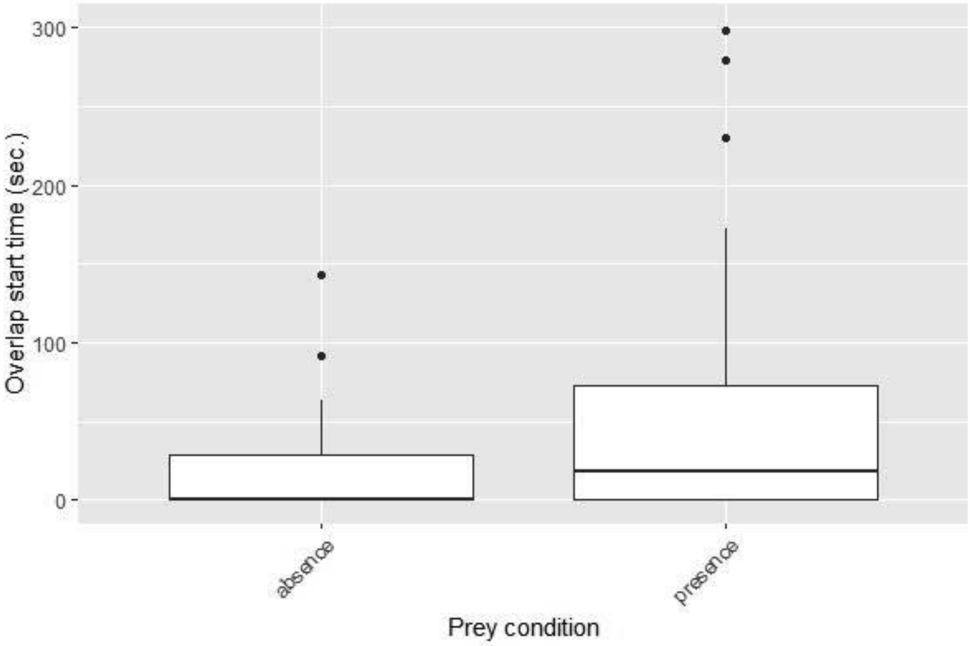
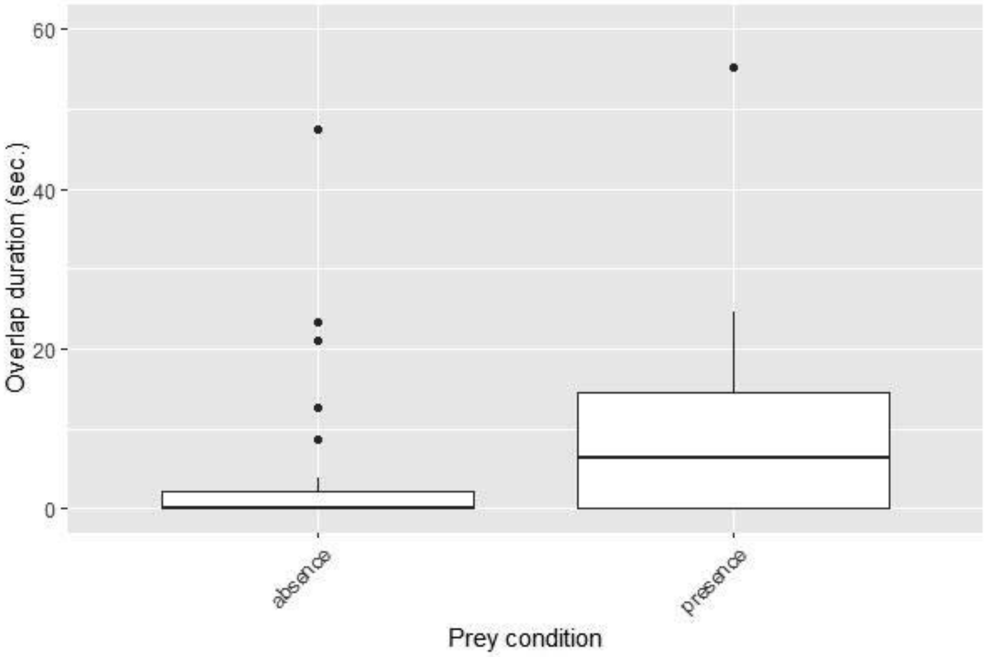


Figure 3. Tank setup and types of bait used to investigate the alternation of lionfish partners. The bait and the three movement pattern assigned for each bait.

A.


300
200
100
0
Overlap start time (sec.)
absence
presence
Prey condition

B.


60
40
20
0
Overlap duration (sec.)
absence
presence
Prey condition

C.

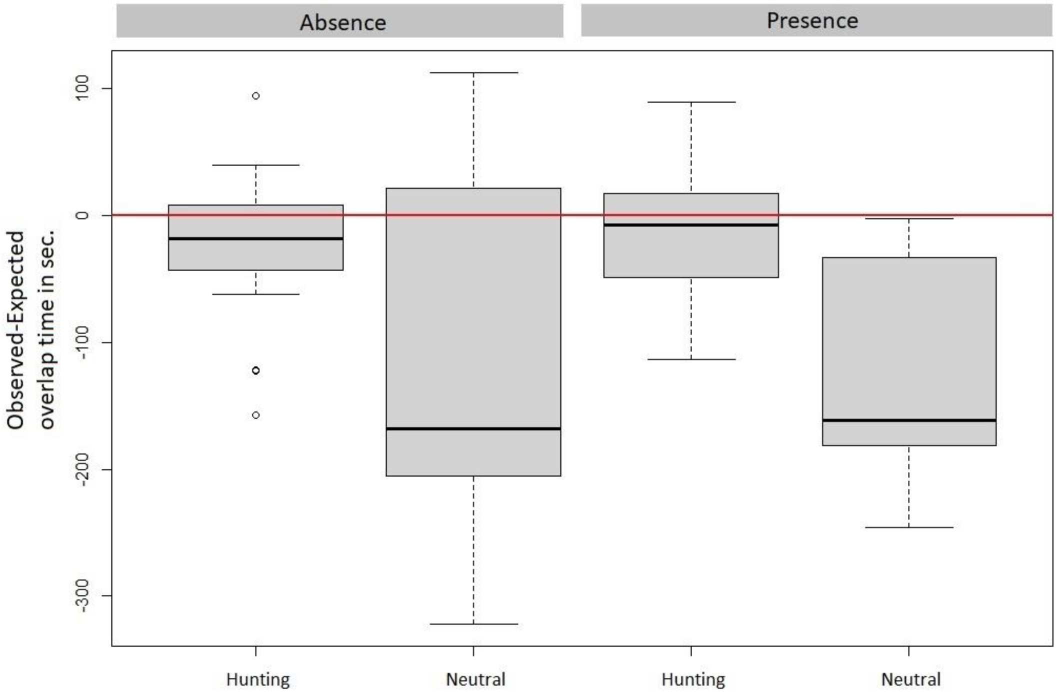


Figure 4. A comparison of time budget spent by *P. miles* in absence and absence of prey conditions. (A) the time spent by individuals till the overlap or associate for the 1st time at any of prey housings. (B) the duration of the 1st overlap by the paired individuals. (C) the expected time to be spent in association in each zone, compared to the actual observed time.

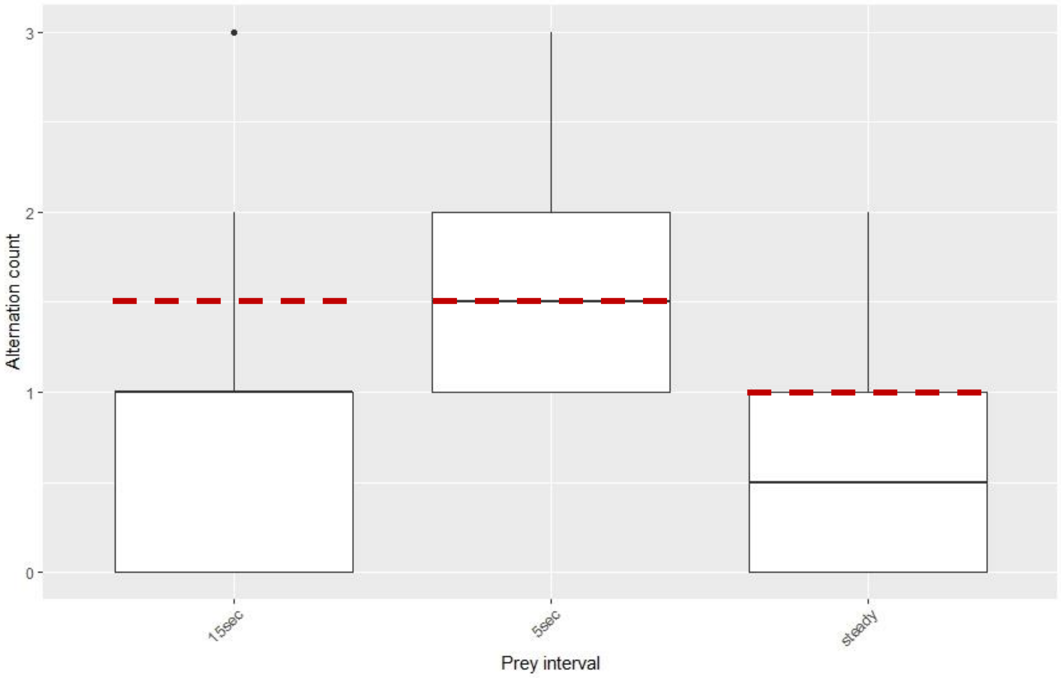


Figure 5. Boxplots of the number of observed alternations of strikes within trials versus the null hypothesis that alternations occur at random (red dashed lines). In the first two treatments, a total of four prey items was offered, alternating between two different locations. In the first treatment, time interval between prey item presentations was 15 s, in the second treatment it was 5 s. In the third treatment, three items were made accessible simultaneously at one location.

CHAPTER 3 | PERSPECTIVES ON PREDATOR INSPECTION IN FISHES

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Status: In Preparation.

3.1. Background

Predation is a major selective force for the evolution of animals' morphological, physiological and behavioural traits. As both sides are under strong selection, predator-prey interactions are often viewed as evolutionary arms races. The importance of predation during evolutionary time is evident; however, animals also have the ability to assess and behaviorally influence their risk of being preyed on during their lifetime (1). Prey needs to use the habitat in a nutrient-efficient way, resolve interaction with competitors, and find suitable mating options (2), but failure in appropriately recognizing predation risks may cause death. Optimizing the balance between predation risk and behavioural activities requires the prey's acquisition of accurate and reliable information about predation risk, and then adjusting to this information (3). An essential part of prey adaptation is hence the evolution of central-nervous systems that allow individuals to obtain, store and use the information for decision-making (4).

Appropriate decisions during direct interactions with predators can enhance trade-off management in various ways. An escalated form of interaction is mobbing of the predator (5). We do not discuss this highly proactive strategy to make predators leave the area. Instead, we focus on the literature on predator inspection behaviour. Following and extending a definition by (6): predator inspection is the approach of a predator or predators by prey that is i) aware that there is an object worthy of inspection; ii) not showing any signs of aggression towards the predator, and iii) returning more or less to its original location prior to the approach. While predator inspection may be widespread in vertebrates (7–10), it has been studied in detail mostly in fishes (6,7,11–16). Therefore, we focus on the fish predator inspection literature.

Predator inspection may have various functions. The most commonly assumed benefit is to obtain information about the risk of a predator attack via the assessment of danger cues (6,11). Suppose for example that certain cues reveal a predator’s hunger state, which in turn correlates with attack probability. In that case, prey could subsequently continue with daily activities if the predator is perceived as neutral or be vigilant and stay close to cover if the predator is perceived as a danger (6,7,11–14,17). Alternatively, prey may approach sit-and-wait predators as a means to signal their detection. This informs the predators that any hunting attempt would be highly likely to fail because the surprise element is gone. Such communication from prey to predator is mutually beneficial as the predator’s best response is to go away to hunt elsewhere, and the prey can hence resume daily activities (18). This form of communication has been termed perception advertisement (19,20). Yet another potential benefit of predator inspections may be that specific individuals approach predators to show their physical quality, either to the predators or to conspecifics. Signals to predators have been termed 'vigour advertisement' that aims at reducing personal attack risk (8). Signals to conspecifics can increase the inspector's 'reputation' that, in turn, may increase, for example, access to mating partners (18,21,22).

Notably, the various potential functions of prey seeking interactions with predators are not mutually exclusive. With this in mind, we evaluate the fish literature on predator inspection behaviour. Prey fish often show peculiar behavioural patterns, i.e. saltatory movements towards a predator, that help to infer that an inspection takes place (6). Furthermore, fish research has focused on shoaling species, where one, two or several individuals leave the shoal to approach a predator (6). Such cases have attracted considerable research because behavioural decisions need to be understood within the cooperation framework between unrelated individuals. As best illustrated with a prisoner's dilemma (23), cooperation may yield benefits that cannot be achieved through isolated individual actions, but cooperators often have to ensure that cheaters do not exploit them. Two aspects are intriguing about predator inspections in shoaling species. First, if inspectors return with information that other shoal members can use, inspectors take an apparent risk of approaching a predator but provide a public good to the shoal. Thus, one has to explain why individuals benefit from inspecting a predator if free-riding and obtaining free information appears to be an alternative option. Second, when two (or more) individuals simultaneously inspect a predator, their relative spatial positions become another issue of cooperation and defection; this is because the individual closer to the predator is likely to incur a greater predation risk than the follower. Therefore, both individuals would prefer to be the follower, which would lead to a 'war of attrition’ (24) and hence to a premature end of a co-inspection unless a solution is found.

In this review, the first part evaluates the existing evidence for the various functional hypotheses: reputation gains, information gains, and communication to the predator by prey fishes (see Figure 1), with examples from laboratory experiments and wild observations. The latter two hypotheses also require understanding predator decision-making regarding attack and retreat. In the second part, we will relate the functional hypotheses to game theory to evaluate what forms of cooperation and conflict underlie predator inspection behaviour. We will show that despite significant research efforts, much crucial information is currently missing for a comprehensive understanding of predator inspection behaviour.

3.2. Functions of Predator inspections:

3.2.1. Prestige communication

In prestige communication, individuals conduct risky behaviours or help others as a means of displaying their quality. Prestige communication is tightly linked to the handicap principle (25), i.e. the idea that only resourceful individuals can provide certain costly signals for a price that is less important than the resulting benefits (26,27). In this context, predator inspection could function like a behavioural peacock’s tail. A very straightforward prediction of the prestige communication hypothesis is hence that males should inspect predators more frequently than females do. If this were the case, one would predict a positive correlation between male physical quality and the frequency of predator inspections and/or the proximity to a predator during an approach. This in turn should be assessed by females and used as a criterion for partner choice. If the showing-off is coupled with important information transfer about the predator state, the scenario would fit the ‘competitive investment’ concept ((22,28), see also (29)).

The hypothesis has been mostly tested in guppies, with rather mixed results. Female guppies are potentially more at risk of predation than males are, due to various predator species apparently preferring their larger size (30,31). As it stands, females shoal more than males, identify possible predators sooner and conduct more predator inspections (30,31). Thus, females largely rely on own actions to manage predation risk (32). Nevertheless, studies demonstrated that female guppies favour males initiating inspections (as described as bolder males (32–35)) over brightly coloured ones when given a chance to witness their inspection behaviour against a prospective fish predator (36). Hence, inspector males may have higher mating success due to female mating preferences, as predicted by the prestige communication hypothesis. Additional studies on mixed-sex groups during predator inspection events showed yet another potential benefit of predator inspections for male guppies: apparently, males tend to join inspecting females to attempt "sneaky matings" rather than to inspect predators (11,37). Thus, the males take advantage of the distracted inspecting females during the inspection trip (38,39), which has hence nothing to do with prestige communication. In conclusion, the prestige communication hypothesis needs further studies on a variety of species for a more thorough evaluation of its merits in the context of predator inspection.

3.2.2. Cue assessment

There is intriguing and suggestive correlational evidence that inspection behaviour is used to gather information about the identity and motivational state of the predator ((40–43), but see also (11,37)), which can reduce the likelihood of future attacks on the shoal. For example, in an experimental test based on a one-way mirror, receiver minnows behind the mirror observed the response of transmitter minnows threatened by a pike model. When the pike model approached the transmitter fish's foraging area, this altered the transmitters’ behavior in a way that made the receiver minnows stop feeding and instead hiding and skittering (40). Most importantly, a comparative experimental study on guppy populations revealed that guppies can discriminate between hungry and satiated predatory pikes, and that the more experienced guppies are the more effective they are in identifying and avoiding the pike (41).

When prey relies just on ***visual cues***, alert or other disruption indicators, the distance between the prey and possible predator and experience with the predator has been found to have a strong association with perceived danger levels. For example, shoals of experienced and naive glowlight tetras were exposed to live cichlids and decreased their inspection rate with decreased distance to the predator. However, the experienced tetras were more sensitive to antipredator responses compared with naive tetras (44). Meanwhile, in minnow populations under unequal predation pressure by pikes, inspection events by transmitters rose when the model distance was reduced (without chemical cues), and inspections by transmitters were closer when in a larger group (6,40). For sticklebacks, the level of danger (measured as the frequency of pike strikes against stickleback) to both single and paired inspectors grew significantly as inspector approached more closely (43), demonstrating that the cost of predator inspection is considered a danger of predation that rises with proximity. Furthermore, minnows visually detected a possible predator sooner and changed their behaviour more as a consequence of the information received during inspection (45), and when they saw a visual attack, they performed more frequent inspections and in bigger inspection groups (16).

A broad range of aquatic species emits ***chemical cues*** when detected predators are disturbed or they emit injury-released warning cues when trapped by a predator (reviews (3,46,47)). Many prey species mainly rely on the diet concentration of the predator to help them assess danger levels (minnows (17,48,49), guppies (41,50), characin fish (44,50), cichlid (15). Furthermore, the quality and quantity of the inspector's diet can influence the antipredator responses of the fellow shoal (51). For example, guppies are known to differentiate between various kinds of donor disruption signals (27,33). When dividing shoals into emitters and receivers, receivers distinguished between emitter's cue treatments, reacting more strongly to behavioural changes from emitters given a protein-rich diet and a larger overall diet (51).

Multiple cues interact in the prey background, and information about local predation risk (and subsequently acquiring the recognition of novel predators) is available to individual inspectors based on not only the predator's visual and chemical cues. For example, (52) shows that chemical, visual, and hydromechanical cues (e.g. the predator's posture, fins movement, and ventilation operculum (41)), when combined with additional visual cues (eg. predator species, size, etc), may be useful sources of information about the presence of predators during predator inspection. In addition, the efficiency of predator-produced cues can be increased when prey fish receive them in combination (52). Consequently, the frequency of predator inspection and inspection groups can be increased (13).

Although chemical cues in the form of prey alarm cues in the predator's diet are the primary source of information regarding local predation risk during inspection behaviour, visual cues have been shown to aid in information acquisition in fish and are used when chemical information is unavailable or ambiguous (12). For example, when exposing shoals of glowlight tetras to a live predator under low (3 lux) or high (50 lux) light levels and in the presence of a cichlid-fed tetra (with an alarm cue) or swordtails (with an alarm cue not recognized by tetras) in a 2x2 experimental design, tetras demonstrated threat-sensitive inspection behaviour (increased inspection latency, decreased inspection frequency, smaller examining group sizes, and higher minimum approach distance) when confronted with a predator combined with a tetra-fed food signal, independent of light levels. Only under high light circumstances were similar threat-sensitive inspection behaviours reported in cichlids with a swordtail-fed food cue.

Interspecific information transfer of cues:

If we look at the literature or predator inspection, it seems to favour the hypothesis that prey tends to gain information about predator behavior (Figure 1). However, studies on the quality and quantity of information gained during the predator inspection of inmates still need further investigation. We list the current relevant research to conceal this gap. As minnows tightened their shoaling in response to disturbance cues from inspectors, disturbance cues from isolated minnows did not elicit a significant shoaling response (49). Moreover, receivers responded to disturbance cues from familiar groups compared to cues from unfamiliar groups or isolated minnows (49). In addition, experienced prey considerably impacts chemically mediated predator inspection behaviour and attack cone avoidance (44). Experienced tetras, for example, had dramatically enhanced antipredator reactions, changed their inspection behaviour, and demonstrated much greater attack cone avoidance than naive tetras, independent of predator feed type and concentration (44). After all, assessing the danger of predation should be balanced against other factors that might impede or enhance anti-predator behaviour.

3.2.3 P-P communication:

When confronted with a predator, certain prey prefers to reveal themselves by acoustical or visual displays, which were traditionally thought to be warning signs given to other prey and targeted at the predator, seemingly to prevent further pursuit (18,20). Many studies on predator inspection functions revealed that it is frequently performed to deter the predator from further attacks and announce their anti-predatory fitness and/or awareness of its existence, warning other group members that a predator is present (5–7,19,20,50,53,54). Predator approaching may be dangerous for prey because they are constrained by the predator's reaction and not entirely under the control of the prey (1). However, the need for more information and fitness on the changes in the environment may be beneficial knowledge for risk assessment and decision-making (1,2). Predators, for example, were less attentive to and less likely to attack and kill inspectors than non-inspectors and were, therefore, more likely to abandon an initial approach towards inspectors than a non-inspecting one (Gobbies:(55), Minnows (37), Gazelles (8)). According to these findings, inspectors have a reduced predation risk than non-inspectors.

One predator approach behaviour technique in certain prey species might be to annoy or chase the predator away, leaving the predator with little choice but to flee after exploitation (8,56). Furthermore, when approaching predator, the prey may indicate that it has been noticed and that the element of surprise has been gone (18). The predator may then decide to quit its present hunting effort and shift its focus to less watchful prey (19,20,56). For example, in an experimental setup, when exposing guppies to cichlid predators, the predators were substantially less attentive and less likely to attack and kill guppies that were inspecting compared to those that were not, and they were more likely to abandon an initial approach towards an inspecting guppy than a non-inspecting guppy. This indicates that inspectors had a considerably reduced chance of assault and mortality when approached by the predator than non-inspectors (55) (and see also (11,37). However, few research on predator inspection has been undertaken in the wild, and many predator experimental aquaria were too small to enable a thorough examination of this specific deterrent mechanism. (1,13,40,46,48,49).

3.3. What motivates inspectors to approach dangerous predators?

Encountering a predator forces the prey individuals into inactive state (depressed mating and foraging habits); hence initiating a predator inspection terminates the prolonged exposure and pursuit with their vital activities or alters their subsequent behaviour. Which raises the question of **what motivates the prey individuals to initiate a risky inspection?.** One motivation can be the *hunger state* of the group. As hunger increases predator inspection events and frequency, as well as their foraging rate (6,17,45), the hungrier fish in a shoal is more willing to take more significant risks to inspect a potential threat at a distance (57,58). Another possible phenotype indicator of their initiation to risky predator inspection is *boldness* (6,17,36,45,57,59). More than a few species of predator inspector fish (32–34,60) were found to conduct significantly more inspections by individuals who were naturally "bold" than individuals who lacked the trait. Also, more *experienced individuals* of the predator were found to perform more frequent inspections than the un-experienced ones (see (31,45,61,62)) in response to experience and boldness when it comes to starting and frequency of inspections). Even if all individuals obtain the same information about a predator at the beginning of an experiment, there may be variation in inspection behavior. More exposed individuals inspect more (37,40,63), and so do individuals with higher social ties (64).

3.4. What type of game is played in the predator inspection?

Understanding predator inspection within a game theoretical framework has arguably been the most prominent goal. Several experimental studies aimed at identifying the payoffs corresponding to inspecting versus staying with the shoal, and leading versus following during an inspection. The emergence of the iterated prisoner's dilemma game as a paradigm to understand cooperation led to a focus on pairwise inspections (43,65–67). The involved empiricists concluded that stickleback and guppy pairs most likely solve an iterated prisoner's dilemma with a Tit-for-Tat-like strategy (66,67). Not all scientists agree with this interpretation, with the most common alternative explanation being that pair inspection represents a snowdrift game (68). In a snowdrift game, mutual defection is not an evolutionarily stable outcome. While it is best to cooperate with a defector and defect on a cooperator in a single interaction, iterated decisions may lead to alternate leading as a cooperative solution. In addition, initiators may generally fall into a volunteer dilemma in the step of initiating an inspection. Here, we explore the potential payoffs in more detail. Most importantly, we distinguish between the payoff distribution i) among shoal members depending on whether individuals inspect or remain with the shoal, and ii) among co-inspecting individuals depending on whether they are leading or following.

3.4.1. Payoff distribution and inspection functionality among shoal members

In principle, one could potentially imagine three scenarios. Leaving the shoal could either represent a N-player prisoner's dilemma (maximal conflict), a volunteer's dilemma (negative frequency dependence), or an N-player 'prisoner's delight' (self-serving). The payoffs depend on which functional explanation for predator inspection applies, and they may also differ between individual shoal members.

One potential function of predator inspection is *prestige and reputation* gain for males. Typically, this function will be linked to sexual selection and states that males rather than females should perform it. Only if inspections serve to signal own quality to other shoal members, the payoff matrix of inspection could fit a N-player prisoner's dilemma. However, inspectors would gain overall fitness benefits because the inspection game is combined with another game, where the gained reputation gains translate into increased payoff.

The classic example involves individual males showing their quality through predator inspection, which then causes females to preferentially mate with them (22,28,29,69). Hence, males who perform it will be more likely to get more mating, which would be considered a selfish act for the males to do the inspection. In this scenario, inspections are indeed risky, and only high-quality males benefit from paying the cost. Inspections could be just a means to show one's quality, or inspectors could obtain information about the predator's state and transfer this information to other shoal members (fitting the concept of reputation-based partner choice;(28)). Males inspectors can still transfer information and be useful to others, but the critical thing is that they get more benefits from mating with females. If predator inspection has a reputation function, females would need some cognitive abilities. They need to observe and evaluate males based on the frequency of inspection, and the proximity to the predator, where high-quality males should perform more often and/or closer to the predator. The question here is will the female acknowledge and rank the approaching different males properly, and choose to mate with the male who highly performed during the predator inspection? Suppose this is a case of reputation benefit, males should be performing more in correlation to their quality level, and females should be able to evaluate their predator inspection performance. Importantly, high-quality males would solve a N-player prisoner dilemma game because the game is combined with benefits occurring in the mating game. This logic is equivalent to humans contributing to public goods because such contributions yield benefits in an indirect reciprocity game (70).

An alternative function to signaling to conspecifics is to conduct inspections to signal to the predator. One possibility is that predator avoid attacking individuals who have signaled their vigour (*vigour advertisement;* (55)). Hence, when performing a vigor advertisement via a predator inspection, the prey reduces its own risk of being attacked by initiating a predator inspection and would directly obtain benefits from inspections. In this scenario, inspections are not cooperative acts. Instead, shoal members are in direct competition with each other over who will most likely become the target of an imminent predator attack. All or most individuals should inspect predators, and compete over who does it most often or approaches the predator closest. As with the intraspecific quality signaling hypothesis, the logic of the handicap principle would apply in that high-quality individuals should show more inspection behavior. An important difference is that females would also compete to inspect in order to avoid becoming the predator’s target. As a result, predator inspection would not be a public goods game. Each individual aims at reducing the probability of the predator attacking it (selfish). Hence, under the vigour advertisement hypothesis, predator inspection would not be a form of cooperation, and it would not be considered a prisoner dilemma (55).

Regarding the game structure, *perception advertisement* differs fundamentally from vigor advertisement, despite they look similar in reducing the risk of attack. The function of *perception advertisement* is to inform the predator about its exposure and the group's vigilance about its existence, which in turn is beneficial to the entire group, not to a specific individual. If inspections serve as perception advertisement, the dynamics most likely to fit a volunteer’s dilemma: someone should signal to the predator that it has been detected to make it leave, but the question is who volunteers? This is because inspecting should be riskier than remaining in the shoal. The resulting war of attrition may be resolved by shoal members facing different levels of trade-offs. High quality individuals and/or hungry individuals may have shorter giving-up intervals and hence inspect. If perception advertisement represented a N-player prisoner’s dilemma, fish should not be able to solve it (unless the game were combined with a mating game; see above). The crucial tests for perception advertisement would be that it alters the predator’s subsequent behavior in a way that benefits the prey, i.e. inspections should reduce attack probability, and ideally even make the predator move away.

Finally, inspections may not serve as signals but to *gain information* about the predator’s state, and the most obvious interpretation is that prey tends gains information about predator states through emitted cues. Suppose the inspection is only about information gain; in that case, the inspector needs to learn whether the predator is hungry or satiated and adjust the behaviour afterwards (the inspector changes the behaviour based on the information obtained). If the predator is hungry and the probability of an attack is high, the prey should adopt conservative behaviour and be more vigilant (e.g. reducing foraging, functioning closer to shelters). However, if the predator is satiated and the attack probability is low, the prey can focus on daily activities like foraging and mating, etc. An additional point to be considered is whether the group quantity will influence the *information gained* while decreasing the risk. From the inspector's point of view, gaining information is a function of distance, and it can act accordingly based on the information that has been individually gained. For example, if one inspector goes to X distance, it can gain more information than when inspecting at 2X distance despite the existence of an inspector partner (Figure 2.A). However, from the shoal point of view, it could receive more information cues from the inspector/s; if there are more inspectors, then the information can be more reliable in correlation to the repeated responses of the inspectors. For example, if 2 inspectors out of 10 are hiding after an inspection, that should lead to a different antipredator response by the other shoal members compared to 9 out of 10 inspectors hiding. There is a possibility of misinterpretation of the risk in the first case than in the second case. Hence, the information transfer can be more accurate when received from a larger inspection group.

For the information gain hypothesis, a crucial question is to what degree the information obtained during own inspection (‘private information’) is transmitted to other shoal members (‘public information’). If public information is as reliable as private information, inspections would represent a volunteer’s dilemma. In that case, a single inspection event would suffice as piscivores do not rapidly change their state: a predator that is satiated now will remain satiated for the next hours. If private information is more reliable than public information, several inspections by different individuals may occur. They would be self-serving for high quality individuals, while for low-quality individuals the benefits of additional information gain would not outweigh the costs of increased predation risk.

After all, we consider the current evidence insufficient to conclusively determine what the function(s) of predator inspection is(are) and hence what game(s) is (are) played on the shoal level during predator inspection events. There is various evidence for condition-dependent inspections: inspection initiators are characterized mainly by being bolder (34), more experienced (44), and larger (71,72). While condition-dependence is most prominent in the concepts of prestige communication and vigor advertisement, condition may also affect inspection decisions when the function is perception advertisement and/or information gain. Furthermore, the current evidence for prestige communication and/or vigor advertisement is rather weak. The latter has been promoted by (55) based on a study that involved a predator with four prey fish, all moving freely in tanks 80 x 30 x 30 cm in size. The authors reported that inspection behavior correlated negatively with predation, but a lack of methodological details made it impossible for us to understand how inspections can be identified in such a setup. Therefore, we consider it an open question whether the negative correlation was based on signaling or simply reflect superior condition. An experimental study by (43), using levers to move dead sticklebacks towards a predator, found no evidence that a close approach as a form of vigour advertisement reduces predation risk.

3.4.2. Payoffs among co- inspection members during predator approach

An important observation from naturally occurring predator inspections is that singleton inspections are frequent. Thus, inspections involving two or more individuals are not needed to overcome any public goods problem within the shoal, i.e. to initiate any predator inspection behavior. As pointed out in the previous section, we consider it highly unlikely that shoals experience a proper N-player prisoner's dilemma when it comes to predator inspections. Thus, the question arises whether it is possible that two co-inspecting fish face a 2-player prisoner's dilemma payoff matrix, as originally argued by (65). In order to address this question, we first need a description of what constitutes a cooperative behavior and a defective behavior, respectively. This is a challenging issue, as actions are quantitative rather than qualitative in nature: individuals can make a saltatory move of variable length, and they can lead/lag behind by variable distance. To categorize actions qualitatively, we consider a forward move that, in the absence of the partner moving, would cause the actor gaining the lead as cooperative. In contrast, we consider a lack of moving despite being at the same distance or lagging behind, as well as a move from behind that does not result in taking the lead as defective. It seems to be pretty logical that co-leading yields a higher payoff than both trying to lag behind, as the latter would result in the breakdown of the predator inspection and hence a lack of the benefits that can achieved from it. The difficult question to answer is whether leading with a laggard yields the lowest possible payoff (the sucker's payoff), and whether lagging behind an inspector yields the highest possible payoff.

We think that the appropriate experiments have not yet been conducted. The key unanswered questions are **how pair inspections affect the likelihood of a predator attacking compared to singleton inspections**, and **how the predator adjusts its focus on a paired inspector depending on whether it is leading or lagging behind**. If at any given distance, a predator is less likely to attack when two fish approach rather than one, pair inspections cannot be a prisoner's dilemma because a permanent laggard would still yield some protection to the leader compared to inspecting alone. Another extreme would be that pair inspections more than double the attack probability, but this scenario can safely be excluded because there would be selection against pair inspections, and hence we should not observe them. An interesting possibility is that pair inspections slightly increase attack probability (but less than double). In that case, mean individual risk would be lower than during singleton inspections but it would be possible that pairs face a prisoner's dilemma if the predator were more likely to attack the leader than it would a singleton. To contrast the scenario with the alternative (Figure 2.B) in which the leading fish faces higher attack probabilities than the laggard but still lower than when inspecting alone (43,73) (Figure 2.C). In the latter case, and more generally in any case in which the leader faces a higher attack probability than the laggard, the co-inspecting partners would face a snowdrift game.

As each inspection involves multiple decisions about moving, and individuals may repeatedly co-inspect, individuals may use reciprocal strategies to achieve high levels of cooperation. Indeed, there is overwhelming evidence that inspection behavior is conditional on the partner's current and past performance (43,64–67,74–76). Moreover, individuals have preferred partners for predator inspection (64,77,78), which is to be expected if individuals consistently differ with respect to inspection behavior, i.e. willingness to lead/reciprocate and typical minimal distance to a predator. As has been pointed out repeatedly (68,79,80), conditional cooperation may also be observed in iterated snowdrift games.

3.5. Why repeated visits?

A generally overlooked element of predator inspection research may give information on the function of predator inspection. Laboratory studies show inspection rates more significant than the number of prey fish (1-2 inspections per minute by 15% of the shoal, (12,32,54)). These results suggest that information gain is of less importance than currently assumed: what information could be gained through multiple inspections within 10-30 min that cannot be gained in a single one? Signaling functions provide more parsimonious explanation for multiple inspections. For example, perception advertisement presumes that in nature, predators would indeed leave the area in response to an inspection (as described for leopards,(20)). In the experimental lab settings, predators cannot leave, which in turn elicits repeated inspections. Natural observations or large test ponds that allow predators to move between several shoals would allow to test the perception advertisement hypothesis in more detail. Perception advertisement most obviously functions if the predator moves, but it could also function by reducing the predator's attack probability. Some piscivorous fish species may tend to stay at a place in order to avoid extra energy expenditures, even if current chances of hunting success are low. Under such conditions, repeated inspections may serve to remind the predator that it is still monitored. Thus, a future study should also quantify attack probability as a function of time since the last predator inspection. More generally, it is necessary to study predator decision-rules in more detail. Surprisingly to us, there is apparently not a single study that tested how predator attack probability varies as a function of predator hunger state. If inspections function to obtain information about the predator, its state should reliably predict attack probabilities.

As a final note, early and late inspections are not necessarily undertaken by the same inspectors (34). Potentially, re-inspection might be a sort of experience enhancer or inspection training in situations in which a predator has been determined to be of low threat. Early inspectors showing no sign of distress might encourage other group members to practice their skills. One could test this idea again by manipulating predator hunger state; hungry predators should cause less additional individuals to inspect than satiated predators would.

3.6. Conclusions

Currently, there is some evidence for all four potential functions of predator inspection, none of them being very conclusive. Predator inspection affects mating successes regardless of the sex of the inspectors, either by communicating prestige or by sneaky mating of less successful males towards unguarded inspecting females. Shoaling prey also tends to inspect the predator to assess potential risk cues, such as visual cues of predator (Species presence and species state), chemical cues (alarm cues, disturbance cues, safety cues, etc.), or in many cases, the combination of both the visual and chemical cues. Furthermore, predation inspection is shown to prevent the predator from additional attacks and enhance prey anti-predatory fitness despite the predator's response that is not totally under the prey's control. By approaching a predator, the prey may signal that it has been observed and that the element of surprise is gone, and the predator may then opt to target less vigilant prey.

As it stands, there are currently too many open questions to provide more conclusive answers. Prominent among these open questions are i) why individual fish inspect predators repeatedly over short time periods; ii) whether predators would swim off in response to prey inspection behavior; iii) how predator hunger state affects attack probabilities; iv) how inspection group size affects predator attack probability on them; and v) how an individual’s position within the inspecting group relative to the predator affects its personal risk.

Only once the function(s) of predator inspection is/are known and the above questions were answered, we can finally tackle the question what games are played by inspector at the two distinctive stages, 1) among group members (initiator selection), and 2) among inspector members.

3.7. Reference

1. Lima SL, Dill LM. Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool. 1990 Apr 1;68(4):619–40.
2. Kelley JL, Brown C. Chapter 16 Predation risk and decision making in poeciliid prey. :11.
3. Kats LB, Dill LM. The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience. 1998;5(3):361–94.
4. Shettleworth SJ. Cognition, Evolution, and Behavior. Oxford University Press; 2009. 715 p.
5. Curio E, Ernst U, Vieth W. The adaptive significance of avian mobbing: II. Cultural transmission of enemy recognition in blackbirds: Effectiveness and some constraints. Zeitschrift für Tierpsychologie. 1978;48(2):184–202.
6. Pitcher TJ, Green DA, Magurran AE. Dicing with death: predator inspection behaviour in minnow shoals. Journal of Fish Biology. 1986;28(4):439–48.
7. Fishman MA. Predator Inspection: Closer Approach as a Way to Improve Assessment of Potential Threats. Journal of Theoretical Biology. 1999 Jan 21;196(2):225–35.
8. FitzGibbon CD. The Costs and Benefits of Predator Inspection Behaviour in Thomson’s Gazelles. Behavioral Ecology and Sociobiology. 1994;34(2):139–48.
9. Nocera JJ, Taylor PD, Ratcliffe LM. Inspection of mob-calls as sources of predator information: response of migrant and resident birds in the Neotropics. Behav Ecol Sociobiol. 2008 May 29;62(11):1769.
10. Blaszczyk MB. Boldness towards novel objects predicts predator inspection in wild vervet monkeys. Animal Behaviour. 2017 Jan 1;123:91–100.

11. Pitcher T. Who Dares, Wins: the Function and Evolution of Predator Inspection Behaviour in Shoaling Fish. Netherlands Journal of Zoology. 1991 Jan 1;42(2–3):371–91.
12. Brown GE, Magnavacca G. Predator Inspection Behaviour in a Characin Fish: an Interaction between Chemical and Visual Information? Ethology. 2003;109(9):739–50.
13. Brown GE, Godin JGJ. Who dares, learns: chemical inspection behaviour and acquired predator recognition in a characin fish. Animal Behaviour. 1999 Feb 1;57(2):475–81.
14. Häberli MA, Aeschlimann PB, Milinski M. Sticklebacks benefit from closer predator inspection: an experimental test of risk assessment. Ethology Ecology & Evolution. 2005 Jul;17(3):249–59.
15. Hesse S, Anaya-Rojas JM, Frommen JG, Thünken T. Social deprivation affects cooperative predator inspection in a cichlid fish. R Soc Open Sci [Internet]. 2015 Mar 25 [cited 2021 Apr 15];2(3). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4448828/
16. Cowan J, Brown G. FORAGING TRADE-OFFS AND PREDATOR INSPECTION IN AN OSTARIOPHYSAN FISH: SWITCHING FROM CHEMICAL TO VISUAL CUES. Behaviour. 2000 Jan 1;137(2):181–95.
17. Murphy KE, Pitcher TJ. Predator attack motivation influences the inspection behaviour of European minnows. Journal of Fish Biology. 1997;50(2):407–17.
18. hasson O. Pursuit-deterrent signals: communication between prey and predator. Trends in Ecology & Evolution. 1991 Oct;6(10):325–9.
19. Caro TM. Pursuit-deterrence revisited. Trends in Ecology & Evolution. 1995 Dec 1;10(12):500–3.
20. Zuberbühler K, Jenny D, Bshary R. The Predator Deterrence Function of Primate Alarm Calls. Ethology. 1999;105(6):477–90.
21. Zahavi A, Zahavi A. The handicap principle: A missing piece of Darwin’s puzzle. Oxford University Press; 1999.

22. Roberts G. Competitive altruism: from reciprocity to the handicap principle. Proceedings of the Royal Society of London Series B: Biological Sciences. 1998;265(1394):427–31.

23. Luce RD, Raiffa H. Games and decisions: Introduction and critical survey. Courier Corporation; 1989.

24. Austad SN. A game theoretical interpretation of male combat in the bowl and doily spider (Frontinella pyramitela). Animal Behaviour. 1983 Feb 1;31(1):59–73.

25. Zahavi A. Mate selection—a selection for a handicap. Journal of theoretical Biology. 1975;53(1):205–14.

26. Grafen A. Biological signals as handicaps. Journal of theoretical biology. 1990;144(4):517–46.

27. Penn DJ, Számadó S. The Handicap Principle: how an erroneous hypothesis became a scientific principle. Biological Reviews. 2020;95(1):267–90.

28. Roberts G, Raihani N, Bshary R, Manrique HM, Farina A, Samu F, et al. The benefits of being seen to help others: indirect reciprocity and reputation-based partner choice. Philosophical Transactions of the Royal Society B. 2021;376(1838):20200290.

29. Lotem A, Fishman MA, Stone L. From reciprocity to unconditional altruism through signalling benefits. Proceedings of the Royal Society of London Series B: Biological Sciences. 2003;270(1511):199–205.

30. Magurran AE, Seghers BH. Population differences in predator recognition and attack cone avoidance in the guppy Poecilia reticulata. Animal Behaviour. 1990;40(3):443–52.

31. Magurran AE, Seghers BH. Predator Inspection Behaviour Covaries with Schooling Tendency Amongst Wild Guppy, Poecilia reticulata, Populations in Trinidad. Behaviour. 1994;128(1/2):121–34.

32. Magurran AE, Nowak MA. Another battle of the sexes: the consequences of sexual asymmetry in mating costs and predation risk in the guppy,

Poecilia reticulata. Proceedings of the Royal Society of London Series B: Biological Sciences. 1991;246(1315):31–8.

33. Wilson DS, Clark AB, Coleman K, Dearstyne T. Shyness and boldness in humans and other animals. Trends in ecology & evolution. 1994;9(11):442–6.

34. Budaev S, Zworykin D. Habituation of Predator Inspection and Boldness in the Guppy (Poecilia reticulata). Journal of Ichthyology. 2003 Apr 15;43:s243–7.

35. Dugatkin LA, Alfieri MS. Boldness, behavioral inhibition and learning. Ethology Ecology & Evolution. 2003 Jan 1;15(1):43–9.

36. Godin JG, Dugatkin LA. Female mating preference for bold males in the guppy, Poecilia reticulata. Proceedings of the National Academy of Sciences. 1996 Sep 17;93(19):10262–7.

37. Magurran AE. The adaptive significance of schooling as an anti-predator defence in fish. In JSTOR; 1990. p. 51–66.

38. Evidence for sperm transfer during sneaky mating in wild Trinidadian guppies - Matthews - 2000 - Journal of Fish Biology - Wiley Online Library [Internet]. [cited 2022 Sep 21]. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1095-8649.2000.tb02150.x

39. Godin JGJ. Predation risk and alternative mating tactics in male Trinidadian guppies (Poecilia reticulata). Oecologia. 1995 Aug 1;103(2):224–9.

40. Magurran AE, Higham A. Information Transfer across Fish Shoals under Predator Threat. Ethology. 1988;78(2):153–8.

41. Licht T. Discriminating between Hungry and Satiated Predators: The Response of Guppies (Poecilia reticulata) from High and Low Predation Sites1. Ethology. 1989;82(3):238–43.

42. Licht T. Discriminating between Hungry and Satiated Predators: The Response of Guppies (Poecilia reticulata) from High and Low Predation Sites1. Ethology. 2010 Apr 26;82(3):238–43.

43. Milinski M, Lüthi JH, Eggler R, Parker GA. Cooperation under predation risk: experiments on costs and benefits. Proceedings of the Royal Society of London Series B: Biological Sciences. 1997 Jun 22;264(1383):831–7.

44. Brown GE, Dreier VM. Predator inspection behaviour and attack cone avoidance in a characin fish: the effects of predator diet and prey experience. Animal Behaviour. 2002 Jun 1;63(6):1175–81.

45. Magurran AE. Predator inspection behaviour in minnow shoals: differences between populations and individuals. Behav Ecol Sociobiol. 1986 Sep 1;19(4):267–73.

46. Chivers DP, Smith RJF. Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. Ecoscience. 1998;5(3):338–52.

47. Summey MR, Mathis A. Alarm responses to chemical stimuli from damaged conspecifics by larval anurans: tests of three neotropical species. Herpetologica. 1998;402–8.

48. Mirza RS, Chivers DP. Fathead Minnows Learn to Recognize Heterospecific Alarm Cues They Detect in the Diet of a Known Predator. Behaviour. 2003;140(11/12):1359–69.

49. Bairos-Novak KR, Ferrari MCO, Chivers DP. A novel alarm signal in aquatic prey: Familiar minnows coordinate group defences against predators through chemical disturbance cues. Journal of Animal Ecology. 2019;88(9):1281–90.

50. Brown GE, Paige JA, Godin JGJ. Chemically mediated predator inspection behaviour in the absence of predator visual cues by a characin fish. Animal Behaviour. 2000 Sep 1;60(3):315–21.

51. Goldman JA, Crane AL, Feyten LEA, Collins E, Brown GE. Disturbance cue communication is shaped by emitter diet and receiver background risk in Trinidadian guppies. Current Zoology [Internet]. 2021 Mar 16 [cited

2021Oct20];(zoab025).Availablefrom:https://doi.org/10.1093/cz/zoab025

52. Mikheev VN, Pasternak AF. Defense behavior of fish against predators and parasites. J Ichthyol. 2006 Dec 1;46(2):S173–9.
53. Brönmark C, Hansson LA. Chemical Ecology in Aquatic Systems. OUP Oxford; 2012. 318 p.
54. Saavedra I, Amo L. The importance of chemical, visual and behavioral cues of predators on the antipredatory behavior of birds. Journal of Avian Biology [Internet]. 2020 [cited 2021 Jul 12];51(7). Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/jav.02431
55. Godin J guy J, Davis SA. Who dares, benefits: predator approach behaviour in the guppy (Poecilia reticulata) deters predator pursuit. Proceedings of the Royal Society of London Series B: Biological Sciences. 1995 Feb 22;259(1355):193–200.
56. hasson O. Pursuit-deterrent signals: communication between prey and predator. Trends in Ecology & Evolution. 1991 Oct;6(10):325–9.
57. Godin JG, Crossman S. Hunger-dependent predator inspection and foraging behaviours in the threespine stickleback (Gasterosteus aculeatus) under predation risk. Behavioral Ecology and Sociobiology. 1994 Feb 1;34:359–66.
58. Bejder L, Samuels A, Whitehead H, Gales N. Interpreting short-term behavioural responses to disturbance within a longitudinal perspective. Animal Behaviour. 2006 Nov 1;72(5):1149–58.
59. Whitham J, Mathis A. Effects of Hunger and Predation Risk on Foraging Behavior of Graybelly Salamanders, Eurycea multiplicata. J Chem Ecol. 2000 Jul 1;26(7):1659–65.
60. Ioannou C, Payne M, Krause J. Ecological consequences of the bold-shy continuum: The effect of predator boldness on prey risk. Oecologia. 2008 Sep 1;157:177–82.

61. Roy T, Bhat A. Population, sex and body size: determinants of behavioural variations and behavioural correlations among wild zebrafish Danio rerio. Royal Society Open Science. 5(1):170978.

62. Tang ZH, Wu Q, Fu SJ. Inspection behaviour and inter-individual cooperation in juvenile qingbo: the effects of prior predator exposure and food deprivation. J Ethol. 2018 May 1;36(2):181–90.

63. Provenance, shoal size and the sociobiology of predator-evasion behaviour in minnow shoals [Internet]. [cited 2022 Jun 3]. Available from: https://royalsocietypublishing.org/doi/epdf/10.1098/rspb.1987.0004

64. Croft DP, Hamilton PB, Darden SK, Jacoby DMP, James R, Bettaney EM, et al. The role of relatedness in structuring the social network of a wild guppy population. Oecologia. 2012 Dec 1;170(4):955–63.

65. Milinski M. Tit for tat in sticklebacks and the evolution of cooperation. nature. 1987;325(6103):433–5.

66. Dugatkin LA. Do guppies play TIT FOR TAT during predator inspection visits? Behav Ecol Sociobiol. 1988 Dec 1;23(6):395–9.

67. Milinski M, K¨lling D, Kettler R. Tit for Tat: sticklebacks (*Gasterosteus aculeatus*) ‘trusting’ a cooperating partner. Behavioral Ecology. 1990;1(1):7–11.

68. Noë R. Cooperation experiments: coordination through communication versus acting apart together. Animal Behaviour. 2006 Jan;71(1):1–18.

69. Zahavi A. Altruism as a handicap: the limitations of kin selection and reciprocity. Journal of Avian Biology. 1995;26(1):1–3.

70. Milinski M, Semmann D, Krambeck H. Donors to charity gain in both indirect reciprocity and political reputation. Proceedings of the Royal Society of London Series B: Biological Sciences. 2002;269(1494):881–3.

71. Külling D, Milinski M. Size-dependent predation risk and partner quality in predator inspection of sticklebacks. Animal Behaviour. 1992 Nov 1;44(5):949–55.

72. Walling CA, Dawnay N, Kazem AJN, Wright J. Predator inspection behaviour in three-spined sticklebacks (Gasterosteus aculeatus): body size, local predation pressure and cooperation. Behav Ecol Sociobiol. 2004 Jun 1;56(2):164–70.

73. Parker GA, Milinski M. Cooperation under predation risk: a data-based ESS analysis. Proceedings of the Royal Society of London Series B: Biological Sciences. 1997 Aug 22;264(1385):1239–47.

74. Dugatkin LA. Dynamics of the TIT FOR TAT strategy during predator inspection in the guppy (Poecilia reticulata). Behav Ecol Sociobiol. 1991 Sep 1;29(2):127–32.

75. Dugatkin LA, Alfieri M. Tit-For-Tat in guppies (Poecilia reticulata): the relative nature of cooperation and defection during predator inspection. Evol Ecol. 1991 Jul 1;5(3):300–9.

76. Croft DP, Krause J, Darden SK, Ramnarine IW, Faria JJ, James R. Behavioural trait assortment in a social network: patterns and implications. Behavioral Ecology and Sociobiology. 2009;63(10):1495–503.

77. Darden SK, James R, Cave JM, Brask JB, Croft DP. Trinidadian guppies use a social heuristic that can support cooperation among non-kin. Proceedings of the Royal Society B: Biological Sciences. 2020 Sep 9;287(1934):20200487.

78. Thomas POR, Croft DP, Morrell LJ, Davis A, Faria JJ, Dyer JRG, et al. Does defection during predator inspection affect social structure in wild shoals of guppies? Animal Behaviour. 2008 Jan 1;75(1):43–53.

79. Raihani NJ, Bshary R. Resolving the iterated prisoner’s dilemma: theory and reality: Resolvings the IPD. Journal of Evolutionary Biology. 2011 Aug;24(8):1628–39.

80. Bshary R, Zuberbühler K, Van Schaik CP. Why mutual helping in most natural systems is neither conflict-free nor based on maximal conflict. Philosophical Transactions of the Royal Society B: Biological Sciences. 2016;371(1687):20150091.

3.8. Figure

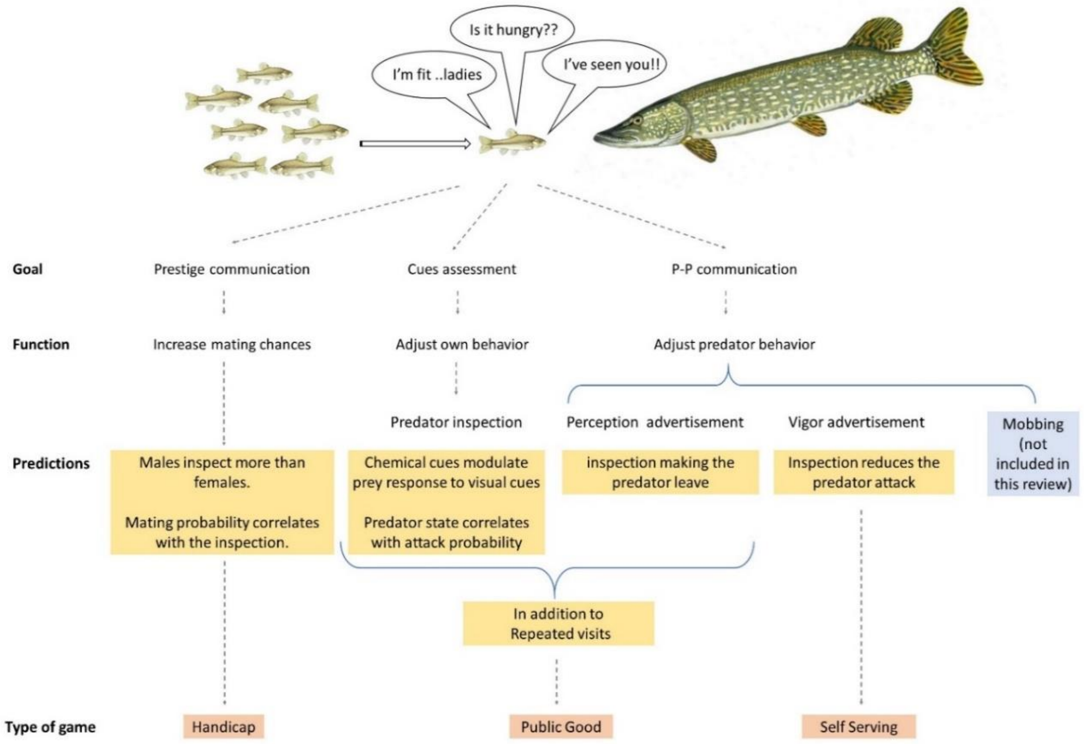
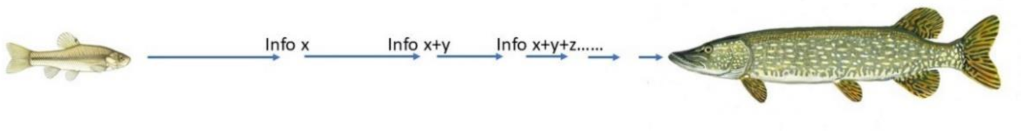


Figure 1. Structure of the current review illustrating each scenario by prey motivating a predator inspection episode. The potential goals and resulting functions lead to the behaviours and strategies performed. The prediction highlighted in yellow, and the types of the game highlighted in orange need further investigation.

A.


Info x
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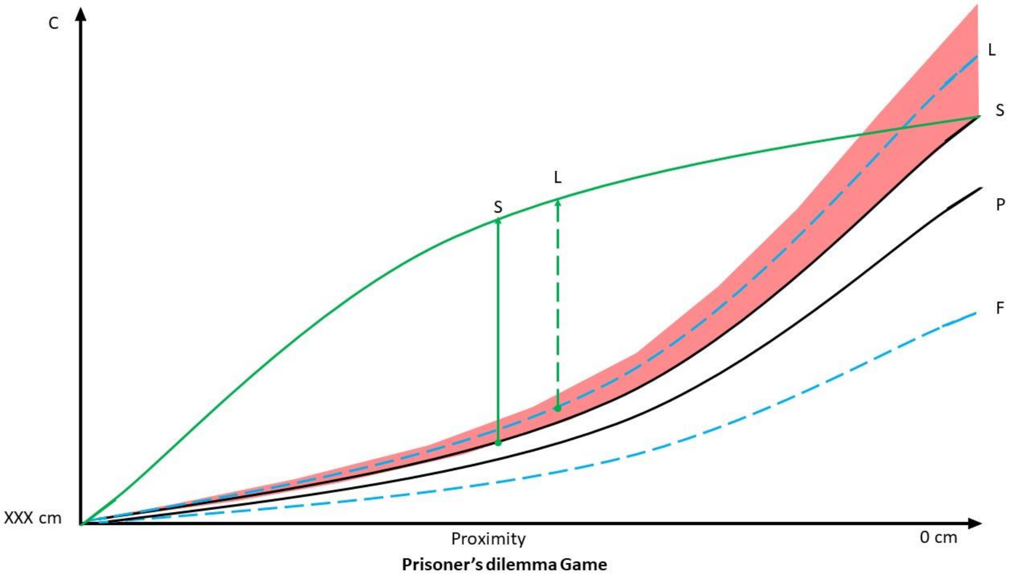
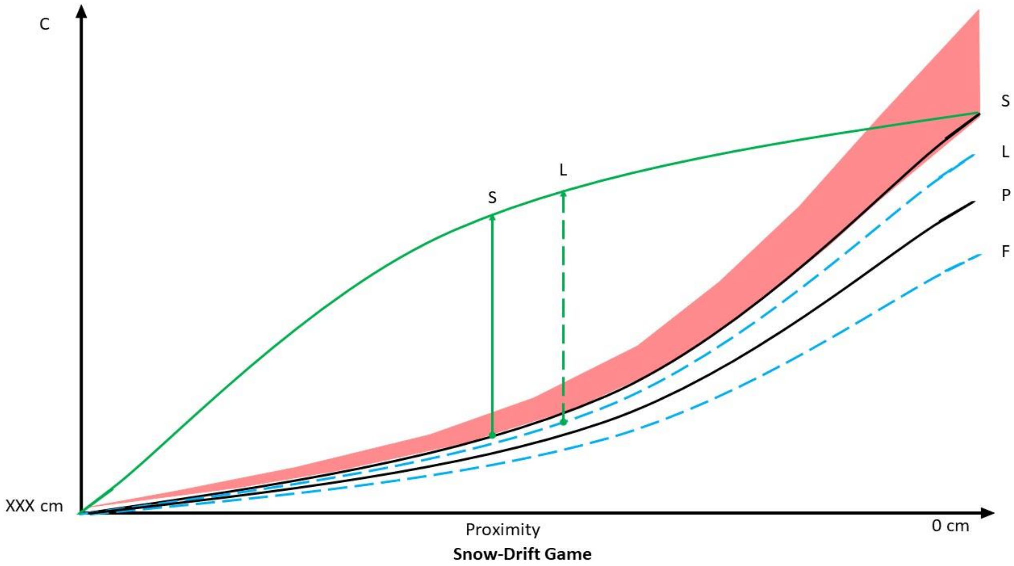
B.


C
L
S
L
S
P
F
XXX cm
Proximity
0 cm
Prisoner's dilemma Game

C.


C
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S
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F
XXX cm
Proximity
0 cm
Snow-Drift Game

Figure 2. The relative comparison in different games, assuming that individuals in closer proximity can acquire more information than at further distance. Abbreviation; C: Cost, B: Benefit, S: Singleton, L: Leader, F: Follower, P: Mean in a pair. The red zone represents risk levels for a pair inspector that would be above the risk of a singleton inspector. A. Illustrating the general idea of information gain as an individual approaches the predator in saltatory movements. B. Illustration of the cost functions fitting a PD game; being a leader in a pair is slightly more risky than when singleton, but extremely risky if compared to the follower (R: L> S > P> F). C. Illustration of the cost functions fitting a SD game; if the risk of capture for the leader in a pair is less than when inspecting alone (R: S> L> P> F).

GENERAL DISCUSSION

D.1 Summary

We dedicated this thesis to investigate and provide reliable results on the cooperative hunting behaviour of lionfish *P. miles* inhabiting the Red Sea. Besides, we provided a research review on predator inspection on prey fishes.

The first chapter of this thesis is based on the functionality of the cooperative hunting strategy and the recruitment signalling of a lionfish co-existing partner. The investigation and data collection were field-based, intended to measure the associations of a joint hunt and involvement of recruiting signals, and then laboratory-based, intended to verify the recruitment tendency of partners in case of inaccessible prey. In the field, lionfish individuals are associated less frequently than the calculated prediction as a chance. During the occasional associations, no fin-flaring sequences were recorded. In laboratory-controlled conditions, contrary to the recruitment hypothesis, there was no evidence for individual recruiting initiatives from the focals towards partners during different conditions. In addition, fin flaring was not in support of signalling initiatives, as it happened most often in the partner's corridor even after the partner was released.

In the second chapter, we continued to investigate the tendency of lionfish partners to coordinate their space and time during the hunt, and to what extent they alternate strikes and if they reciprocate their catch. Individuals took longer to join each other for the first time in the same prey zone in the presence of prey than in its absence. When the prey was present, lionfish tended to stay longer in the hunting zone; but pairs either ignored or avoided each other. In terms of lionfish individuals' tendency to team up with other hunters, we found that the observed duration of overlapping with partners within zones was significantly shorter than the expected duration. We predicted that if the partners were actively overlapping, the observed durations would be greater than the expected durations. However, when the duration spent in hunting zones by lionfishes was examined, there was not a significant difference in the aggregation (overlapping) duration of the expected than the observed duration value, but not in the neutral zones. Moreover, a second experiment revealed that individuals did not alternate their strikes, and the number of alternations appeared to be determined by the time interval between successive presentations: the shorter the interval the more likely alternations occurred. Observed alternations occurred randomly and less frequently but not significantly less frequently than expected by chance.

In the third chapter, we reviewed the predator inspection in more detail, understanding prey decision-making and the game played by prey members during an inspection. Predator inspection contributes to mating success by communicating mate prestige and fitness, or sneaking up on unguarded inspecting females. Another function is that shoaling prey inspects the predator for potential risk cues, such as chemical cues, visual cues, or a combination of both. Predator inspection may also communicate fit body condition -despite the unpredictability of the predator's response-, hence, it could prevent the predator from attacking again and improve the prey's anti-predatory response. However, most research on fish inspection behaviour has been conducted in the lab, where predator and prey separation distances are unrepresentative of the wild or larger semi-experimental tanks. In addition, we investigated the potential games played among group members and inspection initiators. The sort of games played in the first round was determined by the functionality of the inspection. Despite the proposal that predator inspection may be regarded as a prisoner dilemma, the game played by the inspector member in the first round is proposed to be a volunteer dilemma rather than a prisoner dilemma, as the N-player prisoner's dilemma has not yet been solved. Meanwhile, in the second round between inspectors, we can suggest that the game played by the inspector member is more likely a volunteer dilemma or a snowdrift game rather than a prisoner dilemma.

D.2 Lionfish cooperative hunting

Animals communicate, coordinate their movement, recruit and signal in cooperation to increase their collective chances of hunting, and may also need to regulate how to share information such as the availability and location of food (1,2). A signal between individuals can be defined as encoding and transferring information by an organism - the sender - to other organisms - the receivers. Hence, active signalling between partners to recruit a hunting partner can be identified as clear cognitive achievement interspecifically or intraspecifically (3,4). However, it is vital term that this information provided can alter the behaviour of the receiver in the direction that benefits the signaller (5–7). Despite the diverse forms of communication signals in animals, we did not find any evidence that lionfish *P. miles* communicate through performing fin flaring signals. As communication through signalling is an action which provides information to another animal, not all actions by one animal that provides information to another peer could qualify as signals.

On the other hand, recruitment is another type of communication in some animals, and can be defined as a collective system when organisms effectively communicate toward their inter and/or intra-specific to the location of a resource (8,9). In many animals, various methods are used to recruit inter/intraspecific for food acquisition, which can also be through vocalisations or movement patterns (3,10). There are two steps to the recruitment process: 1) the "invitation" of a partner, i.e., the recruitment initiation, which takes place mostly where the potential partner is located, and 2) the "orientation" process, i.e., the transmission of directional information toward the resource (11). Here, we found no evidence that *P. miles* hunt together in the wild, which makes sense since we found no evidence that *P. miles* recruits, and if so, such associations are random. In addition, we found no evidence of time and space coordination, taking turns, or alternating hunting strikes. Furthermore, regarding the comparison of lionfish hunting techniques between the native and invasive range, the field observations by Zhioua 2020 (unpublished master thesis) did not identify a) any significant differences and b) did not report a single event of collaborative hunting. Hence, our results suggest that *P. miles*' success in the Caribbean is not due to cooperative hunting strategies but to other factors.

D.3 The General picture

D.3.1 Lionfish hunting techniques

Predators may actively search and pursue the prey or quietly hide and wait for the chance to attack, and the physical capabilities of a predator determine the mode of pursuit (e.g., ambush or chase) (12,13). Lionfish are nocturnal hunters; their predation rates and activity levels were substantially greater during crepuscular times (14). In addition, lionfish predation activity is not restricted only by prey availability (14,15), which can be related to their sit-and-wait and opportunistic hunting modes. Lionfish in the native range exhibited slower life histories and narrower resource use (16), in addition to a high degree of hunting efficiency when hunting solitarily (17). In both the native and invaded ranges, the following adaptations in lionfish have been proposed to increase hunting success: striped colouration for crypsis (18), slow movement to avoid prey flight responses (14,19,20), water blows' jets' to make prey orient towards lionfish (21).

In both the native and invaded range, lionfish aggregations were considered a coincidence (22,23) caused by individuals' mutual attraction to comparable reef structures (23), and the tendency to co-exist around the same hunting spots but without any sophisticated huntings observed (22). In addition, despite significant geographical variance, lionfish had identical hunting behaviour in both the native and invaded range ((19), and Zhioua 2020 unpublished master thesis). However, the invasive consumes higher daily prey biomass than in the native range ((19), Wermeille 2020 unpublished master thesis). Furthermore, lionfish on invaded reefs depended less on 'blowing' behaviour for prey acquisition due to significant prey naiveté in the invaded region (19). On the other hand, if we look at the lionfish ecology in both native and invaded ranges, lionfish would prefer to target and consume solitary prey that are small and shallow-bodied, and lie close to the reef (14,24). This type of prey in nature is mainly singular and free to forage and only flee if the predator is detected. In this case, detecting lionfish is unlikely, due to their hunting techniques like slow approaching and cryptic body pattern(14), in addition to jet blowing (17). Hence, the favourable traits of the lionfish prey are vulnerability rather than availability or quantity (24).

D.3.2 Do lionfish need recruitment and coordinated hunting in nature?

During our personal field observation in both the native and the invaded range, we did not record any events of single or grouped individuals herding a shoal of prey for a simultaneous hunt. However, assuming that lionfish are in a situation where cooperative hunting results in the best interests, then lionfishes are under the selection of their ability to cooperate and coordinate so they would conduct better hunts. If this is a possible case, the highlighted constraints are: 1) does cooperative hunt provide more per capita?. Lionfish might face a situation where the prey shoal is more than one, or big enough to be sharable. Shoal prey can be reasonable prey to target, considering that they are mostly slow and swim in respect to interspecific proximity, which limits their escaping response -due to the lack of a central brain responsible for such decisions (25). However, based on (24), lionfish tend to observe and swim close to shoaling prey and not consume them, but consume solitary ones instead. In addition, lionfish do not tend to perform a cooperative or coordinated hunt (14,19,22,23), despite the vulnerability of shoaling prey. In this case, the lack of cooperative hunting might not be entirely due to prey vigilance or vulnerability but more because conducting a solitary and opportunistic hunt provides more self-sufficiency per capita for the lionfish individual rather than the conditional obligation to share or alternate the outcome.

In cases of lionfish aggregation, another constraint is that 2) is recruiting overcome prey vigilance?. Whether a focal lionfish would decide to guide unknowing conspecific, where both partners are more successful than a singleton. However, this is not the case in nature; lionfish aggregation was proven to be due to habitat complexity rather than for feeding or hunting (23).

In addition, during hunting in the wild, lionfish hover above or close to their possible victim, flare, spread their large, interconnected pectoral fins, and then strike (19, 27). Hence, aggregation, in general, can lead the prey to detect the concentrated different types of cues of lionfish and feel the vibration from the fin movement (27) or a previous attack, which can limit their success in capturing the prey.

In conclusion, solitary hunting for lionfish seems more gainful regarding the certainty of hunting success and prey capture. In addition, paired conspecifics might lead to an unsuccessful hunt, where multiple recruitments can constrain the hunt's success. For example, prey might move or escape; recruiting a partner can, to some extent, enhance the shoal vigilance and distract the attention toward any of the hunters to allow the partner to strike and vice versa.

D.4 Restrictions of the original plan

The primary aim of this thesis was to investigate and compare the cooperative hunting of lionfish *P. miles* between its native range in the Red Sea and its invaded range in the Caribbean. Furthermore, we wanted to investigate and compare how far prey evasive responses differ in both native and invaded range populations. In principle, the main idea behind the original plan was to be able to replicate Lönnstedt et al., 2014 (28) and to see how important cooperative hunting is for the success of lionfish populations in the Caribbean range. Since we could not replicate the findings, then we can expect lionfishes not to cooperate and reciprocate as a default in the Caribbean.

In addition, we encountered significant difficulties in data collection in the Caribbean range. The primary location to study the lionfish was planned on Barbados island, on the west coast of Bellaire research institution. However, the study species was rare to find on the west coast due to frequent and aggressive culling, and they only existed at depths over 50 meters, where it was dangerous to collect due to safety regulation measures. In addition, diving on the east coast was not a safe option due to the sea condition of high waves against rocky cliffs. Afterwards, the next attempt took place in a private research laboratory at Alligator head foundation in Jamaica. However, after establishing solid communication regarding our research requirements, we ran into an unrealistic representation by the hosting research station regarding the available resources and unsuitability of the water system to allow the habitation and survival of fish in the lab, which led to the failure to meet the research logistics.

To summarise, we could not conduct experiments on lionfish or its common prey in the invaded range and did not find any evidence of lionfish performing cooperative hunting in the native range. Eventually, an observation field comparison of lionfish hunting techniques between the native and invasive range by Zhioua 2020 (unpublished master thesis) indicated that there were no significant differences, and did not report a single event of collaborative hunting in either the native range nor the invaded one. Thus, we consider it unlikely that laboratory experiments at both ranges would reveal any significant differences in hunting strategies.

In addition, the Covid-19 pandemic limited the time available for data collection. Given all together, we changed our plan to validate the cooperative hunting behaviour of lionfish *P. miles* inhabiting the Red Sea in detail, and to write a review on predator inspection by shoaling prey was deemed a suitable alternative to include research on predator-prey interactions from the prey perspective. A review would at least provide a conceptual contribution to prey behaviour and underlying strategies.

D.5 Conclusion and outlook

As the reproducability crisis has beed repeadetly reported in science, this thesis deals with a consequences of a scientific miscondict research by Lönnstedt and co-authers. Lönnstedt was found guilty of scientific misconduct, including data fabrication, in a Science paper that shares all the same data fabrication as the Biology Letters article (29). Co-authors of Lönnstedt submitted a collage of lionfish to provide evidence that Lönnstedt collected enough lionfish to conduct the experiments described in their paper, but the collage was subsequently found to contain manipulated images of the same lionfish in an attempt to be passed off as different individuals (30). Furthermore, the papers of Lönnstedt that faced an accusation of misconduct and proof of fabrication are still with the support of the 'biology letter journal', and their rejection to publish our correcting outcome articles. Even though this PhD studied a sister species, *P. miles* , the results should help to lay to rest a study that editors of a journal resist to remove. However, and after all, an correcting study of zebra lionfish *Dendrochirus zebra* is needed with more suitable experimental design.

My studies on *P. miles* fail to replicate the results of (28), and our finding is that there is no evidence for active recruitment, coordination in time and space, or reciprocity-like alternation in strikes. This could be due to the fact that lionfish species and/or study sites differ in how they hunt together. Lionfish prefer shoaling prey, but they target and consume solitary ones. This is mostly due to shoals' conspicuousness, which reduces predator search time. However, when assuming that lionfish are capable of conducting cooperative hunting, then they are under the decision to cooperate and coordinate so they would conduct better hunts. To decide that, could depend on prey size or individual per capita, the time consumed, and the probability of success when recruiting one or multiple partners, where paired lionfish must be double as successful as a singleton. As a result, lionfish are not generally sophisticated cooperative hunters. The success of the genus Pterois in the Caribbean appears to be related to other characteristics rather than those in (28,31).

In regard to the experiment designs, the indicated ability of lionfish to communicate, coordinate and reciprocate by taking turns in group activities was impressive, despite their small brains (32). So, assuming the Lönnstedt study describes some actual behaviours, my findings imply that careful attention to experimental design with respect to the lionfish body and its appendix size is required to avoid obtaining seemingly sophisticated results. The 'signalling behaviour' occurred in narrow spots that require manoeuvring with the pectoral fins. The turn-taking may have resulted from evading one predator and coming to rest in front of the other in a confined area.

A crucial point to add, while this PhD focused on the cooperative hunting of lionfish, an additional study needs to be carried out regarding their prey respends in the native and the invasive range. As stated in previous studies, lionfish are opportunistic hunters that target shoal prey but eventually consume solitary prey, which could be due to the high level of vigilance of prey toward the predator. Hence, we can raise the question of what are antipredator responses and strategies of the preferred shoals of lionfish to target. Moreover, to what extent is the prey antipredator response differ between the native and the invaded range.

In extension to investigate prey anti-predator responses, an intensified study is also needed to investigate the predator-inspection behaviour of prey shoals. The previous studies of predator inspection behaviour did not include any indications of controlled predator states used in the experiments (e.g., hunger level, stress level, …etc.), which contributes to the cues transferred to the inspecting prey, to the information gained and their following responses. In addition, the multiple (repeated) inspections require revisiting, with an altered experimental setup that allows the live predator to freely leave and distance from the prey once it has been inspected. Furthermore, regarding cooperation behaviour occurring in predator inspection by shoal members, the main unsolved concerns are how paired inspections impact the chance of a predator attacking in comparison to singleton inspections, and how the predator adjusts its focus on a paired inspector depending on whether it is leading or lagging behind. We believe that the necessary experiments have not yet been carried out. Eventually, filling the gaps in the cooperative behaviours by the prey will develop the understanding of the necessity of cooperation among predator species, and to what extent it contributes to the predator-prey interaction' arms race.

D.6. References

1. King AJ, Cowlishaw G. When to use social information: the advantage of large group size in individual decision making. Biology letters. 2007;3(2):137–9.
2. Franks NR, Partridge LW. Lanchester battles and the evolution of combat in ants. Animal Behaviour. 1993;
3. Bshary R, Hohner A, Ait-el-Djoudi K, Fricke H. Interspecific communicative and coordinated hunting between groupers and giant moray eels in the Red Sea. PLoS Biol. 2006;4(12):e431.
4. Bednarz JC. Cooperative hunting Harris' hawks (Parabuteo unicinctus). Science. 1988;239(4847):1525–7.
5. Manser MB, Seyfarth RM, Cheney DL. Suricate alarm calls signal predator class and urgency. Trends in cognitive sciences. 2002;6(2):55–7.
6. Smith JM, Harper D. Animal signals. Oxford University Press; 2003.
7. Otte D. Effects and functions in the evolution of signaling systems. Annual Review of Ecology and Systematics. 1974;385–417.
8. Deneubourg JL, Goss S. Collective patterns and decision-making. Ethology Ecology & Evolution. 1989;1(4):295–311.
9. Bonabeau E, Henaux F, Guérin S, Snyers D, Kuntz P, Theraulaz G. Routing in telecommunications networks with ant-like agents. In Springer; 1998. p. 60–71.
10. Strübin C, Steinegger M, Bshary R. On group living and collaborative hunting in the yellow saddle goatfish (Parupeneus cyclostomus) 1. Ethology. 2011;117(11):961–9.
11. Liefke C, Hölldobler B, Maschwitz U. Recruitment behavior in the ant genus Polyrhachis (Hymenoptera, Formicidae). Journal of Insect Behavior. 2001;14(5):637–57.
12. Pianka ER. Evolutionary ecology. Eric R. Pianka; 2011.

13. MacArthur RH. Geographical ecology: patterns in the distribution of species. Princeton University Press; 1984.

14. Green S, Akins J, Côté I. Foraging behaviour and prey consumption in the Indo-Pacific lionfish on Bahamian coral reefs. Mar Ecol Prog Ser. 2011 Jul 18;433:159–67.

15. Ritger AL, Fountain CT, Bourne K, Martín-Fernández JA, Pierotti ME. Diet choice in a generalist predator, the invasive lionfish (Pterois volitans/miles). Journal of Experimental Marine Biology and Ecology. 2020;524:151311.

16. Côté I, Green S, Morris J, Akins J, Steinke D. Diet richness of invasive Indo-Pacific lionfish revealed by DNA barcoding. Mar Ecol Prog Ser. 2013 Jan 9;472:249–56.

17. Albins MA, Lyons PJ. Invasive red lionfish Pterois volitans blow directed jets of water at prey fish. Marine Ecology Progress Series. 2012;448:1–5.

18. Albins MA, Hixon MA. Worst case scenario: potential long-term effects of invasive predatory lionfish (Pterois volitans) on Atlantic and Caribbean coral-reef communities. Environ Biol Fish. 2013 Nov;96(10–11):1151–7.

19. Cure K, Benkwitt C, Kindinger T, Pickering E, Pusack T, McIlwain J, et al. Comparative behavior of red lionfish Pterois volitans on native Pacific versus invaded Atlantic coral reefs. Mar Ecol Prog Ser. 2012 Oct 25;467:181–92.

20. Randall JE. Reef and shore fishes of the South Pacific. University of Hawai’i Press, Honolulu. 2005;

21. Albins MA. Effects of invasive Pacific red lionfish Pterois volitans versus a native predator on Bahamian coral-reef fish communities. Biological Invasions. 2013;15(1):29–43.

22. Sarhan H, Bshary R. No evidence for conspecific recruitment for cooperative hunting in lionfish Pterois miles. Royal Society open science. 2021;8(9):210828.

23. Hunt CL, Kelly GR, Windmill H, Curtis-Quick J, Conlon H, Bodmer MDV, et al. Aggregating behaviour in invasive Caribbean lionfish is driven by habitat complexity. Sci Rep. 2019 Jan 28;9(1):783.

24. Green SJ, Côté IM. Trait-based diet selection: prey behaviour and morphology predict vulnerability to predation in reef fish communities. Journal of Animal Ecology. 2014;83(6):1451–60.

25. Fernald RD. Chapter 28 - Decision-Making in Fish: Genetics and Social Behavior. In: Dreher JC, Tremblay L, editors. Decision Neuroscience [Internet]. San Diego: Academic Press; 2017 [cited 2022 Dec 3]. p. 351–9.Availablefrom:https://www.sciencedirect.com/science/article/pii/B9780128053089000282

26. Allen G, Eschmeyer W. Turkeyfishes at Eniwetok. Pac Discovery. 1973;26:3–11.

27. Anton Gamazo A. Ecology and evolution of the lionfish invasion of Caribbean coral reefs: resistance, adaptation and impacts. 2013;

28. Lönnstedt OM, Ferrari MCO, Chivers DP. Lionfish predators use flared fin displays to initiate cooperative hunting. Biol Lett. 2014 Jun;10(6).

29. Researcher in Swedish fraud case speaks out: ‘I’m very disappointed by my colleague’ [Internet]. [cited 2022 Dec 8]. Available from: https://www.science.org/content/article/researcher-swedish-fraud-case-speaks-out-i-m-very-disappointed-my-colleague

30. Journal stands by paper facing misconduct allegations, but expresses concern about related images [Internet]. [cited 2022 Dec 8]. Available from: https://www.science.org/content/article/journal-stands-paper-facing-misconduct-allegations-expresses-concern-about-related

31. Lönnstedt OM, McCormick MI. Ultimate Predators: Lionfish Have Evolved to Circumvent Prey Risk Assessment Abilities. Browman HI, editor. PLoS ONE. 2013 Oct 16;8(10):e75781.
32. Becker M. Using relative brain size to better understand trophic interactions and phenotypic plasticity of invasive lionfish (Pterois volitans). bioRxiv. 2020;