Electronic Supplementary Material

Lionfish predators use flared fin displays to initiate cooperative hunting

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Appendix S1. Additional information on Material and Methods and Results

Detailed information on Materials and Methods

a) Study species

Dendrochirus zebra (figure S1a), a native to the Pacific, is usually found living in small (2-4 individuals) groups on or around coral bommies and caves in 3-40 meters of water. They are slow moving ambush predators that feed on small crustaceans and fish [1, 2]. During the day *D. zebra* are found hiding in crevices or under corals, but they become highly mobile at night, with most activity seen between 09.00-03.00 (Lönnstedt, personal observation). In the field, they have been observed feeding together in groups of up to four individuals, surrounding schools of small prey fish with their large fins preventing escape, individuals taking turns to swim in and strike at the prey [2]. Cooperative hunting has also been observed in other scorpaenid fishes, both in *Pterois volitans* [3] and *Pterois miles* [4, 5]. *D. zebra* co-occurs with the spotfin lionfish (*Pterois antennata*; figure S1b), another non-aggressive, nocturnal pteroine species often found living in small groups with conspecifics. Both species were collected on the reefs surrounding Lizard Island Research Station (14°40’S, 145°28’E), on the northern Great Barrier Reef (Australia) by divers using hand-nets and clove oil. As a

control species we used the freckled grouper (*Cephalophalis microprion*). It has a broad overlapping distribution with both the pteroine species but is a solitary, crepuscular hunter [6]. Groupers were collected using baited hook and line on SCUBA. All three species are often found lurking around schools of juvenile fish in the summer recruitment months. Prey (juvenile cardinalfish; *Apogon doerderlini*) were collected using light traps that had been moored along the reef crest overnight (for design see [7]).

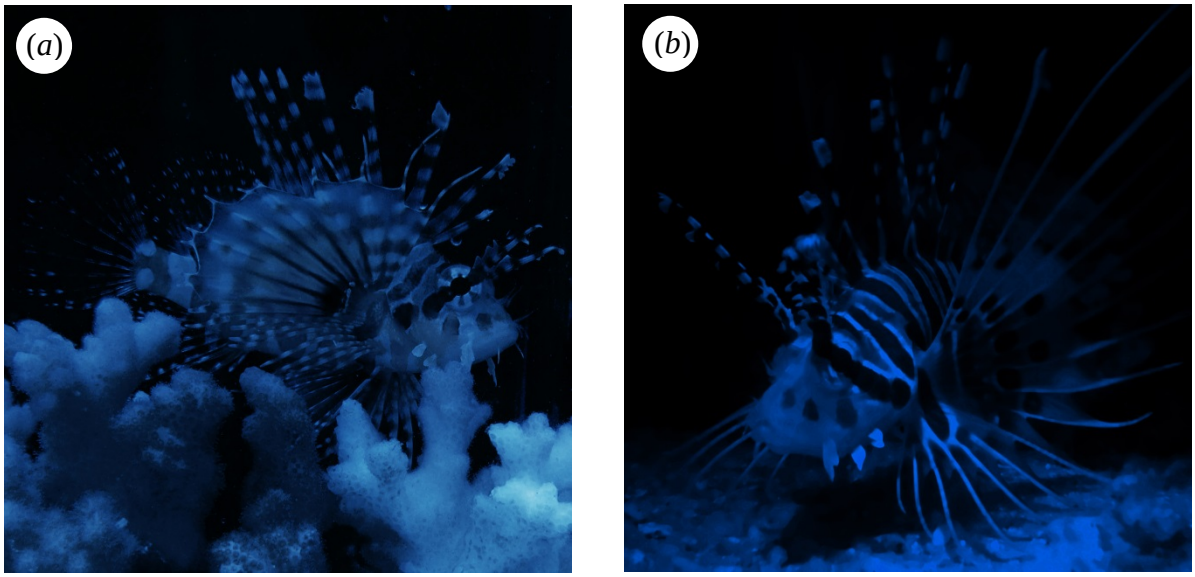

Figure S1. The two Pteroine species used in the study, both nocturnally active predators, (a) *Dendrochirus zebra* and (b) *Pterois antennata*.

Details of the experimental procedure

(i) Initiation of Cooperative Hunting

The testing chamber was designed as a maze and consisted of two compartments, separated by two opaque barriers ensuring the two compartments were obscured from the others view. The chamber design ensured test fish (hunt initiators), had to swim through a labyrinth to locate potential hunting partners (hunt responders), held in predator holding areas. *D. zebra* were placed in the experimental chambers and allowed to acclimate before observations commenced. Trials were conducted between 09.00pm and 03.00am as *D. zebra* are nocturnal predators [1]. A mirror was suspended over each tank at 45° so that fish could be viewed undisturbed from above (as per established protocols see McCormick & Lönnstedt [8]). An

underwater LED torch fitted with a No. 29 Kodak Wratten optical filter was used to illuminate the chamber. The filters only allow the transmission of light with wavelengths above 620 nm, and as the spectral sensitivity of reef fish rapidly declines above 600nm [9], the light did not visibly interfere with fish behaviours. At the start of the trial a potential partner predator (either *D. zebra* [9.46 ± 0.29 cm Standard Length SL ± SE], *P. antennata* [9.53 ± 0.52 cm SL], or *C. microprion* [9.48 ± 0.41 cm SL]) was added to the predator holding area, or empty compartments in control treatments (N = 10). To maintain statistical independence each individual predator was used for only one trial. Behaviours of test fish and any interactions with partner predators were then viewed for five minutes. After the pre-stimulus period, six prey fish [*A. doerderlini*; 1.26 ± 0.13 cm SL] were added to the prey compartment. The behaviour of focal individuals was then viewed for a further five minutes. Behavioural variables included; proportion of time spent in front of prey compartment, proportion of time spent in front of predator holding compartments, and number of flared fin displays seen from initiator predators and responder predators. A video (filmed in 'nightshot function') has been added as a supplementary file showing the lionfish initiator displaying a hunting posture followed by the fin waving and the ensuing response by the partner predator (Movie S1).

ii) Hunting efficiency

In these sets of trials a test fish (*D. zebra*) was placed in the test arena either a) alone (N=10), or together with another b) *D. zebra* (N=10) or a c) *P. antennata* (N=6) inside the predator holding area. Prey shelters consisted of a rounded coral skeleton (*Gonipora sp*.), the shelters ensure the schooling prey stick around the area but they easily evade a solitary slow moving lionfish. In the control treatment the transparent partitioning separating predators from prey was removed after one minute so test fish could reach the prey. In treatments b and c, the prey partitioning was removed when *D. zebra* initiators swam to predator holding compartments and displayed fin signals, after which partner predators were released into the testing chamber (figure S2). Trials ran for ten minutes after which the remaining prey fish were counted, collected and returned to the reef. The number of fish successfully caught per individual predator, time to first prey was captured (s) time to initiate cooperative hunt (s), the order of strikes (initiator vs. responder) and time to all fish were caught (s) was recorded.

Figure S2. The two different species of lionfish (*Dendrochirus zebra* to the left and *Pterois antennata* to the right) displaying flared fins while hunting together in the experimental labyrinth chamber.

iii) Description of communication signal

The flared fin display consists of two stages: the first is the hunting posture stage when lionfish lower their head and display flared pectoral fins and a rapidly undulating caudal fin (figure S3a; Movie S1). At this signal potential partners respond with increased activity levels. The hunting posture stage lasts from three to nine seconds and is followed by a slow waving of the pectoral fins (flared fin display stage; figure S3b-c). Lionfish initiators (*D. zebra*) would slowly undulate each fin back and forth (one at a time), while oriented in front of the potential partner predator (see Movie S1). Pectoral fins would be undulated up to four times each. After this partner predators (*D. zebra* and *P. antennata*) often responded by undulating their fins, then they would vigorously attempt to leave the predator holding area. After displaying the signal, initiators would swim off to the prey area, wait for a while and then return to the predator holding compartment and recreate the communication signal.

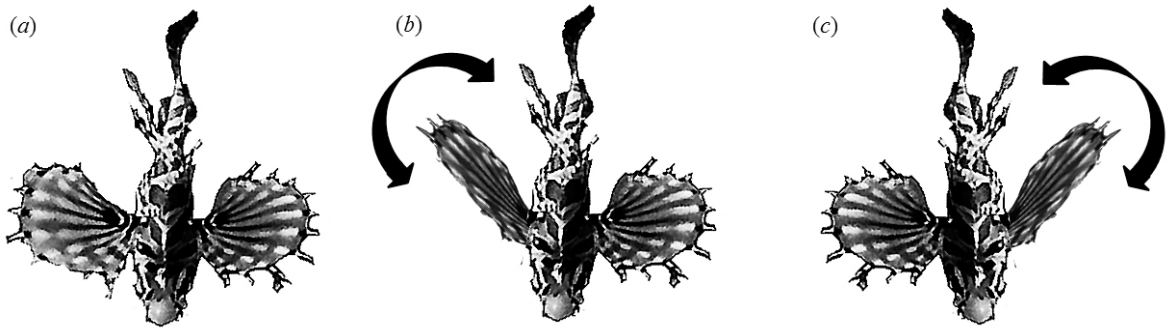

Figure S3. The communication signal displayed by the initiator predator, *Dendrochirus zebra*. This signal consisted of two stages, first initiators would display a hunting posture idiosyncratic to the pteroine predators; the head is lowered, pectoral fins are flared wide and the caudal fin is undulated repeatedly and rapidly (a). Following this stage, *D. zebra* would slowly undulate each of its pectoral fins back and forth up to four times each (b-c).

iv) Statistical analysis

Change in behavioural variables (pre-post) before and after prey were added were tested using MANOVA. Variables included in the analysis were; change in proportion of time spent in front of prey compartment, change in proportion of time spent in front of predator holding compartments, and number of flared fin displays seen from initiator predators in the four different treatments. Post hoc ANOVAs were undertaken to determine the nature of the differences in individual dependent variables found by MANOVA. Differences in time to first capture and number of prey caught between solitary individuals, two *D. zebra,* and *D. zebra* and *P. antennata* hunting partners were tested using ANOVAs. Assumptions of homogeneity of variance and normality were examined with residual analysis.

For the analysis of taking turns, we calculated the number of switches (from 0 (only one fish ate all the prey) to 5 (they diligently took turns every single time)), and compared the average number of switches to a theoretical value of 2.5 (if this was random) using a one-sample t-test (2-tailed). To compare which fish in the cooperation (initiator or responder) took the first strike at prey we combined the data for both groups and performed a $\chi 2$ test to see if the identity of the fish to first consume a prey was random or not. Independent t-test compared initiation time between conspecfic and heterospecific hunts and performed a paired t-test to compare the number of prey eaten by both fish.

Results

The switching pattern was significantly different from random for both types of cooperative hunting, with fish showing a strong alternating pattern in their foraging order (conspecific hunting: t_9=8.5, p<0.001; heterospecific hunting: t_5=5.8, p=0.002). The two hunting partners did not differ in their overall foraging success, indicating an equal share of the resources (conspecific hunting: t_9=1.4, p=0.2; heterospecific hunting: t_5=0.4, p=0.7). The order for first attack was not random: the initiator fish was most often the first one to attack a prey (χ^2_1 = 6.25, p=0.012). Conspecific and heterospecific hunting do not differ in time to initiate cooperation (t_{14}=-0.6, p=0.5), time to first attack (t_{14}=-0.2, p=0.8), time to first capture (t_{14}=-0.5, p=0.6) or total time foraging (t_{14}=-0.4, p=0.7) indicating that the species identity (*D. zebra* or *P. antennata*) of the partner predator does not influence the efficacy of cooperative hunts.

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