Damsel in distress: captured damselfish prey emit chemical cues that attract secondary predators and improve escape chances

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In aquatic environments, many prey animals possess damage-released chemical alarm cues that elicit antipredator behaviours in responsive conspecifics and heterospecifics. Despite considerable study, the selective advantage of alarm cues remains unclear. In an attempt to investigate one of the more promising hypotheses concerning the evolution of alarm cues, we examined whether the cue functions in a fashion analogous to the distress vocalizations emitted by many terrestrial animals. Our results suggest that chemical alarm cues in damselfish (Pomacentridae) may have evolved to benefit the cue sender by attracting secondary predators who disrupt the predation event, allowing the prey a greater chance to escape. The coral reef piscivore, the dusky dottyback (Pseudochromis fuscus), chemically eavesdrops on predation events and uses chemical alarm cues from fish prey (lemon damselfish; Pomacentrus moluccensis) in an attempt to find and steal prey from primary predators. Field studies showed that P. fuscus aggregate at sites where prey alarm cue has been experimentally released. Furthermore, secondary predators attempted to steal captured prey of primary predators in laboratory trials and enhanced prey escape chances by 35–40%. These results are the first, to the best of our knowledge, to demonstrate a mechanism by which marine fish may benefit from the production and release of alarm cues, and highlight the complex and important role that semiochemicals play in marine predator–prey interactions.

1. Introduction

Alarm cues are emitted by animals subjected to extreme danger [1] and many aquatic prey animals, including fishes, amphibians, molluscs, crustaceans and insects involuntarily release a chemical alarm cue when captured by a predator. These chemical cues are located in the epidermal layer and are only released upon mechanical damage (for review, see [2]). Conspecifics that detect and respond to the alarm cue benefit by obtaining an early warning of an active predator; however, it is difficult to see any direct advantage for the captured individual that releases the semiochemical [3,4]. This poses interesting questions about the evolutionary origin of chemical alarm cue systems in aquatic vertebrates [5,6]. If the alarm cue evolved as an altruistic act aimed at warning closely related individuals (kin selection [2]), then a voluntary release mechanism would be more efficient. Instead, it has been suggested that the involuntary release of chemical alarm cues in fish may have evolved analogously to the actively released distress vocalizations made by many terrestrial animals following capture (secondary predator hypothesis [4,5]).

In birds and mammals, actively released alarm calls function to attract a secondary, kleptoparasitic predator that interferes with the predation sequence, thereby providing the prey with a chance to escape [1,5,7]. Indeed, when detecting aerial predators, Belding’s ground squirrels (Spermophilus beldingi) that emit alarm vocalizations have a significantly higher chance of survival than
individuals that fail to give alarm calls [8]. Chivers et al. [9] similarly demonstrated that survival of fathead minnows, *Pimephales promelas* (a species of freshwater fish), increased in the presence of secondary predators in staged predator encounters; however, there is no evidence of selection of chemical alarm cues enhancing the survival of cue senders in fishes. While aquatic prey could potentially benefit from the alarm cues released from their injured tissues by inciting predator–predator interactions (i.e. prey may effectively be exploiting the behaviour of predators [10]), this might best be interpreted as incipient communication, and requires further study in order to understand the underlying mechanisms that may be involved in cue evolution [2].

If predatory fishes use chemical alarm cues to locate and attempt to steal already caught prey when foraging (kleptoparasitism [11]), an efficient or optimally foraging piscivore should be able to differentiate between edible and inedible prey using these cues [12]. Recently, Lönnstedt et al. [13] provided empirical evidence that a coral reef fish predator (*Pseudochromis fuscus*) was attracted to, and could distinguish size and quality of caught prey (*Pomacentrus amboinensis*) using damage-released prey chemicals. Other marine predators similarly use their olfactory sense and chemical cues to locate prey when foraging: blacktip reef sharks (*Carcharhinus melanopterus*) and grey sharks (*Carcharhinus m_equatorialis*) are attracted to the disturbance cues released from distressed grouper (*Epinephelus morio*) [14]. Furthermore, Klimley et al. [15] showed that white sharks (*Carcharodon carcharias*) hunt in relatively close proximity to conspecifics in order to ‘eavesdrop’ on predation events and kleptoparasitize caught prey, which may lead to increased feeding rates and reduced hunting costs. However, whether chemical cues released from injured prey function to attract a secondary, kleptoparasitic predator that disrupts the predation sequence thereby increasing escape chances of caught prey has never before been explicitly tested in a marine predator–prey system. Because consuming a prey can be a rapid process after a successful capture, response to these chemical alarm cues must be swift to be effective [5]. One would predict that the density of predators within an area will influence the time it takes before an interfering predator arrives [16]. Coral reefs are rich in the diversity and total number of predators and many juvenile reef fish exhibit scars (presumably from failed predator attempts), suggesting they may regularly escape from predators following an attack [13,17]. Furthermore, coral reefs are topographically complex habitats where potential kleptoparasites are more likely to detect feeding opportunities based on chemical, rather than on visual cues [18]. Also, many coral reef predators are active during crepuscular periods or at night when visual cues are limited [13,17]. Here, prey who emit chemical alarm cues may attract predators that, in their attempt to steal prey, inadvertently assist in the escape of captured individuals [5,19]. In this study, we used field and laboratory experiments to examine if chemical alarm cues released from injured damselfish prey may have evolved in accordance with the secondary predator hypothesis [4–6]. First, we tested whether secondary predators were attracted to damage-released chemical alarm cues from prey injected into territories of primary predators in the wild. Second, we examined if secondary, kleptoparasitic predators enhanced the survival of captured prey by interfering with the original predation sequence under laboratory conditions.

2. Material and methods

(a) Study species and collections

The experimental study was conducted at Lizard Island Research Station (14°40′ S, 145°28′ E), on the northern Great Barrier Reef, Australia, during three months (October–December) in 2011 and 2012. Here, the predatory dusky dottyback *Ps. fuscus* (figure 1a) can occur at densities of 35.33 per 100 m², making up a significant proportion of the predator assemblage in some areas [19]. *Ps. fuscus* is an active and cryptic mesopredator that exhibits a strong preference for preying upon juvenile damselfish (*Pomacentridae*) of a range of sizes, many large enough to prevent direct consumption [20]. *Ps. fuscus* are highly territorial, with each individual having a distinct home range site often in the vicinity of their small damselfish prey [21]. Wild adult *Ps. fuscus* [66.28 ± 3.41 mm; mean standard length (SL) ± s.e.] were collected by SCUBA divers from the shallow fringing reefs surrounding the island using a dilute solution of anaesthetic clove oil and hand nets. Individuals were kept separately inside mesh baskets in 60 l flow-through seawater tanks and fed daily with fish pellets. Prey (juvenile lemon damselfish; *Pomacentrus moluccensis* [13.5 ± 0.54 mm SL]; figure 1b) used in both experiments were collected as newly settled juveniles from the reef and maintained in groups of 20 in 60 l flow-through aquaria with shelter and fed *Artemia* nauplii twice a day. The size of *Po. moluccensis* was the same across all experiments and was chosen based on the feeding preferences of *Ps. fuscus*, ensuring prey were large enough to not be swallowed whole [20].

(b) Field experiment: chemical alarm cues as secondary predator attractants

To examine if secondary predators were attracted to chemical alarm cues released from within home ranges of resident *Ps. fuscus*, we performed in situ behavioural observations of predators within the coral reef matrix. Individual *Ps. fuscus* (60–80 mm SL) were located at different sites in the shallow reefs surrounding Lizard Island (water depth: 2–6 m). Home range territories were defined for each focal individual prior to the start of each trial (average size: 3.53 m²). To enable the introduction of chemical cues into the predator’s territory a 3 m transparent plastic tube was attached in the centre of the territory using metal skewers. This ensured minimal disturbance by observers as divers were located at least 3.5 m away from the site of cue injection. To prepare cues underwater two juvenile *Po. moluccensis* were placed in sealed plastic bags that had been filled with 300 ml of seawater. Fish inside the bags were euthanized by a quick blow to the brain case, and the fish’s epidermis was superficially cut using a scalpel blade placed inside of the plastic bag (see [13]). The prepared stimulus was relatively dilute compared with previous experiments [13,18,22], but was made to imitate concentrations that mesopredators are likely to encounter during natural predation events. A large plastic syringe equipped with a fine needle was used to perforate the bag and extract the contents of the bag. At the start of the experiment, 60 ml of the stimulus preparation was injected into the tube and flushed through with 60 ml of previously obtained seawater (to ensure that the entire stimulus cue had been flushed through). Treatments were (i) extracts from the damaged skin of juvenile *Po. moluccensis* and (ii) seawater (controls; n = 18 per treatment). At 1 min intervals, 60 ml of the preparation was then re-injected into the tube and flushed, in this way, introducing the chemical cue or seawater control in pulses over a total period of 5 min. All substances were transparent and were not visually detectable by the observer when released. Before trials commenced the current speed was estimated from the movement of neutral density particles moving over a 0.5 m distance and found not to exceed 0.05 m s⁻². Dye trials suggest that
Predator behaviours were examined when combinations between predators and prey. The light grey predator represents the primary predator that had initial access to the prey after an acclimation period. The mirror ensured that the primary predator was unaware of the secondary predator’s presence, while it received both chemical and visual cues from the primary predator’s compartment (image is not to scale). Photo credits: Oona Lönsted. (Online version in colour.)

Figure 1. (a) The predatory species, the dusky dottyback *Ps. fuscus* and (b) the prey species, the lemon damselfish *Po. moluccensis*. (c–e) The different experimental combinations between predators and prey. The light grey predator represents the primary predator that had initial access to the prey after an acclimation period. Predator behaviours were examined when (c) alone, (d) where the primary predator and secondary predator were aware of each other and (e) when a one-way mirror ensured that the primary predator was unaware of the secondary predator’s presence, while it received both chemical and visual cues from the primary predator’s compartment (image is not to scale). Photo credits: Oona Lönstedt. (Online version in colour.)

The in situ behavioural response of *Ps. fuscus* to two different treatments was tested. The response to experimental stimuli was quantified by recording: activity level of focal individuals recorded as a variable on a scale from 0 to 3 at 0.1 increments, where 0 is hiding in shelter; 1 is often retreating to shelter and taking more than 5 s to re-emerge, weakly or tentatively striking at cue source/any additional predators; 2 is shying to shelter when additional predators enter the area but quickly emerging, purposeful strikes at cue source/additional predators; and 3 is eliciting hunt and search behaviours, and striking aggressively at cue source/additional predators. Such scales have been shown to be efficient and repeatable ways of summarizing the activity of fish in the field [22,24]; percentage time that focal fish spent within 10 cm of the cue injection site; number of additional *Ps. fuscus* individuals attracted to within the focal fish’s home range territory; and number of aggressive interactions (chases or direct attacks) between focal fish and any additional conspecifics.

(c) Laboratory experiment: effects of secondary predators on escape probabilities of prey

We investigated the effects of secondary predators on escape probabilities of captured prey in a series of mesocosm experiments. *Ps. fuscus* individuals were placed in separate compartments in flow-through seawater tanks (790 × 400 × 380 mm) partitioned by a removable mid-section and allowed to acclimate overnight (figure 1c–e). A series of nylon strings enabled the observer (sitting behind a screen) to carefully release prey by removing a small tank, and also remove the main tank partition as the experiment progressed. The tank floor was covered in a 2 cm layer of coral sand, and each compartment contained a *Pocillopora damicornis* hard coral skeleton shelter (100 × 150 × 150 mm). Three identical tank set-ups were used (*n* = 20 per treatment). In the first treatment (single predator; figure 1c), an individual *Ps. fuscus* was placed in one of the compartments together with a prey fish (juvenile *Po. moluccensis*) that was located inside of a small transparent holding tank (60 × 60 × 80 mm). The predator was observed for 4 min before the prey holding tank was raised and prey released. Three seconds after the predator had caught the prey (i.e. held it in its mouth), the mid-section partitioning was removed, and the predator was observed for a further 4 min or until the prey was completely swallowed. In the second treatment, a second predator was placed in the adjacent compartment (figure 1d). The primary predator, who was aware of the presence of the second predator owing to the transparent mid-section, was again observed for 4 min prior to prey release. Immediately after the primary predator had caught the prey (i.e. held it in its mouth), the mid-section partitioning was removed, and the second predator was allowed to enter the primary predator’s compartment and ensuing interactions were recorded for a further 4 min, or until the prey was swallowed. The flow-through aquaria ensured the second predator could both see and smell the primary predator and the caught prey prior to partitioning removal. In the last experimental treatment, the mid-section partitioning was covered in a one-way mirrored tint ensuring the second predator could both view and receive olfactory cues from the adjacent compartment, whereas the primary predator was unaware of the additional predator’s presence prior to removal of the partitioning (figure 1e). Behaviours recorded during each trial included: number of times primary predators struck at the prey inside the holding tank.
during the 4 min pre-release period; average distance (cm) from the coral shelter after prey capture; successful or unsuccessful stealing attempts; and whether *P. moluccensis* successfully escaped from predators. For a *P. moluccensis* to be considered as having escaped, it had to free itself from the predator’s grasp and be within the safety of shelter for a minimum of 20 s [6]. Given that coral reefs are naturally topographically complex habitats [18], prey escaping from a failed predator attack would have a good chance of reaching the shelter of coral, thereby being safe from further attacks [17,22].

(d) Data analysis

All data were analysed using *Statistica* v. 9. A one-factor multivariate analysis of variance (MANOVA) was carried out to test whether there was a difference in overall behaviour of the primary predator in the field (experiment 1) between the two stimulus treatments (chemical alarm cue, seawater control). Variables included in the analysis were: activity level; distance from cue source; number of additional *P. fuscus* attracted; and any aggressive interactions. To further explore the significant differences in behaviour revealed by the MANOVA, one-factor ANOVAs were performed on the behavioural variables, followed by Tukey’s HSD means comparison tests.

One-factor ANOVAs were performed to test the equality of means between primary predator behaviours for the two variables measured in prey stealing trials in the laboratory (experiment 2): the number of attacks on prey inside holding compartments and distance from shelter after prey had been caught (cm). A Fisher’s exact probability test was used to compare the total number of trials in which prey escaped in control trials (single predator) versus experimental treatments (aware primary predator or unaware primary predator) in the laboratory. Prior to analysis, all data were examined for normality and homogeneity of variance using residual analysis.

3. Results

(a) Predator attraction in the field

In the wild, *P. fuscus* individuals were differentially attracted to the chemical alarm cue from damaged prey (*P. moluccensis*) and the seawater control (MANOVA: Pillai’s trace = 0.85, d.f.4,31, p < 0.0001). Focal *P. fuscus* increased activity levels in response to damage-released cues from *P. moluccensis* (*F*1,31 = 74.602, *p* < 0.0001; figure 2a) and spent a greater proportion of their time within the vicinity of the cue source outlet compared with fish exposed to control cues (*F*1,31 = 116.62, *p* < 0.0001; figure 2b).

The prey chemical alarm cue also acted as an attractant for additional mesopredators. Chemical alarm cues attracted more mesopredators to the home range of focal *P. fuscus* than when seawater alone was released (*F*1,31 = 57.11, *p* < 0.0001; figure 2c). With additional mesopredators came an
increase in the mean number of aggressive interactions between focal Ps. fuscus and secondary predators attracted to the chemical alarm cue, but not to the seawater controls ($F_{1,31} = 43.51, p < 0.0001$; figure 2f).

(b) Prey escape probabilities in the laboratory
Interactions between primary predators and prey in holding tanks prior to partition removal differed significantly depending on treatment. When alone or unaware of the presence of the second predator, primary predators struck at the prey holding tank only a few times before giving up; however, when hosts were aware of a second predator, the primary predators continually struck at the prey holding compartments (ANOVA: $F_{2,57} = 38.75, p < 0.0001$; figure 3a). When aware of the presence of a second predator, hosts were also more aggressive and oriented themselves away from the secondary predator’s compartment and towards the coral shelter (ANOVA, $F_{2,57} = 34.98, p < 0.0001$; figure 3b).

In 19 of the 20 trials, the solitary Ps. fuscus predator caught and consumed the prey individuals after prey release. However, a greater number of prey escaped the primary predator’s initial grasp when a secondary predator was allowed to directly interact with the primary predator (see electronic supplementary material, movie S1). Captured Po. moluccensis escaped in 8 of 20 trials when primary predators were aware of the presence of additional predators (Fisher’s exact probability test, $p = 0.0098$; figure 3c). Secondary predators were more likely to steal prey from unaware primary predators (electronic supplementary material, movie S2). Owing to the interference by kleptoparasitic predators, prey were able to escape in 7 of 20 trials where the primary predator was unaware of the second predator, which was significantly more often than when no secondary predator was present (Fisher’s exact probability test, $p = 0.0218$; figure 3c). Once the partitioning was removed, secondary Ps. fuscus used the element of surprise in an attempt to steal prey captured by the primary predator, but several times the damselfish successfully escaped again owing to direct interference by the primary predator.

4. Discussion
This is the first study, to the best of our knowledge, to provide direct empirical evidence that a marine predator is attracted to and aggregates around prey chemical alarm cues under natural conditions. Furthermore, survival results from our laboratory trials show that the probability that captured damselfish prey will escape from a predator increases through the interference by an additional, kleptoparasitic predator. These results highlight the potential for alarm cue senders to directly benefit by attracting secondary kleptoparasitic predators that may enhance the survival of the alarm sender by interfering with the primary predator. In our laboratory experiments, additional predators arriving at the capture site tried to snatch prey from the mouth of the first predator, which often caused the primary predator to release its prey. This allowed Po. moluccensis individuals to escape to the safety of the nearest coral shelter. The results are the first to demonstrate a mechanism by which coral reef fish prey may directly benefit from the production and release of chemical alarm cues and highlights the complex role semiochemicals play in marine predator–prey interactions.

The ecological conditions present on coral reefs are likely to have favoured the evolution of chemical distress cues. Piscivores are diverse and common on tropical reefs [25]. Here, juvenile fishes settle from the larval phase in huge numbers during the recruitment season [26], and mortality through predation is very high and selective [25–27]. Moreover, a number of studies have shown that these juveniles possess damage-released alarm cues that are important for triggering antipredator behaviours and learning the identity of predators [13,22,28]. It is likely that the prey alarm cue functions as a primary cue source that alerts secondary predators to the presence of a captured prey item, therefore attracting several opportunistic predators over larger distances. However, once in the direct vicinity of the predation event, the visual system is more likely to take over as the kleptoparasitic individuals attempt to steal the captured prey. In the current laboratory experiment, visual cues were always present which limits the conclusions we can draw about secondary predators enhancing prey escape chances solely due to alarm cue
release. However, our field study shows that predators are attracted to alarm cues, which may thus have evolved in part as a distress signal. Indeed, if the release of alarm cues gives the donor a direct survival benefit, as suggested by this study, then there will be a strong selective force to maintain these traits in the population.

The use of distress cues to attract secondary predators is not restricted to fishes. A number of animal taxa vocalize upon capture by predators (e.g. alligators [29], mammals [8], frogs [30], fishes [31] and birds [11]). Högstedt [1] illustrated that a number of terrestrial and aerial predators were attracted to the alarm call of the European starling (Sturnus vulgaris) and concluded that alarm vocalization functions to attract additional animals capable of interfering with the predation sequence (be it a parent or a secondary predator). In our study, prey escape chances increased from 5% to 35–40% when a secondary, kleptoparasitic predator was present. So while attracting additional predators may be risky, it provides the captured prey with a greater chance of survival [8,9]. The use of acoustic or chemical alarm cues to attract secondary predators upon capture may be a widespread phenomenon that has been important in the evolution of alarm calling in many predator–prey systems [3].

In species-diverse marine habitats such as coral reefs, the high abundance of predators and prey would be expected to favour prey stealing, yet examples of piscine kleptoparasites are rare [32]. In this study, the large number of attacks on the unattainable prey item by predator-aware Ps. fusca suggests they may know the likely reaction of the observing predator and are consequently more motivated to obtain and ingest the prey rapidly, before interference from the second predator. When unaware of any additional conspecifics, Ps. fusca primary predators often lost the prey owing to interference by the secondary predator. Opportunistic kleptoparasites are typically found in taxa that include vertebrate prey in their diet as they provide high energy content and can be difficult to locate and/or capture [33]. Most gape-limited piscivores attempt to swallow their captured prey fish whole; however, prior to ingestion, they often manipulate the prey in their mouth for a varying period of time in order to be able to swallow the prey head first [17,20]. The lengthy handling times often associated with many prey fish may therefore allow kleptoparasites ample time to sneak in and attempt to steal the prey, unwittingly aiding in its escape [9].

Chemical alarm cues from prey not only provide information to predators in the vicinity of a successful strike by another predator, but also provide valuable information that allows potential kleptoparasites to judge whether or not to interfere with the prey ingestion sequence. Lönstedt et al. [13] found that Ps. fuscus were differentially attracted to chemical alarm cues based on the size and body condition of the prey (Po. amboinensis), with the most attractive prey cues coming from fish that were an appropriate size for the predator’s gape and in good body condition. While it would be beneficial to the captured prey to attract any predator, regardless of size, to the site of capture to disrupt the predation sequence, the secondary predators need to balance the costs of interference against the benefits of the stolen prey item. For the potential kleptoparasites, the chemical alarm cue from captured fishes provides critical information with which to weigh up the benefits of a potentially dangerous interaction with another predator against the caloric benefit of the captured food item.

The attraction to prey chemicals is likely to have evolved in opportunistic predators as a mechanism to locate and, through surprise, steal a low-cost meal [16]. Capturing prey is a time-consuming process with a low probability of success, making stealing a low-cost alternative. A field study found that Ps. fuscus has one of the highest strike success rates of any marine predator examined to date (26% when using an ambush strategy [21]), but typically strike success rates of piscivores are low and many predators have empty guts [34]. Ps. fuscus must fuel a high metabolism and has a high predicted ingestion rate, but despite this a study of 52 individuals found that 8% of their guts were empty [21]. In this study, several Ps. fuscus predators rapidly aggregated around the sites of the experimental release of damselfish alarm cues in situ; up to three additional Ps. fuscus were attracted to the home range of focal individuals within 2 min of cue release, with the quickest predator moving into the alarm cue area within 30 s of alarm cue discharge. This suggests that being informed (passively through chemical information) about the presence of a captured prey is information that often must lead to a beneficial outcome for the secondary predator, suggesting that kleptoparasitism, though hard to observe in the field, may be commonplace.

While our evidence suggests that there are distinct benefits to the production and sequestration of compounds that act as a distress cue through the enhanced escape probability it affords via predator disruption, this advantage is unlikely to be the sole driver underlying the development of these chemicals that are only released through skin damage. The antipathogen hypothesis suggests that the alarm substance cells in Ostario- physan fish produce an antibacterial substance and initially evolved to deal with parasites or bacterial infections following injury [6]. A fish that escapes a predator attack with injuries would benefit from a damage-released antipathogen that facilitates quick wound healing [2,5]. A recent study testing this hypothesis found that alarm cell production is stimulated by exposure to skin-penetrating pathogens and parasites in fathead minnows, Po. promelas [6]. However, the predator attraction hypothesis and antipathogen hypothesis are not mutually exclusive. The alarm substance cells could initially have evolved to protect fish against bacterial infections following injury and its function as an alarm cue and predator attractant may have been secondarily acquired [5]. Selection based on the predator attraction function may drive the evolution of the senders alarm cue secretion, whereas selection on responsive kin would favour those that respond to the cue with an antipredator response.

Although damage-released chemical alarm cues have primarily been studied in the context of selection, our data suggest that predators can chemically eavesdrop on predation events and may improve alarm cue sender survival through their direct interference. Our study further strengthens the hypothesis that damage-released chemicals may originally have evolved as a secondary predator attractant aiding prey escape in aquatic environments [4,6]. Ps. fuscus used chemical alarm cues released from damaged Ps. moluccensis as aggregation cues and repeatedly attempted to steal caught prey of primary predators, significantly increasing the chances of escape for the signal sender. Taken more broadly, this work introduces a new way of viewing predatory foraging tactics in marine environments and highlights the important and complex role chemical cues play in predator–prey interactions in coral reef environments.
Ethics. This research corresponds to Australian laws and regulations under the animal ethics guidelines at James Cook University (permit number: A5193).

Authors’ contributions. M.I.M. and O.M.L. designed the experiment. O.M.L. carried out the data collection and analysis. O.M.L. wrote the paper and M.I.M. substantially contributed to manuscript revisions.

Data accessibility. The raw data are provided as electronic supplementary material.

Competing interests. We declare we have no competing interests.

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