Sensory cues of a top-predator indirectly control a reef fish mesopredator

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Behavioural trophic cascades highlight the importance of indirect/risk effects in the maintenance of healthy trophic-level links in complex ecosystems. However, there is limited understanding on how the loss of indirect top–down control can cascade through the food-web to modify lower level predator–prey interactions. Using a reef fish food-web, our study examines behavioural interactions among predators to assess how fear elicited by top-predator cues (visual and chemical stimuli) can alter mesopredator behaviour and modify their interaction with resource prey. Under experimental conditions, the presence of any cue (visual, chemical, or both) from the top-predator (coral trout *Plectropomus leopardus*) strongly restricted the distance swum, area explored and foraging activity of the mesopredator (dottyback *Pseudochromis fuscus*), while indirectly triggering a behavioural release of the resource prey (recruits of the damselfish *Pomacentrus chrysurus*). Interestingly, the presence of a large non-predator species (thicklip wrasse *Hemigymnus melapterus*) also mediated the impact of the mesopredator on prey, as it provoked mesopredators to engage in an 'inspection' behaviour, while significantly reducing their feeding activity. Our study describes for the first time a three-level behavioural cascade of coral reef fish and stresses the importance of indirect interactions in marine food-webs.

The indirect effects of predators influence the structure and dynamics of ecological communities by affecting fear and risk landscapes for prey (Schmitz et al. 2004, Creel and Christianson 2008). Evidence from both terrestrial and aquatic ecosystems, suggests that risk imposed by top-predators is strong enough to cascade through food-webs, controlling the behaviour of mid trophic-level species (i.e. mesopredators, mesoconsumers) and mediating their impact on resource prey (reviewed by Vance-Chalcraft et al. 2007). Under risk of predation, mesopredators undergo costly behavioural changes (antipredator response) in which they attempt to avoid a top-predator at the expense of foraging or other fitness-enhancing activities (Sih 1980, Lima and Dill 1990). For example, research on the wolf/ungulate/aspen system in North America shows that the removal of predation risk by depletion of large carnivores triggers an ecological and behavioural 'release' of mesopredators, which often leads to a depletion of resource prey species (reviews by Prugh et al. 2009, Ritchie and Johnson 2009, Estes et al. 2011, Ripple et al. 2014). While the theory of tropic cascades is well developed, there is limited field evidence on the connections among trophic levels and how populations change in response to perturbations in the trophic links.

Coral reefs are among the most diverse ecosystems, with up to 200 species of fish macro-carnivores interacting in complex food-webs (Bellwood et al. 2004). The balance of the coral reef food-web could be, however, under threat as the overexploitation of large-predatory fishes (Jackson et al. 2001, Myers and Worm 2003, Robbins et al. 2006) may eliminate the predation risk that behaviourally controls many marine mesopredators (reviewed by Heithaus et al. 2008). Recent field-evidence already shows that in the absence of top-predators, mid-size fishes increase their feeding rates, foraging area and activity periods, likely having negative effects on the population of resource prey (Stallings 2008, Madin et al. 2010a, McCauley et al. 2010, Ruppert et al. 2013, Rizzari et al. 2014). Nevertheless, because much of the research has focused on mid-level herbivores (which feed on behaviourally simple organisms such as plants or algae; Madin et al. 2010b, Rizzari et al. 2014), we have limited understanding on how indirect effects can be transmitted though the fish food-web, or how mesopredator release can lead to strong behavioural suppression of prey fish populations. Despite many authors highlighting the strength of behavioural cascades and the importance of trait-mediated indirect interactions (reviewed by Werner and Peacor 2003, Schmitz et al. 2004), most evidence comes from terrestrial and freshwater ecosystems. Marine ecologists have seldom studied the behavioural linkages between more than two-trophic levels simultaneously (reviewed by Preisser et al. 2005), and never (to our knowledge) using a three-level food chain of fish. Detailed studies of mesopredator fish behaviour are needed to elucidate

cascading indirect risk effects in coral reefs and understand the indirect consequences of large-carnivore declines in marine systems (Heithaus et al. 2008, Baum and Worm 2009).

To understand the nature of the linkages among trophic groups it is necessary to first comprehend how predators and prey interact. Aquatic vertebrates typically assess predation risk through chemical and visual stimuli (Helfman 1989, Murphy and Pitcher 1997, Kats and Dill 1998, Brown et al. 2011) which contain different information on the type, temporal resolution and intensity of the threat (Smith and Belk 2001). Chemical cues, such as predator-released odours (i.e. kairomones) or conspecific damage-release alarm cues inform prey of the characteristics of the threat over large spatio-temporal scales (Kats and Dill 1998, Wisenden 2000, Ferrari et al. 2010, Brown et al. 2011). These cues are usually complemented by visual stimuli (e.g. sight of predator) which are indicative of immediate threat and give more accurate information on the location, behaviour and motivation of the predator (Helfman 1989, Murphy and Pitcher 1997, Hartman and Abrahams 2000, Smith and Belk 2001). The dominance of one or the other cue depends on both the environment and context of the cue transition (McCormick and Lönnstedt 2013). Determining how meso-predators respond to sensory cues from top-predators is key to understanding the behavioural mechanisms that underlie indirect risk effects.

To extend our understanding of the indirect top–down control of fish community dynamics, we studied how risk elicited by top-predator cues (chemical and visual stimuli) affected mesopredator behaviour and modified their interaction with resource prey. Using experimental mesocosms, we used a three-level food-web of coral reef fishes (coral trout/dottyback/damselfish recruits) to address the following questions: 1) how is the activity, space use and feeding behaviour of a mesopredator modified by cues from a top-predator? 2) does the magnitude of the antipredator response by meso-predators vary with the type of cue present (chemical or visual stimuli) or the interaction amongst cues? 3) are top-predator cues enough to behaviourally control the mesopredator foraging activity? And 4) how is the behaviour of resource prey modified by changes in mesopredators behaviour that results from the presence of top-predator cues? Answering these questions allowed us to describe for the first time a three-level behavioural cascade of coral reef fish and stress the importance of indirect interactions in marine food-webs.

Methods

Study species and fish handling

Fish were collected from the lagoon of Lizard Island (14°40′S, 145°28′E), northern Great Barrier Reef (GBR), during the austral summer months (October–January). *Pseudochromis fuscus* (brown dottyback) was used as the focal mesopredator species, as it is a small site-attached carnivore (up to 10 cm total length; TL) known to voraciously consume newly settled fishes in both the laboratory and the field (Beukers and Jones 1998, Feeney et al. 2012). Adult dottybacks (6.9 ± 0.09 cm, mean TL ± SE, n = 50) were captured from shallow patch reefs by SCUBA divers using hand nets and an anesthetic clove oil solution. Resource prey consisted of planktivorous juveniles of the whitetail damselfish, *Pomacentrus chrysurus* (12.7 ± 0.03 mm, mean standard length SL ± SE, n = 300), collected with light traps just before their recruitment on the reef. Traps were moored overnight at least 100 m away from the reef edge and collected at dawn. The top-predator used was the leopard coral trout *Plectropomus leopardus*. This large carnivore is relatively common on the GBR (3.5 to 9.0 fish 1000 m²; Ayling et al. 2000), reaches more than 50 cm SL, and feeds predominantly on small sized (< 9 cm) reef-associated fish (St John 2001). The thicklip wrasse *Hemigymnus melapterus*, was used as the non-predator species. Despite its large size (> 30 cm TL) it feeds mainly on small invertebrates like crustaceans, polychaete worms and mollusks (Randall 2013). Individuals of *P. leopardus* (39.3 ± 2.6 cm, mean TL ± SE, n = 4) and *H. melapterus* (33.4 cm TL) were caught using hand lines (with barbless hooks) and barrier nets, respectively.

Fishes were maintained in separate tanks at the Lizard Island Research Station, in a flow-through seawater system at ambient temperatures and light photoperiods. Damselfish recruits were fed *Artemia* spp. twice daily, while the rest of the collected fishes (dottybacks, coral trouts and the thicklip wrasse) were daily fed thawed prawn or squid. All fish were released at the end of the experiment back to their collection site.

Experimental overview

Changes in the behaviour of the focal mesopredator (dottybacks) to five experimental treatments were quantified: 1) visual cue of the top-predator; 2) chemical cue (odour) of the top-predator; 3) simultaneous chemical and visual cues of the top-predator; 4) simultaneous chemical and visual cues of the non-predator; and 5) chemical and visual cues of an empty tank. The first three treatments (1, 2 and 3) corresponded to top-predator treatments, while the last two (4 and 5) served as controls. Behavioural observations were recorded on six resource prey (damselfish recruits) while the mesopredators were exposed to the experimental treatments. Ten replicate trials were undertaken for each cue treatment, with all fish being tested only once to maintain independence among trials.

Observation mesocosms

The experiments were undertaken in five replicate 368-l pools (referred hereafter as mesopredator arena; 112.5 cm diameter × 40 cm height; water height ~ 28 cm), containing a piece of rubble head (20 × 20 × 15 cm), an air stone, a 2 cm layer of sand, a 30 cm ruler (used for reference in the video recording) and two glass tanks located on opposite ends (a rectangular for the top-predator and a cylindrical for the resource prey; Appendix 1 Fig. A1). The top-predator tank (also used for the non-predator; 36 × 47 × 40 cm) was screened on all sides with removable black Perspex panes, while the cylindrical prey tank (24 cm diameter × 38 cm height) was clear plastic with 32 small holes (> 0.5 cm) to allow exchange of chemical cues between the mesopredators and the resource prey. Two video cameras were positioned on tripods over the observational mesocosms; one was high above the meso-

predator arena to record the movements of the dottybacks, while the other was placed over the prey tank to document the behaviour of the damselfish recruits and the feeding strikes of the dottybacks on the tank. The five experimental treatments were randomly assigned daily to each of replicate observational pools. After running each trial the seawater from the pool was completely exchanged and all the objects (e.g. rubble head, ruler) were thoroughly rinsed with saltwater to remove residual chemical cues.

Experimental protocol

Before running experimental trials, damselfish recruits were trained to recognize cues from the dottybacks as their collection prior to reef settlement may have prevented them from learning the visual and chemical identity of reef-associated predators. Naïve juvenile fishes can learn the identity of a novel predator by pairing simultaneously conspecific damage-release chemical cues (indicative of threat) with visual and/ or chemical cues of a predator (Brown and Chivers 2005, Ferrari et al. 2010, Lönnstedt et al. 2013). To prepare the damage-released chemical cues, three damselfish per training session (12–14 mm SL) were euthanised with a quick blow to the head (following James Cook University animal ethics permit: A1720) and placed in a petri dish where 10 superficial cuts were made to the skin of each donor fish (5 cuts per flank). Fish were then rinsed with 10 ml of sea water (previously obtained from their tank) creating a solution of damage-release alarm cues. To obtain the mesopredator odour (kairomones), four dottybacks were randomly selected and kept for at least 12 h in a tank containing 4-l of aerated seawater. Training of the damselfish recruits occurred by introducing simultaneously in their 22-l holding tank (with water flow suspended): 10 ml of the conspecifics damaged-released chemical cues; 30 ml of the dottyback odour; and a live dottyback placed in a sealed ziploc bag (serving as a visual cue). After 10 min all cues were removed from the tank, and water flow was restored. Three training sessions were carried out in total, each one comprising the training of ~ 100 damselfish recruits.

Experimental trials were initiated with a 5 min acclimation period of the coral trout and the six damselfish recruits (instead of just one, to further stimulate the activity of the dottyback) in their respective observation tanks. A dottyback (previously starved for 48 h) was then introduced in to the arena and its pre-stimulus behaviour was video recorded for 10 min. The assigned stimuli was revealed afterwards by: 1) removing the black panes from the top-predator tank thereby allowing the mesopredator sight of the top-predator; 2) overflowing the top-predator tank to spill coral trout odour (chemical cue) into the mesopredator arena; or 3) simultaneously performing (1) and (2) to expose the dottybacks to both visual and chemical cues from the top-predator (or the non-predator species or the empty tank). Immediately after exposure to the cues, the post-stimuli behaviour of the dottybacks was video recorded for 10 min. During this last sampling period, a second video camera positioned over the damselfish recruits recorded their activity. A total of 50 dottybacks and 300 damselfish recruits were used in the experiments. Due to the availability of only four coral trouts, these were randomly assigned to the three top-predator treatments.

Behavioural assessment

Five behavioural attributes of the dottybacks foraging activity were quantified from the pre- and post-stimulus 10 min video recordings: 1) percentage of time spent in shelter; 2) total distance moved (cm); 3) distance swum per trip outside the shelter (cm); 4) total area explored (cm²); and 5) number of feeding strikes (no. / 10 min). Measures of space use (2, 3 and 4) by the dottyback were obtained from the recordings using ImageJ software to calculate the length of each trip (distance swum per trip outside the shelter), the total distance swum (sum of all the individual trips) and the area explored (area of the convex polygon formed when joining the outermost locations ventured).

The antipredator behaviour of the six damselfish recruits was assessed during the 10 min post-stimulus period (while dottybacks were under the effect of the experimental treatments) by estimating their use of space under maximum predation risk. For videos in which the dottybacks struck the damselfish tank (e.g. during post-stimulus periods without top-predator cues), the exact moments of the strikes were designated as instants of maximum predation risk, and among these, 10 frames were chosen haphazardly for analysis. Conversely, for videos that lacked dottyback strikes (e.g. during many post-stimulus periods where top-predator cues were present), we assumed that the maximum predation risk was constant throughout the time, and we selected haphazardly 10 frames to analyze. For each video frame assessed, the position of each damselfish was recorded in relation to the source of risk (location of the dottyback or its strike) and classified into seven distance bands (–12 cm, –9 cm, –6 cm, 0 cm, + 6 cm, + 9 cm, + 12 cm) radiating from the center of the cylindrical prey tank (24 cm diameter). Fish located from the center of the tank (0 cm band) towards the source of risk were assigned to a positive (+) band, with the '+ 12 cm band' being indicative of a specimen located at the minimum distance from the source of risk. In contrast, a damselfish within the '–12 cm band' was positioned the furthest away from the dottyback and considered to have the strongest antipredator behaviour.

This approach was specifically chosen as it allowed a comparison of the damselfish antipredator behaviour (measured as space use) across all treatments while indirectly including the inherent variations in the predation pressure between trials. For trials in which the dottyback was exerting high predation pressure (due to the lack of top-predator cues) the 'maximum predation risk' was when the dottyback struck, however, for trials in which the dottyback was exerting low predation pressure and never struck (due to top-predator cues), the predation risk (exerted by the activity and presence of the dottyback alone) was constant throughout all frames and any could be selected as a moment of maximum risk.

Statistical analysis

Changes in the dottyback behaviours between the pre- and post-stimulus observation periods were calculated and used as raw data in the analyses. The five behaviours were not independent from each other so they were compared among treatments using a one-way multivariate analysis of variance (MANOVA). Subsequently, ANOVAs and orthogonal

planned comparisons were used to contrast the behaviours between specific pairs of treatments: Non-predator treatment (4) versus Empty tank treatment (5); Control treatments (4, 5) versus Top-predator treatments (1, 2, 3); Visual cue treatment (1) versus Chemical cue treatment (2); Isolated top-predator cue treatments (1, 2) versus Combined chemical and visual cue treatment (3). The antipredator response of the damselfish recruits was compared among treatments with a one-way analysis of covariance (ANCOVA) using the number of feeding strikes during the pre-stimulus period as a covariate. This approach was used to reduce within-treatment error variance and to take into account any effect that pre-stimulus strikes could have on the post-stimulus behaviour (through conditioning or learnt behaviour of the damselfish recruits). The ANCOVA was followed by the same planned comparisons used for the dottybacks. Residual analyses were used to examine if the data satisfied the assumptions of normality and homoscedasticity. All the behavioural data from the dottybacks were normal and homoscedastic, however, data from the damselfish recruits was square root transformed to meet the assumptions of parametric tests.

Data available from the Tropical Data Hub Research Data repository: doi: 10.4225/28/5507A0EF761F7 (Palacios et al. 2015).

Results

The behaviour of the dottybacks was significantly affected by the experimental cue treatments (MANOVA, $F_{6,40} = 3.23$, $p < 0.001$) and all five variables measured, were in part, responsible for this difference (ANOVAs, $p < 0.001$; Table 1). Dottybacks exposed to cues from the top-predator had contrasting behavioural changes to fish exposed to both control treatments. During the pre-stimulus period, all dottybacks were very active exploring the arena and striking at the damselfish recruits. However, dottybacks exposed to top-predator cues during the post-stimulus period exhibited a significant increase in the percentage of time spent sheltering as well as a reduction in the total distance swum, the total

area explored, the distance swum per trip and the number of feeding strikes in relation to those exposed to control treatments (Fig. 1a, c, e, g, i; planned comparisons, $p < 0.001$; Table 1). It was noteworthy, however, that both control treatments showed different patterns for certain behaviours. Dottybacks exposed to cues from the non-predator significantly reduced the distance swum per trip and the number of feeding strikes in comparison to those exposed to the empty tank (Fig. 1g, i; planned comparisons, $p > 0.05$; Table 1).

Dottybacks had very similar behavioural changes when exposed independently to chemical or visual cues from the top-predator (Fig. 1b, d, f, j; planned comparisons, $p > 0.05$; except for distance/trip, Fig. 1h, Table 1). The presence of any of these predatory-cues restricted the activity and foraging of the dottybacks, and increased the time spent in shelter by almost 40%. For three out of the five behaviours the response of the dottybacks differed between the isolated cue treatments and the combined visual and chemical cue treatment. Pairing of both sources of risk elicited a stronger behavioural response measured as a significant increase in the percentage time spent sheltering, and significant reductions in the total area explored and the distance swum per trip (Fig. 1b, f, h; planned comparisons, $p > 0.05$, Table 1).

The space use of the damselfish recruits during the post-stimulus period was not explained by the number of pre-stimulus strikes ($F_{1,44} = 0.08$, $p = 0.77$), but it significantly differed among the five experimental treatments (ANCOVA, $F_{4,44} = 16.08$, $p < 0.001$). During the control treatments damselfish recruits stayed far away from the strikes of the dottybacks (using mostly the negative bands; -4.7 ± 0.3 cm), while in the top-predator treatments they remained significantly closer to the source of risk ($+1.5 \pm 0.7$ cm; planned comparison, $p < 0.001$, Fig. 2a, Table 1). Although the space use of the damselfish was relatively similar among the three top-predator treatments (planned comparisons, $p > 0.05$, Fig. 2b, Table 1), we did detect differences between the two control treatments (planned comparison, $p > 0.05$, Fig. 2a; Table 1). Damselfish positioned themselves significantly closer to the source of risk when the dottybacks were being exposed

Table 1. Summary of F-values for the ANOVAs, ANCOVA and planned comparisons examining five behaviours of the dottybacks (mesopredators) and the space use (distance from source of risk) of the damselfish recruits (resource prey). ANOVAs were ran to test the behaviours of the dottybacks among the five treatments, while an ANCOVA was used to test the space use of the damselfish recruits. The number of feeding strikes in the pre-stimulus period was used as the covariate in the ANCOVA, however, this variable did not significantly explain the space use of the damselfish recruits during the post-stimulus period ($F_{1,44} = 0.08$, $p = 0.77$). Degrees of freedom: ANOVAs (4, 45); ANCOVA (4, 44); Planned comparisons (1, 45). Treatments: C = Chemical cues (top-predator released odours), V = visual cues (sight of the top-predator). Asterisks indicate significant differences where * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$.

|		Mesopredator: dottyback					Resource prey: damselfish
Analysis		Time in shelter (%)	Total distance (cm)	Total area (cm²)	Distance/ trip (cm)	Feeding strikes (no. / 10 min)	Distance from the source of risk (cm)
	ANOVA/ ANCOVA	12.72***	7.04***	9.59***	11.06***	7.29***	16.08***
Planned comparisons	Empty tank versus Non-predator treatment	0.1	1.06	3.86	4.76*	12.13**	4.11*
	Control treatments versus Top-predator treatments	44.11***	24.70***	23.2***	24.42***	14.77***	57.02***
	C versus V	0.71	1.81	3.95	10.74**	0.29	0.06
	C, V versus C and V	5.97*	0.59	7.35**	4.33*	1.72	0.45
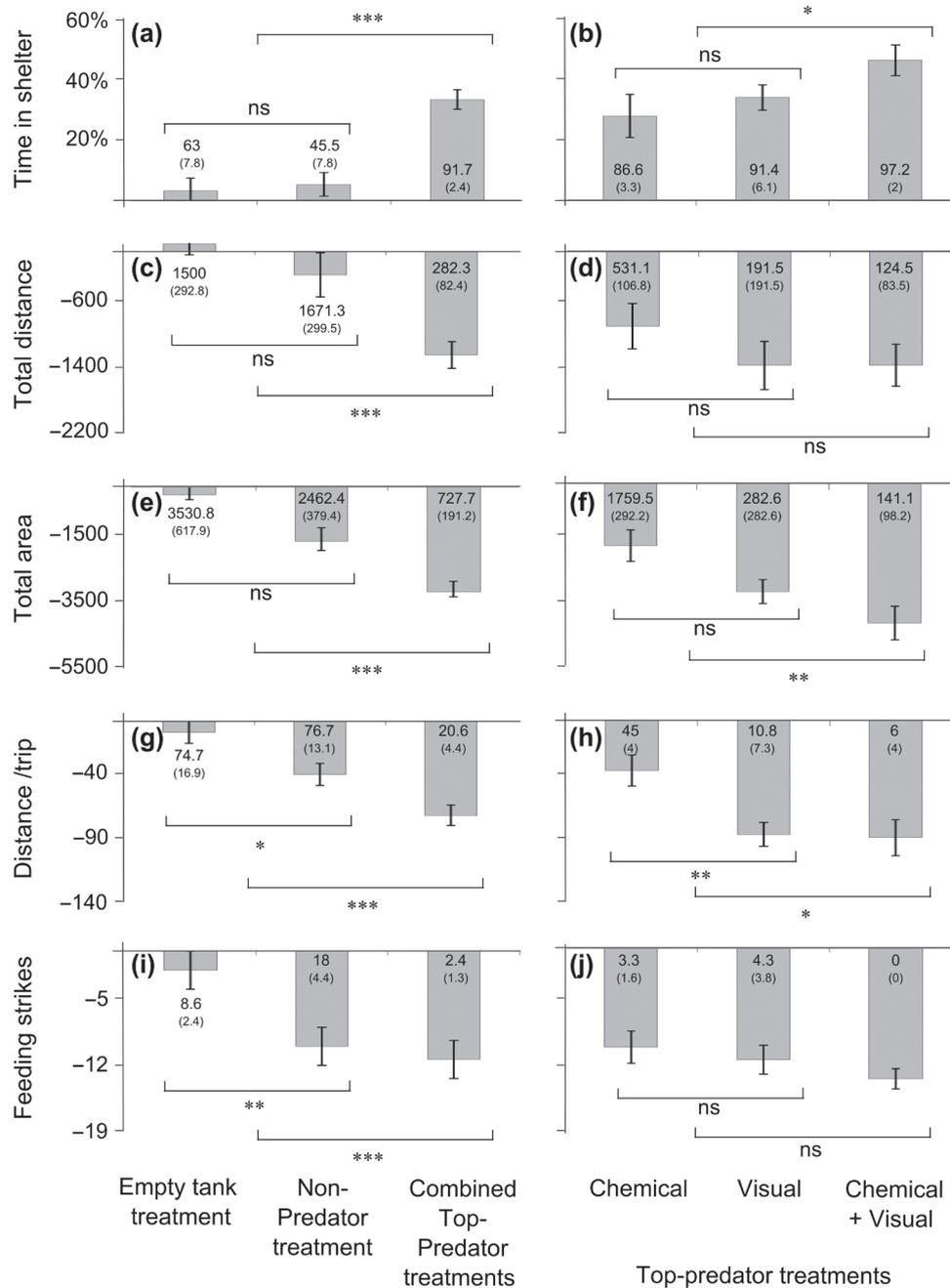
Figure 1. Change (mean ± SE) in (a, b) time spent in shelter (%), (c, d) total distance moved (cm), (e, f) total area explored (cm²), (g, h) distance swum per trip (cm), and (i, j) feeding strikes (no./10 min) by dottybacks (mesopredators) between the 10-min pre and post-stimulus periods. Fish were exposed to: the visual cue of a top-predator (coral trout); the chemical cue (odour) of a top-predator; the co-occurrence of chemical and visual cues of a top-predator; the co-occurrence of chemical and visual cues of a non-predator (thicklip wrasse); and the co-occurrence of chemical and visual cues of an empty tank. Feeding strikes were directed towards six damselfish recruits (resource prey) within the mesocosm. A positive value indicates an increase in activity and a negative value indicates a decrease in activity. Asterisks above or below the bars represent significant differences between the planned comparisons (* = p > 0.05; ** = p < 0.01; *** = p < 0.001). Numbers inside the bars indicate the values recorded during the post-stimulus period [mean (± SE)].

to the non-predator (thicklip wrasse) than when exposed to the empty tank.

Discussion

Our study demonstrated a cascade of indirect risk effects in which the behaviour of mid- and lower-trophic-level species was determined by the risk of predation from top-predators. When experimentally exposed to predatory-cues from a macro-carnivore (coral trout), dottybacks altered their foraging behaviour, decreasing by more than 70% the distance swum, area explored and feeding attacks on damselfish recruits (resource prey). Suppression of the mesopredator activity correspondingly led to a decline in the level of risk they posed to damselfish, as the recruits
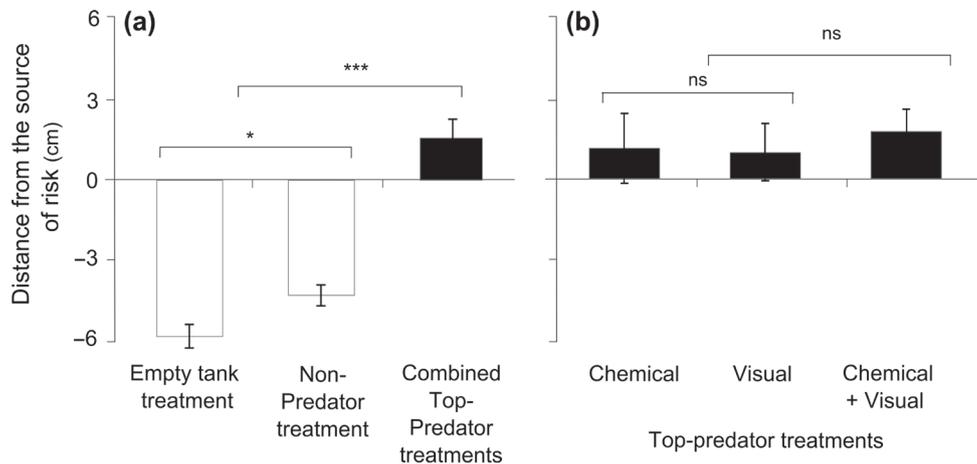
Figure 2. Distance (mean ± SE) from the source of risk (location of the dottyback or its strike) at which damselfish recruits (resource prey) were positioned during moments of "maximum predation risk" within the post-stimulus period. Dottybacks (mesopredators) were under the effect of the five experimental treatments: the visual cue of a top-predator (coral trout); the chemical cue (odour) of a top-predator; the co-occurrence of chemical and visual cues of a top-predator; the co-occurrence of chemical and visual cues of a non-predator (thicklip wrasse); and the co-occurrence of chemical and visual cues of an empty tank. Positive values are indicative of fish with low predator avoidance behaviour (black bars) located close to the risk source, while negative ones are indicative of fish with higher predator avoidance behaviour (white bars) located far away from the source of risk. Asterisks above the bars represent significant differences between the planned comparisons (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

altered their behaviour by coming closer to the source of risk (location of the dottyback or its strike) by 37%. While a previous study illustrated links between risk-mediated mesopredator behavioural changes and the recruitment of reef fishes (possibly through direct, lethal effects; Stallings 2008), the present study is the first to provide evidence that such behavioural alterations can simultaneously modify the behaviour of lower trophic-level species.

Visual or chemical cues from the predatory coral trout triggered a strong antipredator response in the dottybacks, suggesting the presence of indirect top–down control over this mvesopredator. Exposure to either of the predatory-cues independently was enough to reduce the overall activity and feeding rate of the dottybacks, however, the strongest response occurred when visual and chemical cues were presented simultaneously. Many studies on marine and fresh water fishes have shown that multiple sensory cues provide organisms more complete information than any one cue in isolation (Smith and Belk 2001, Amo et al. 2004, Dalesman and Inchley 2008, McCormick and Manassa 2008). Although visual cues, such as the sight of the predator, are indicative of high risk of predation (Helfman 1989, Murphy and Pitcher 1997, Hartman and Abrahams 2000), two laboratory experiments also show no clear difference in the magnitude of behavioural changes elicited by isolated visual and chemical cues (McCormick and Manassa 2008, Milano et al. 2010). The magnitude of the antipredator response is usually dependent on the concentration of risk cues (Dupuch et al. 2004, Zhao et al. 2006, Holmes and McCormick 2011), so it is likely that the relatively strong behavioural changes triggered by chemical cues in these latter studies are due to a high concentration of predator-odour or alarm cues. Chemical cues appear to be resembling a threat as eminent as the sight of the top-predator.

Unexpectedly, the large non-predator also modified the behaviours of the mesopredator. The thicklip wrasse triggered changes in the feeding rate and the distance swum per trip of dottybacks. This was because the dottybacks without reducing their overall activity, shifted their interest from attacking the damselfish recruits to engaging in an 'inspection behaviour' with the large non-predator. Such behaviour is frequently observed in freshwater fish as they tentatively approach a novel predator or potential threat to acquire extra information (Murphy and Pitcher 1997, Smith and Belk 2001, Brown and Magnavacca 2003). Although non-predator species not always trigger behavioural changes on their prey (McCormick and Manassa 2008), a recent study also found that non-predators can reduce the prey foraging activity. Marsh-Hunkin et al. (2013) report that although their non-predator species (French grunt *Haemulon flavolineatum*) did not trigger a reduction in the movement of prey gobies (in comparison to predators like the red lionfish and the Nassau grouper), it did cause a decrease of their feeding activity similar to the one caused by the predators. More research is needed to understand the behavioural effects that non-predators have on lower-trophic levels, however, recent evidence suggests that large non-predators might be playing an important role distracting mesopredator fish from their foraging activities. In this way, large organisms (despite their trophic status) could also be mediating the impact of mesopredators on resource prey. Although in the present study their effect was not as strong as the presence of the top-predator, the wrasse led indirectly to positive changes in the behaviour of the damselfish recruits. These results have important implications for management as they show that not only can the loss of macro-carnivores trigger behaviourally-induced cascades, but the loss of macro-invertivores or herbivores may also lead to the release of mesopredators. To judge the importance of indirect effects of predators on lower-trophic groups we also require an understanding of the relative magnitude of the behavioural effects of non-predators.

Changes in the mesopredator behaviour due to top-predator cues, allowed damselfish recruits to modify their space use and exhibit significantly lower antipredator behaviour. Although the presence of the non-predator did not have an effect as strong as the top-predator, it also led indirectly to positive changes in the space use of the damselfish recruits. Taking into account that organisms make tradeoffs between predator avoidance and other fitness-enhancing activities (Sih 1980, Lima and Dill 1990), the reduction of the antipredator response could be particularly beneficial to the fitness of individuals in vulnerable early ontogenetic stages (Almany and Webster 2006). They could potentially increase their space use, activity and foraging which should lead to a higher energy allocation to growth and later reproduction.

Our results must be considered in the context of previous studies, as the strength of the mesopredator's antipredator response to predatory-cues may be influenced by the species, the behavioural history of the individuals, the habitat conditions, and even the personality of the fish (Chivers et al. 2001, Lima and Steury 2005, Ferrari et al. 2010, Martin et al. 2010, Marsh-Hunkin et al. 2013). Laboratory-based experiments have limitations, such as the ecological relevance of the concentrations of cues, the context of the trials (e.g. procedure, size of the tanks), and the stress of captivity, which can all influence the overall outcome and result in exaggerated manipulations of the natural systems (Irving and Magurran 1997, Blanchet et al. 2007, Kim et al. 2009, Rizzarri et al. 2014). For the present experiment, the concentration of the top-predator cues and the stress that fish normally undergo in captivity may have affected the results. Although our experimental setup could have allowed a potential indirect effect of the coral trouts on the damselfish recruits (Appendix 1 Fig. A1), the coral trouts here employed (~ 40 cm TL) usually prey on bigger damselfish (> 50 mm SL; Kingsford 1992, St John 2001) and do not represent a major threat to the recruits used in our study (< 14 mm SL). Furthermore, because the damselfish recruits were predator-naïve and only conditioned to recognize the smell and sight of dottybacks, it is unlikely they identified the coral trout as their predator. Despite their shortcomings, laboratory experiments are an important tool to study trophic cascades of indirect effects as they allow detailed behavioural observations and strict control over the risk of predation. We consider that only through the combination of field- and laboratory-based approaches will we be able to understand the mechanisms underlying the indirect effects of mesopredator control (Schmitz et al. 2004) and the community dynamics in ecosystems as complex as coral reefs.

Mesopredators constitute an important functional group in most food-webs due to their high abundances, population growth rates, voracity and activity levels (Prugh et al. 2009, Ritchie and Johnson 2009, Ripple et al. 2014). In coral reef systems, their behavioural release could represent a major threat to the assemblage of post-settlement fishes (resource prey) as mesopredators have strong negative effects on the persistence (direct effects; Carr and Hixon 1995, Almany and Webster 2006, Feeney et al. 2012), phenotypic selection (Gagliano et al. 2007) and behaviour of low trophic-level species (indirect effects; McCormick and Manassa 2008, Holmes and McCormick 2011, Marsh-Hunkin et al. 2013). Although intra- and interspecific interactions (i.e. hunting synergies or interference competition; Sih et al. 1998) will affect how mesopredator release is manifest, our results suggest that the reduction of top-predators may have important effects on coral reef fish interactions, as even odours from top-predators can suppress the activity of mesopredators and lower their impact on juvenile fishes. Although top–down processes and trophic cascades are difficult to detect and study in natural marine ecosystems (Heithaus et al. 2008, Baum and Worm 2009), comparable behavioural links to those found here have already been reported in the field. For example, tiger sharks are known to indirectly influence the habitat selection of mesopredators (dolphins) and herbivores (dugongs), whose spatial distributions are sensitive to densities of top-predators (Heithaus and Dill 2006, Wirsing et al. 2007). Evidence also shows that the presence of fur seals reduces the foraging effort of morwong fish on turf algae (Connell 2002) and that the presence of Pacific sleeper sharks indirectly protects the walleye pollock from predation by the harbor seals (Frid et al. 2007). Further studies are required to fully understand indirect risk effects in marine ecosystems, however, evidence is starting to reveal the importance of top-predators in the protection of low-trophic level guilds and the key role that risk effects might play in marine trophic cascades.

Given the speed at which populations of large predators are being depleted (Jackson et al. 2001, Ceballos and Ehrlich 2002, Myers and Worm 2003, Robbins et al. 2006) and at which phase shifts are occurring worldwide (Hughes et al. 2007, Estes et al. 2011) it is imperative to understand the effects of predator removal on community dynamics. The indirect release of mid-ranked herbivores from both terrestrial and marine ecosystems has been relatively well studied in comparison to the release of mid-ranked carnivores (Ritchie and Johnson 2009). Our study expands the work of Stallings (2008) and, suggests that in marine systems even isolated predatory-cues, such as the sight or the odour of a macro-carnivore, can suppress the foraging behaviour of mid-ranked fish and relax their indirect effect on low trophic-level species. Strong indirect effects, such as the ones found here, have the potential of strengthening trophic cascades triggered by the loss of top-predators. Understanding intraguild dynamics among predators will not only serve to better predict the consequences of predator loss, but also aid the management of coral reef fisheries (Dill et al. 2003, Heithaus et al. 2008).

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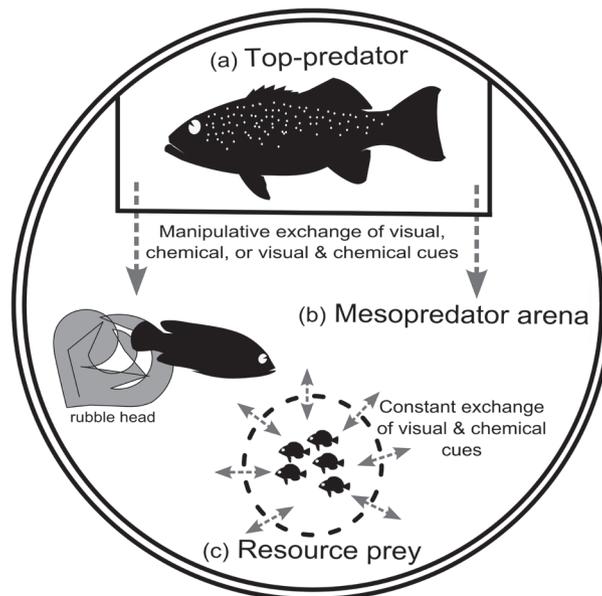
Appendix 1

Figure A1. Illustration of the observation mesocosms constructed to assess the indirect interactions between a three level food-web of coral reef fish. (a) rectangular tank to hold the top-predator (coral trout *Plectropomus leopardus*) or the non-predator (thicklip wrasse *Hemigymnus melapterus*); (b) mesopredator arena where *Pseudochromis fuscus* (dottybacks) could swim freely and indirectly interact with top-predator and/or prey; and (c) cylindrical tank to hold the resource prey (damselfish recruits, *Pomacentrus chrysurus*). The top-predator tank (also used for the non-predator; $36 \times 47 \times 40$ cm) was screened on all sides with removable black Perspex panes, while the cylindrical prey tank (24 cm diameter $\times$ 38 cm height) was clear plastic with 32 small holes (< 0.5 cm) to allow exchange of chemical cues between the mesopredators and the resource prey.