Primary and secondary defences of squid to cruising and ambush fish predators: variable tactics and their survival value

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ABSTRACT

Longfin squid (*Loligo pealeii*) were exposed to two predators, bluefish (*Pomatomus saltatrix*) and summer flounder (*Paralichthys dentatus*), representing cruising and ambush foraging tactics, respectively. During 35 trials, 86 predator–prey interactions were evaluated between bluefish and squid, and in 29 trials, 92 interactions were assessed between flounder and squid. With bluefish, squid predominantly used stay tactics (68.6%, 59/86) as initial responses. The most common stay response was to drop to the bottom, while showing a disruptive body pattern, and remain motionless. In 37.0% (34/92) of interactions with flounder, squid did not detect predators camouflaging on the bottom and showed no reaction prior to being attacked. Squid that did react, used flee tactics more often as initial responses (43.5%, 40/92), including flight with or without inking. When all defence behaviours were considered concurrently, flight was identified as the strongest predictor of squid survival during interactions with each predator. Squid that used flight at any time during an attack sequence had high probabilities of survival with bluefish (65%, 20/31) and flounder (51%, 18/35). The most important deimatic/protean behaviour used by squid was inking. Inking caused bluefish to startle (deimatic) and abandon attacks (probability of survival = 61%, 11/18) and caused flounder to misdirect (protean) attacks towards ink plumes rather than towards squid (probability of survival = 56%, 14/25). These are the first published laboratory experiments to evaluate the survival value of antipredator behaviours in a cephalopod. Results demonstrate that squid vary their defence tactics in response to different predators and that the effectiveness of antipredator behaviours is contingent upon the behavioural characteristics of the predator encountered.

Keywords:

antipredator defence

cephalopod

deimatic behaviour

fish

foraging tactic

ink

Loligo pealeii

prey

protean behaviour

INTRODUCTION

Predation is a constant source of risk for most animals. To maximize survival, prey have developed a wide repertoire of defences ranging from physical armor, toxic chemicals and behavioural displays to evasive manoeuvres (Cott 1940; Edmunds 1974; Bryan et al. 1997; Lenzi-Mattos et al. 2005; Speed & Ruxton 2005). Primary defences are generally characterized by camouflage and cryptic behaviours and are used to avoid detection or recognition and decrease encounter rates with potential predators (Endler 1991). When attack is imminent, secondary defences are deployed to delay, inhibit or escape from an approaching predator. The most common secondary defence is to flee (Humphries & Driver 1970; Eibl-Eibesfeldt 1975); however, direct interactions with predators are often unavoidable (Lingle & Pellis 2002; Edut & Eilam 2004). As a result, prey may attempt to startle, threaten or confuse a predator with defensive postures and erratic, unpredictable escape sequences known as protean behaviour (Humphries & Driver 1970; Edmunds 1974; Driver & Humphries 1988). Deimatic defences are sounds, displays and postures that intimidate or bluff (Young 1950; Edmunds 1974). Defensive eyespots are one example of a deimatic display found in frogs (Martins 1989; Lenzi-Mattos et al. 2005), cephalopods (Hanlon & Messenger 1996), butterflies and moths (Vallin et al. 2005, 2007; Stevens et al. 2008a, b, 2009). Depending on the capabilities of the prey, deimatic displays may signal a warning of true danger or an attempt to deceive a predator into believing prey are larger or more dangerous than they really are; either way, the intention of such displays is to cause predators to hesitate or abandon their attacks (Humphries & Driver 1970; Hanlon & Messenger 1996).

Escape tactics that are erratic and unpredictable are known as protean behaviours and function by confusing approaching predators and impairing their ability to predict prey escape trajectories or positions (Humphries & Driver 1967; Driver & Humphries 1988). Protean defences include the use of colour or body patterns to change appearance as seen in cephalopods (Hanlon & Messenger 1996), irregular movements such as freezing and fleeing in rodents (Edut & Eilam 2004), unsystematic escape trajectories in insects (Domenici et al. 2008), and similar examples in many taxa (Driver & Humphries 1988). Since predator–prey encounters may be incidental (e.g. the predator is not hungry) (Stankowitch & Coss 2006), deimatic and protean behaviours may also be effective in assessing risk and testing predator motivation (Edmunds 1974). The decision of which defence tactic to use presumably depends on the type of predator, the severity of the threat and the environmental factors surrounding the encounter (Lima 1992; Lingle & Pellis 2002; Hoverman & Relyea 2007).

Coleoid cephalopods are prey to numerous marine vertebrates including fish, mammals and diving seabirds (Packard 1972; Clarke 1996; Croxall & Prince 1996; Smale 1996). Because they are soft-bodied and lack hard protective structures such as spines and shells, coleoid cephalopods have evolved a diverse array of other primary and secondary defence behaviours (Packard 1972; Hanlon & Messenger 1996). Most notable are their advanced abilities to colour change and camouflage (Hanlon 2007; Barbosa et al. 2008; Mathger et al. 2008), exhibit postural displays (Hanlon et al. 1999; Huffard 2006; Bush et al. 2009) and use ink to confuse both menacing predators and conspecifics (Wood et al. 2008). While cephalopods are known to use deimatic and protean displays towards predators (Moynihan & Rodaniche 1982; Hanlon & Messenger 1988, 1996; Adamo et al. 2006; Langridge 2009), few studies have evaluated the conditions and types of predators that evoke these defences during predator–prey interactions.

Direct observations of predator responses to cephalopod deimatic and protean displays and the survival value of these defences have been described only once in the field (Hanlon & Messenger 1988) but have not been measured experimentally (Hanlon & Messenger 1996).

When confronted with a predator, prey must make an initial decision to flee or stay (Edmunds 1974). The existing working hypothesis for cephalopods assumes that flee and stay tactics are shown in equal proportions to most predators (Fig. 1). Cryptic and deimatic behaviours are typical stay tactics in cephalopods and are thought to be followed by protean defences in a combined effort to prevent, misdirect or delay an impending attack (Hanlon & Messenger 1996). In this study, a laboratory-based approach was used to test this model and assess the survival value of antipredator defence behaviours shown by squid in response to two predators representing contrasting foraging tactics. Bluefish (*Pomatomus saltatrix*) and summer flounder (*Paralichthys dentatus*) are natural predators of longfin inshore squid (*Loligo pealeii*) in North Atlantic waters (Staudinger 2006) and were chosen to represent cruising and ambush foraging modes, respectively. Predator–prey interactions were evaluated to address the following four questions. (1) What is the initial response (flee or stay) shown by squid towards predators when threatened? (2) When all antipredator defences are considered concurrently, what behaviour or sequence of behaviours best predicts survival in the presence of each predator? (3) Are deimatic or protean behaviours better predictors of squid survival? (4) Do squid vary their responses depending on the type of predator encountered?

METHODS

Animal Care

Animals were cared for and experiments were conducted in accordance with the regulations of the University of Massachusetts Amherst and the Marine Biological Laboratory Institutional Animal Care and Use Committees. Behavioural trials were conducted over a 2-year period at the Marine Resources Center (MRC) of the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts, U.S.A. We collected bluefish (size range 31–63 cm total length, TL) and summer flounder (size range 36–47 cm TL) from local waters and transported them back to the laboratory. Predators acclimated to captivity for approximately 1 month prior to use in behavioural experiments and were maintained on a diet of frozen and live fish and squid. We collected longfin inshore squid (size range 2–21 cm mantle length, ML) from Vineyard and Nantucket Sounds using a modified trawl net. Squid were transported back to the MBL in a live-well tank and transferred either directly into the experimental tank or into a temporary holding tank. When it was necessary to hold squid overnight, they were fed live fish and small squid. Squid were handled as little as possible to avoid imposing further stress postcapture and transferred between tanks in containers filled with sea water to minimize exposure to air (Moltschaniwskyj et al. 2007). No squid was held in captivity for more than 48 h, and only individuals that were robust and showed little to no obvious physical distress were chosen for behavioural trials.

After experiments were completed, all bluefish and flounder were released into local bays and estuaries near their original point of capture. Squid were not used in multiple trials and were euthanized by being placed in salt water and then in a freezer (Moltschaniwskyj et al. 2007). These squid were then used to feed captive animals in the MRC.

Experimental Design

All trials were conducted in a 28 x 10^3 litre, 3.1 x 0.8 m (diameter, height) round tank filled with recirculating, and biofiltered sea water maintained between 16° and 20 °C. The bottom of the experimental tank was lined with a mixed gravel and sand substrate approximately 2–4 cm deep. This allowed squid to rest on the bottom and camouflage, and allowed flounder to bury. The area surrounding the experimental tank was lined with black plastic sheeting to prevent disturbance to acclimating animals as well as during filming. The tank was illuminated by natural light from adjacent windows and during filming by two 500 W lights positioned above the tank.

Twenty-four hours prior to each trial, three predators of similar total lengths were introduced into the experimental tank and food was withheld to standardize hunger levels. Three hours prior to the start of each trial, an opaque PVC cylinder (1.5 m in diameter, 1 m in height) was lowered into the experimental tank, and 15 squid were placed into the inner area of the cylinder and allowed to acclimate. A trial commenced when the cylinder was hoisted above the tank using a pulley system, and each trial lasted 30 min. Behavioural interactions between squid and fish were recorded using Panasonic miniDV PV GS500 video cameras that were manually operated at two lateral viewing windows on either side of the tank, and from a third camera mounted above the experimental tank. This arrangement of cameras ensured that the entire tank was in view at all times and that most behavioural interactions could be viewed from multiple angles.

Data Analyses

Repeated use of predators was necessary because of the difficulties of obtaining and maintaining large numbers of fish in the laboratory. Using a randomized complete block design, we performed a Friedman's two-way ANOVA (Zar 1984) to test whether successive use of fish over the course of all trials affected the number of attacks made on squid or the number of capture efficiencies of bluefish and flounder. There were no significant differences in feeding behaviours of fish (all tests yielded Ps ≥ 0.18), suggesting that repeated use of predators in trials did not influence behavioural results.

Individual squid could not be identified within trials, and a large proportion of squid was removed during trials by predation events (bluefish 45%, 39/86; flounder 42%, 39/92); therefore, to examine the influence of multiple attacks on squid within trials, trends in escape probabilities over consecutive attacks (e.g. first, second, third attack) were evaluated using linear regression analysis. We hypothesized that if the probability of escape decreased with consecutive attacks, deterioration in squid health or responsiveness would be evidenced; if the probability of escape increased with consecutive attacks, there was evidence of habituation or improved avoidance abilities of squid in response to predators. Escape probabilities did not vary across attacks for trials using bluefish ($R^2 = 0.09$, $P = 0.36$) or flounder ($R^2 = 0.02$, $P = 0.74$), suggesting that exposure to multiple attacks within a trial did not significantly influence squid behaviour. Nevertheless, because individual squid could not be identified for all events, the statistical probability values of chi-square tests and classification tree analyses described below should be interpreted with caution since there may be a lack of independence among behavioural responses and thus, the degree of pseudoreplication is unknown.

All predator–prey behaviours were assessed using frame-by-frame analysis of video recorded during each trial. Behavioural responses (initial and subsequent) shown by the specific squid targeted during each predator–prey interaction were recorded, classified as primary or secondary defences, grouped into subcategories including flight, deimatic and protean behaviours, and compiled in an ethogram. The survival value of each interaction was classified as either (1) mortality due to predation, (2) an escape whereby a predator executed an attack but the squid was successful in evading the predator, or (3) an attack abandoned by the predator. In abandoned attacks, predators initiated an aggressive movement towards prey, but did not open their mouths or complete their attacks. For example, bluefish were observed to orient towards squid and then turn away during the final approach. Additionally, summer flounder often reduced swimming speeds and sometimes glided through the water several body lengths away from a targeted squid.

Initial behaviours displayed by squid in response to an aggressive approach by a predator were designated as either a stay or a flee tactic. A chi-square test was used to determine whether squid used stay or flee tactics more frequently as initial responses when threatened by each predator. The null model was based on the existing working hypothesis for cephalopods (Hanlon

& Messenger 1996) that stay and flee tactics would be shown in equal proportions (50:50) towards each predator (Fig. 1).

Classification tree analysis was used to determine the behaviour or sequence of behaviours that were most influential on squid survival overall, and whether deimatic or protean defences were better predictors of squid survival in the presence of each predator. Classification tree analysis is a nonparametric, rank-based discrimination procedure that explains differences among prespecified groups and has the ability to test the significance of overall group classification (McGarigal et al. 2000). Classification tree analysis works by recursively partitioning data into groups that are increasingly more homogeneous using split-values of the explanatory values, and that maximize within-group homogeneity and among-group heterogeneity according to a prespecified information index. Trees are typically overgrown and then 'pruned' back to a smaller tree size (according to the number of terminal nodes, also called 'leaves') that has the minimum honest estimate of true (prediction) error, which is determined by a cross-validation procedure.

Classification tree analyses were used in this study for several reasons. First, the data contained both continuous and categorical explanatory (defence behaviours) and response variables (mortality, escape, abandoned attack), which many parametric statistical methods are unable to manage. Second, classification tree analyses have the ability to explain nonhomogenous relationships between explanatory and response variables, which are a common occurrence in studies such as this. Third, results from classification tree analyses are easily interpretable and can be used effectively and efficiently for predictive purposes.

Classification tree analyses were conducted using the ‘cartware’ package under the ‘rpart’ library in the R computing environment (version 2.9.2, Free Software Foundation, Inc., Boston, MA, U.S.A.). Specifically, trees were built using splits as determined by the Gini information index, and final trees were pruned using a 10-fold cross-validation procedure by employing the 1-S.E. rule (De'ath & Fabricius 2000). The statistical significance of each classification tree was assessed using a Monte Carlo permutation procedure using 100 permutations. Classification trees analysis was chosen over more traditional parametric techniques because it is able to account for and describe the influence of multiple explanatory variables concurrently.

RESULTS

Bluefish and flounder used different predatory behaviours when hunting squid. Bluefish swam around the tank in an organized school and actively searched for squid in the water column and on the substrate. When squid were attacked in the water column, bluefish increased their swimming speed and burst towards individuals or shoals of squid. When squid were attacked on the substrate, bluefish oriented their bodies in a head-down posture, and grabbed squid off the bottom. Conversely, solitary flounder were scattered around the tank and either were buried beneath the substrate or were camouflaged while resting on top of the substrate (Fig. 2a). Flounder would often lie-in-wait and ambush squid as they swam overhead. Although ambush attacks were most common, flounder also stalked squid on the substrate and used active attacks to pursue squid swimming in the water column.

Over the course of 35 trials, 86 predator–prey interactions were evaluated between squid and bluefish, and during 29 trials, 92 interactions were assessed between flounder and squid. Each interaction was representative of the behaviours shown by a single squid and a single predator. We identified three primary defence behaviours and 15 secondary defence behaviours (Table 1). During bluefish trials, both the predator and the prey were in plain view and aware of each other's presence the moment the partition was raised and trials commenced, so we considered all squid defensive behaviours during these trials to be secondary defence tactics. In experiments with flounder, primary defence was observed during 10 trials. Squid were considered to be using primary defence if during the initial period after the partition was raised (1) the squid showed no alarm behaviours or (2) showed disruptive body patterns either while resting on the substrate or swimming, and (3) flounder remained motionless and camouflaged on the bottom. We considered squid to show a switch to secondary defence tactics the moment their behaviour indicated alarm or awareness of the flounder's presence (e.g. tightening of school formation, moving to the surface) or when the flounder moved around the tank.

Initial Response (Flee or Stay) of Squid towards Predators When Threatened

With bluefish predators, squid predominantly (68.6%, 59/86) used stay tactics (χ^2_1 = 11.91, $P = 0.0006$) as their initial response (Fig. 3). Dropping to the substrate with a disruptive body pattern was the most common tactic (Table 2); squid held this position for long periods of time (> 10 s), remaining motionless even when bluefish swam directly overhead (Fig. 2b). Stay tactics displayed in the water column included tightening of school formation and deimatic arm postures such as upward V-curl. Flee tactics were used less often (31.4%, 27/86) and generally included flight with or without inking, and moving to the surface.

In 37.0% (34/92) of interactions with flounder, squid did not detect predators camouflaging on the bottom and showed no reaction prior to being attacked. When squid were aware of an impending attack by flounder, the most common initial response (43.5% (40/92) of all interactions) was to flee ($\chi^2_1 = 8.35$, $P = 0.004$; Fig. 3). Flee tactics included flight with or without inking, scattering and moving to the surface (Table 2). Stay tactics were shown less often (19.6% (18/92) of all interactions) and included primary defence (camouflaging on the bottom), upward V-curl displays and orienting towards flounder while holding a stationary position at the water's surface.

After the initial tactic of stay or flee, squid showed varying sequences of behaviours towards predators in an attempt to avoid or deter attacks. With bluefish, squid that initially used stay tactics, almost always followed with flight (37%, 11/30) and to a lesser extent protean locomotor behaviours (7%, 2/30). When stay tactics were shown as initial responses to flounder, squid were slightly more likely to follow with protean locomotor behaviours (28%, 5/18) than with flight (17%, 3/18). Body pattern changes and inking were frequently shown to both predators subsequent to the initial decision to flee (Fig. 4). Percentage occurrence and mortality rates of individual behaviours are reported in the Appendix.

Behaviours That Best Predicted Survival

Squid behavioural defences either disrupted the attack sequence causing predators to abandon their pursuit; or if an attack was completed, squid escaped and survived, or were captured and consumed (Table 3). When all behaviours listed in Table 1 were considered concurrently, classification tree analysis selected flight as the best predictor of squid survival during bluefish trials (Fig. 5a). The classification tree included two leaves (Kappa = 0.36, $P < 0.01$) corresponding to a high probability (65%, 20/31) of survival due to abandoned attacks when squid fled at least 10 body lengths away, and a high probability (64%, 35/55) of mortality when squid did not flee from bluefish (Fig. 5a).

When all defence behaviours (Table 1) shown towards flounder were evaluated, survival was generally predicted by whether or not squid reacted to flounder prior to being attacked (Fig. 5b). The classification tree formed a three-leaved tree (Kappa = 0.44, $P < 0.01$) corresponding to a high probability (78%, 29/37) of mortality if squid did not react. Squid that did react had a higher probability (88%, 7/8) of survival if attacked while showing a disruptive body pattern in the water column than when attacked while showing a disruptive body pattern near the bottom (probability of an abandoned attack 45%, 21/47). We suspected that reaction was masking the influence of other behaviours of interest; to further explore the effectiveness of behavioural tactics on squid survival, an alternative tree was built excluding the reaction variable. The resulting tree formed two leaves (Kappa = 0.32, $P = 0.03$) and selected flight as the primary splitting variable (tree not shown); not surprisingly, squid had a high probability of escape (51%, 18/35) when they fled compared to a high probability of mortality (63%, 35/57) when they did not.

Deimatic versus Protean Behaviours as Predictors of Squid Survival

The classification tree built using only deimatic and protean behaviours (e.g. postural, locomotor, chromatic and inking displays) shown towards bluefish formed three leaves (Kappa = 0.32, $P = 0.03$), and deimatic postural displays were selected as the primary splitting variable (Fig. 6a). Squid had the highest probability (88%, 7/8) of surviving an attack by bluefish when the tentacles extended display was shown. When upward V-curl and vertical hanging postures were combined with inking, squid had a higher probability (61%, 11/18) of survival due to abandoned attacks than when arm postures were used alone (58% probability of mortality, 35/60).

When only deimatic and protean behaviours shown towards flounder were evaluated, a two-leaved classification tree was formed (Kappa = 0.24, $P < 0.0005$) and inking was selected as the primary splitting variable (Fig. 6b). Squid that inked had a higher probability of escaping (56%, 14/25) attacks made by flounder compared to when squid did not ink (probability of mortality 54%, 36/67).

DISCUSSION

Our results show that longfin squid vary their defence behaviours in response to different types of predators. When confronted with bluefish, a pelagic fish that actively searches for prey while swimming in the water column, squid primarily used stay tactics as initial responses. The most common stay response shown during bluefish trials was to drop to the bottom while displaying a disruptive body pattern. Squid remained motionless and camouflaged on the bottom even if bluefish passed directly above, but would often flee if bluefish oriented downwards in an attack posture. Similar reactions to other cruising fish predators such as striped bass (*Morone saxatilis*), and sudden movements or disturbances from overhead have been reported in wild squid (Stevenson 1934; Macy 1982) as well as in cuttlefish (*Sepia officinalis*) (Hanlon & Messenger 1988). Dropping to the bottom and camouflaging against the substrate was never observed as an initial response towards flounder, suggesting that this behavioural defence is specifically used by squid to avoid pelagic or cruising predators such as bluefish, tunas and mackerel that commonly feed on squid in continental shelf waters of the northwest Atlantic (Bowman et al. 2000; Staudinger 2006).

Conversely, squid primarily used flee tactics as initial responses to approaches by flounder, a benthic ambush predator. Unlike predator–prey interactions with bluefish, there was no single defence behaviour that was shown repeatedly towards flounder over the course of all trials. Squid alternated among flight with or without inking and showed various protean locomotor behaviours as initial responses to flounder attacks; however, erratic jetting, explosive scattering and the blanch-ink-jet manoeuvre were shown almost exclusively towards flounder.

Prey that are subjected to attack by multiple species of predators will often use different modes of defence against each type of predator (Edmunds 1974; Driver & Humphries 1988; Sherbrooke 2008). The different behavioural tactics shown by squid to bluefish and flounder indicate that squid have evolved recognition and response skills that have led to species-specific or type- specific (ambush, cruising) antipredator responses (Edmunds 1974; Sherbrooke 2008). Given these results, we suggest that the existing model for cephalopod defence presented in Hanlon & Messenger (1996) be refined to reflect two distinct sequences of antipredator responses (Fig. 7). During interactions with bluefish, which are chase predators, squid showed a disproportionally large fraction of stay tactics as initial responses, followed mainly by flight, and less often by protean behaviours. During interactions with flounder, which are ambush predators, squid used flight and protean behaviours as initial and subsequent responses; stay tactics, which were rarely shown, were followed by flight and protean behaviours in relatively equal proportions.

Previous studies have shown that coleoid cephalopods respond to potential predators with a variety of antipredator behaviours (Moynihan & Rodaniche 1982; Hanlon & Messenger 1996; Adamo et al. 2006; Langridge 2009), but to the best of our knowledge this is the first study to predict and measure the survival values of key defence behaviours based on actual predation. When all antipredator defence behaviours were considered concurrently, flight was selected by classification tree analyses as the best predictor of squid survival with bluefish and also with flounder when squid reacted to attacks. Flight is the most common secondary defence among animals (Edmunds 1974), and the decision of when to flee is subject to a high degree of natural selection (Stankowitch & Coss 2006). Squid primarily used stay behaviours as their initial response to bluefish, which is a good tactic to avoid visual, cruising predators that are attracted to movement (Neill & Cullen 1974; Keenleyside 1979; Scharf et al. 2002). When attack was imminent, the use of flight during any stage (e.g. detection, approach) of an interaction with bluefish led to higher survival rates by disrupting the attack sequence and causing bluefish to abandon pursuit. Flight was not as influential in deterring attacks by flounder but instead was important if flounder were unsuccessful in their initial attempts to ambush and capture squid. In these instances, squid evaded capture by outmanoeuvring flounder as they fled. Flounder have compressed body forms, and are less agile and slower swimmers than bluefish (Olla et al. 1997; Collette & Klein-MacPhee 2002); consequently, squid may be more likely to outswim flounder when pursued. Squid were more vigilant of bluefish than they were of flounder; hence, they were able to use stay behaviours, including deimatic displays, to assess the motivational state and risk of an approaching bluefish prior to resorting to flight (Edmunds 1974; Stankowitch & Coss 2006).

Deimatic behaviours are thought to startle or frighten predators into hesitating or abandoning their attacks (Edmunds 1974). Studies have shown that prey do not always perform deimatic displays towards all types of predators (Hanlon & Messenger 1988; Sherbrooke 2008; Langridge 2009), and displays vary in their effectiveness against different predators (Vallin et al. 2005, 2007). Deimatic postural displays had a positive and significant impact on squid survival with bluefish but were not as important with flounder. The ‘tentacles extended’ posture was the most effective display because it was used in conjunction with flight, whereas upward V-curl and vertical hanging postures were displayed from stationary positions near the surface. Deimatic postural displays probably function to make squid appear larger and may be more effective against gape-limited predators.

Evaluations of predator–prey responses were limited to interactions where fish behaviours could be interpreted as reliable signals of predatory intent, such as when fish showed some form of orientation or approach towards squid . In addition to behaviours shown in direct response to an approaching predator, deimatic postures were also displayed towards bluefish and flounder that were swimming or resting on the substrate below squid, and thus, that demonstrated no immediate threat to the squid. Since deimatic displays were successful in deterring direct attacks, it is possible that these behaviours may also have influenced predators at times that were not obvious to us. For example, cuttlefish show deimatic displays towards nonpredators that swim nearby (Hanlon & Messenger 1988; Langridge 2009). In the present study, deimatic displays caused both predators to abort some attacks, suggesting that deimatic displays are an effective antipredator strategy in longfin squid. Our findings with squid contrast with previous conclusions (based on cuttlefish) that coleoid cephalopods do not display deimatic responses towards highly dangerous predators (Langridge 2009).

Despite the highly developed visual and mechanosensory systems of cephalopods (Hanlon & Messenger 1996), in over one-third of all predator–prey interactions with flounder, the squid showed no reaction prior to being attacked. Ambush attacks are the primary tactic used by flounder to capture large mobile prey including squid and fish (Staudinger & Juanes 2010). Squid appeared to be incapable of detecting camouflaged flounders below them at short distances, and squid that were ambushed by flounder were clearly at a disadvantage and responded using strong protean locomotor displays such as explosive scattering and erratic jetting. Similar behaviours have been reported in studies when predators were not detected by prey until they were in close proximity (Edmunds 1974; Driver & Humphries 1988).

Protean locomotor defences were not selected as key predictors of squid survival by classification tree analyses even though they were frequently used during interactions with flounder. This is probably because these tactics were not used until an attack was already in progress and reaction times were shorter than when squid were able to detect flounder from a distance. Protean defence manoeuvres were also used when fleeing from flounder. For example, when squid use flight they often vary the angle, speed and direction of their trajectory (Driver & Humphries 1988). Under natural conditions, protean escape behaviours may be more effective when squid are not restricted in the distance they can flee and reaction times are longer (Stankowitch & Coss 2006). When flounder were detected, squid swam near the surface and spent less time on or near the substrate overall in comparison to trials with bluefish. Squid behaviour was probably influenced by flounder's strong association with the bottom and the higher predicted risk of mortality when attacked in the lowest portion of the water column. Under natural conditions, juvenile cuttlefish (*S. officinalis*) showed similar behaviours, and consistently swam towards the surface when confronted with the benthic predator *Serranus cabrilla*; the fish never followed beyond 1m from the benthos (Hanlon & Messenger 1988).

In cephalopods that inhabit shallow-water environments, inking is thought to act primarily as a visual display to distract, hide or escape from predators and as an alarm cue to conspecifics (Hanlon & Messenger 1996; Wood et al. 2008, 2010). In a recent study, ink of the Caribbean reef squid, *Sepioteuthis sepioidea*, was found to have deterrent properties against predatory fish (Wood et al. 2010). Our results support the conclusions put forth by Wood et al. (2010) that inking protects squid and provides the first direct experimental evidence that inking enhances squid survival during interactions with fish predators. When used in conjunction with deimatic arm postures, inking increased the probability of abandoned attacks by bluefish and was selected as the most influential deimatic/protean display overall with flounder. Although inking has been referred to as a protean defence (Wood et al. 2008), predator reactions in this study demonstrated that inking functions as both a protean and a deimatic defence. Bluefish were observed to startle (deimatic response) and turn at acute angles away from ink plumes ejected by retreating squid (Supplementary Material, Video S1). Flounder were less affected by inking but were observed to misdirect (protean response) some of their attacks towards ink plumes rather than towards squid. Cephalopod ink contains the chemical properties to disrupt predator sensory systems and may also act as a chemical deterrent (Derby 2007; Derby et al. 2007; Wood et al. 2010). Although inking clearly affected predatory behaviour, it did not cause bluefish and flounder to avoid squid entirely. Since predators show aversion towards other molluscs such as sea hares that produce unpalatable secretions (Derby 2007), it seems unlikely that ink provided chemical protection from the fish in this study. It is possible that different species vary in their sensitivity to squid ink and that inking may inhibit predation by some species, as has been observed with other cephalopods (Caldwell 2005).

Hanlon & Messenger (1996) suggested that because coleoid cephalopods lack physical defensive structures, the primary defence of camouflage in its many manifestations is used extensively to avoid visual predators. In this study, camouflage via banded body patterns that presumably act as disruptive coloration, used in combination with dropping to the bottom, was frequently shown by longfin squid in response to bluefish but was designated as a secondary defence tactic since bluefish were aware of squid’s presence in the tank at all times. The restricted space, artificially high light levels and lack of structural complexity of the laboratory setting probably facilitated predator detections of squid and increased prey detection and capture rates (Michel & Adams 2009). Under natural conditions, countershading by squids as they hover above the substrate or in the water column probably provides some degree of camouflage as primary defence, especially in the mostly turbid waters of the northwest Atlantic.

Because multiple attacks occurred within some trials, it is possible that squid behaviour was affected by prior experiences. However, squid used in behavioural trials were collected from the wild and acclimated to captivity for only a few hours, and experiments were brief (30 min). Prior to being brought into the laboratory, squid probably experienced predatory events with natural predators, including bluefish and flounder, which co-occur in regional waters (Staudinger 2006); therefore, squid used in laboratory trials were not naïve to predators, and it is unlikely that squid had sufficient time to adapt their behaviour to the laboratory setting or to the predators within each experiment.

Conclusions

Squid and other coleoid cephalopods have evolved a range of primary and secondary behaviours to protect themselves against predators. Avoiding detection via crypsis is thought to be the first line of defence in cephalopods (Hanlon & Messenger 1996) as well as in many other marine and terrestrial animals (Edmunds 1974). In this study, predators were generally aware of prey as soon as trials began, making evaluations of primary defence difficult; consequently, our conclusions are limited to the effectiveness of secondary defences. Although no single behaviour guaranteed survival, there were clear advantages of using certain defences over others during confrontations with each predator. Predators that chase, such as bluefish, rely on speed and endurance to pursue and overcome prey, while ambush predators such as flounder rely on rapid and sudden strikes to catch prey and rarely pursue prey over long distances (Neill & Cullen 1974). Squid recognized the threats posed by each predator and adapted their behaviours accordingly, by using slow and subtle movements or deimatic behaviours during interactions with bluefish, and by using protean behaviours with flounder. Overall, squid survival was significantly improved when flight or inking was used as part of their escape. Flight represents the most common defence in all animals (Eibl-Eibesfeldt 1975; Driver & Humphries 1988), and inking is arguably one of the most unique behaviours inherent to cephalopods. Inking and other unusual antipredator behaviours may function as more than one type of defence depending on how predators react; regardless, inking clearly affected predatory behaviour and increased the probability of squid survival, and, overall, may give squid an advantage in the predator–prey arms race. Differences in the behavioural sequences of squid towards bluefish and flounder suggest that some aspects of the cephalopod model proposed by Hanlon & Messenger (1996; their Figure 5.1) may not apply to this species of cephalopod. We offer a refined model for squid that describes two antipredator defence sequences that we suggest have evolved as responses to ambush and cruising predators (Fig. 7); however, future studies that test additional squid–predator combinations are needed to verify whether the antipredator responses shown towards bluefish and flounder are species specific or universal to all ambush and cruising predators. It is noteworthy that laboratory experiments may provide an oversimplified view of species interactions and are only a first step to understanding the survival value of cephalopod defence tactics. Additional studies conducted in natural habitats are necessary to gain further insight into antipredator behaviours of cephalopods and predator responses.

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Table 1

Ethogram of squid behavioural responses to bluefish and flounder predators

Category	Behaviour	Description
Prey awareness		
	No reaction	Squid appears unaware of predator(s); shows no response prior to attack
	Reaction	Squid reacts to approaching predator using defence behaviours
Primary defence		
	Camouflage on substrate	Disruptive body pattern shown while squid rests on the substrate
	Camouflage in the water column	Disruptive body pattern shown while squid is swimming
	Organized school with a countershading pattern	Squids exhibit a countershading body pattern; swim facing the same direction, parallel to each other, and close together
Secondary defence		
Flight		Squid rapidly moves away from threat via jet propulsion
Deimatic		
	Orient towards predator	Squid face the direction of approaching predator while maintaining position in water column near the surface
Postural	Upward V-curl	Arms flared upwards, exposing beak
	Vertical hanging	Squid hangs vertically in water column near the surface, arms and sometimes tentacles droop downwards
	Tentacles extended	Both tentacles are extended as squid is swimming
Chromatic	Disruptive body pattern	Amber and pink coloration with brown banding; used to camouflage against substrate while dropping to the bottom, resting on the bottom and while swimming in the water column
	All-dark body pattern	Rapid change in body colour to deep brown or red
Protean		
	Drop to substrate	Squid slowly drops to the substrate and shows a disruptive banded body pattern; once on the substrate, squid remains motionless
	Move to surface	Squid rises to the water's surface (usually accompanied by a chromatic change) and orients towards predator
	Tighten school formation	Distance between individuals decreases, squids face same direction and swim parallel to each other
Locomotor via jet propulsion		
	Scatter	Group of squids disperses in multiple and random directions
	Erratic jetting	Squid jets randomly in multiple directions, sometimes alternating between freezing and fleeing
	Blanch-ink-jet	Squid turns all clear, ejects ink cloud, and rapidly jets away
	Jet out of water	Squid jets out of water in opposite direction of threat
Ink		Expulsion of ink either in dense plumes, diffuse clouds, or as pseudomorphs. Typically observed in combination with flight

Table 2

Initial response behaviours (stay or flee) of squid towards bluefish and flounder when threatened

Initial response to predator	Bluefish		Flounder	
	N	%	*N*	%
No reaction	0	-	34	37.0
Stay tactics (Total)	**25**	**68.6**	**17**	**19.6**
Primary defence*	0	-	3	3.3
Deimatic				
Orient towards predator	2	2.3	3	3.3
Postural				
Upward V-curl	4	4.7	9	9.8
Vertical hanging	2	2.3	0	-
Chromatic				
Disruptive body pattern*	15	17.4	0	-
All-dark body pattern	2	2.3	2	2.2
Protean				
Drop to substrate and camouflage	24	27.9	0	-
Tighten school formation	10	11.6	1	1.1
Flee tactics (Total)	**27**	**31.4**	**40**	**43.5**
Flight	11	12.8	17	18.5
Ink and flight	5	5.8	10	10.9
Protean				
Move to surface	8	9.3	5	5.4
Locomotor via jet propulsion				
Scatter	1	1.2	7	7.6
Erratic jetting	2	2.3	1	1.1

N = number of observations; % = percentage of all interactions where a behaviour was shown as an initial response. Results correspond to Fig. 3.

* Squid held disruptive banded body pattern and remained motionless on substrate as predators approached or passed overhead.

Table 3

Outcomes of predator–prey interactions between longfin squid and bluefish or flounder

	N	%
Bluefish		
Escapes	12	14.0
Mortalities	39	45.3
Abandoned attacks	35	40.7
Total interactions	86	
Summer flounder		
Escapes	31	33.7
Mortalities	39	42.4
Abandoned attacks	22	23.9
Total interactions	92	

N = the number of observations; % = percentage of all observations resulting in survival or mortality of squid.

FIGURES

Figure 1. Hypothesized sequence of cephalopod defences during interaction with predators. Modified from Hanlon & Messenger (1996).

Figure 2. (a) Summer flounder (*Paralichthys dentatus*) camouflaging on the substrate. (b) Longfin squid (*Loligo pealeii*) showing a disruptive banded body pattern on the substrate after dropping to the bottom, with bluefish (*Pomatomus saltatrix*) swimming overhead. Arrows point to camouflaging squid.

Figure 3. Percentage frequency of stay and flee tactics shown as initial responses by squid towards bluefish and summer flounder during predator–prey behavioural trials. Results correspond to Table 2.

Figure 4. Percentage frequency of occurrence of all squid behavioural defences shown during predator–prey interactions with bluefish and flounder. Includes initial responses and subsequent reactions. Percentage occurrence and mortality rates of individual behaviours are reported in the Appendix.

Figure 5. Classification trees describing the outcomes of predatoreprey interactions in behavioural trials. Classification trees depict recursive partitioning of observations into a final set of leaves that best explained differences in squid survival based on all primary and secondary behavioural variables shown towards (a) bluefish (model correct classification rate (CCR) = 0.64, Kappa = 0.36, $P < 0.01$) and (b) flounder (model CCR = 0.6196, Kappa = 0.44, $P > 0.01$).

Observations or statements that were ‘true’ for each splitting variable are presented in the left branch; all other responses are presented in the right branch. Values at the base of each leaf correspond to the percentage of observations in all trials classified as the dominant response variable (escape, mortality, abandoned attacks) in that leaf. Values in parentheses are the total number of observations in each leaf.

Figure 6. Classification trees describing the outcomes of predatoreprey interactions in behavioural trials. Classification trees depict recursive partitioning of observations into a final set of leaves that best explained differences in squid survival based only on deimatic and protean behavioural variables shown towards (a) bluefish (model CCR = 0.62, Kappa = 0.32, $P = 0.03$), and (b) flounder (model CCR = 0.54, Kappa = 0.24, $P < 0.0005$). Observations or statements that were ‘true’ for each splitting variable are presented in the left branch; all other responses are presented in the right branch. Values at the base of each leaf correspond to the percentage of observations in all trials classified as the dominant response variable (escape, mortality, abandoned attacks) in that leaf. Values in parentheses are the total number of observations in each leaf.

Figure 7. Refined model of antipredator defence sequences shown by squid during interactions with (a) pelagic cruising predators and (b) benthic ambush predators. Arrow thickness varies according to the importance of each type of response shown by squid towards each predator.

Figure 1

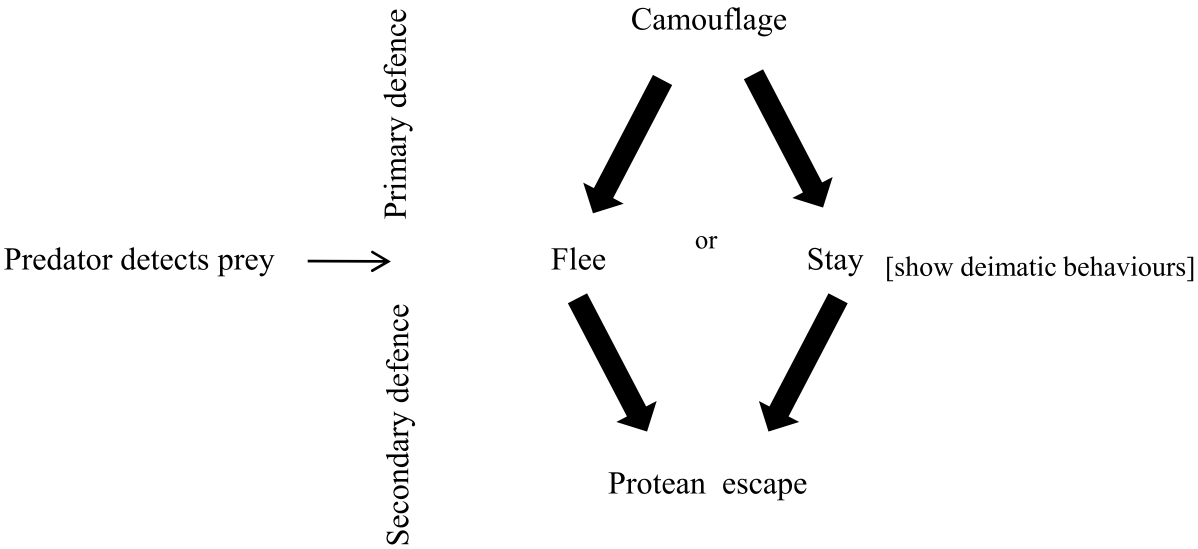

Predator detects prey
Primary defence
Secondary defence
Camouflage
Flee
or
Stay
[show deimatic behaviours]
Protean escape

Figure 2

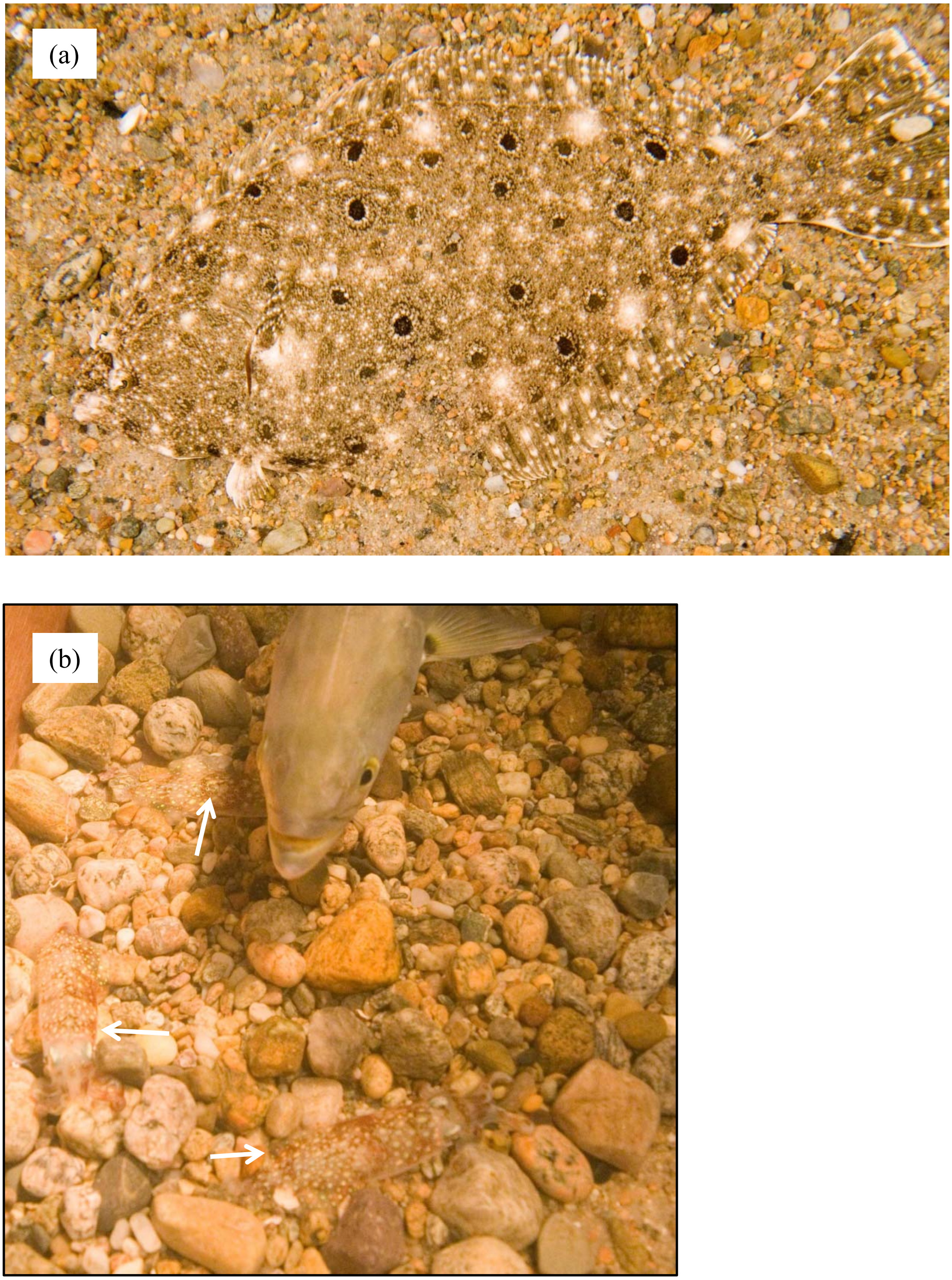
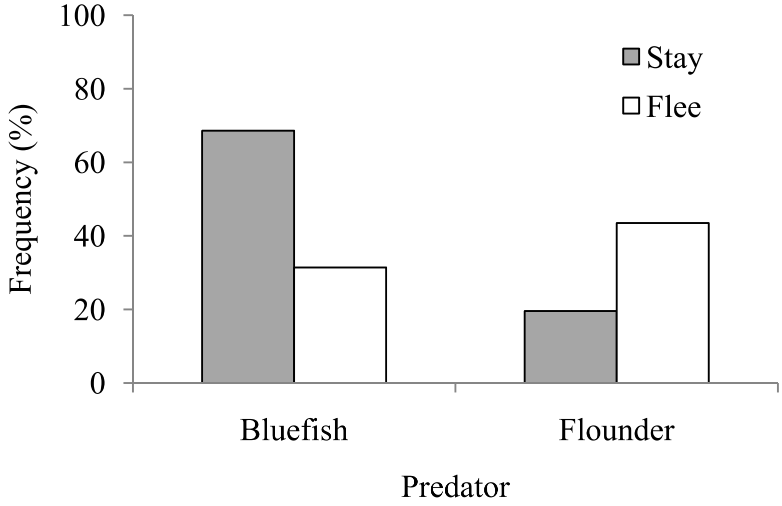
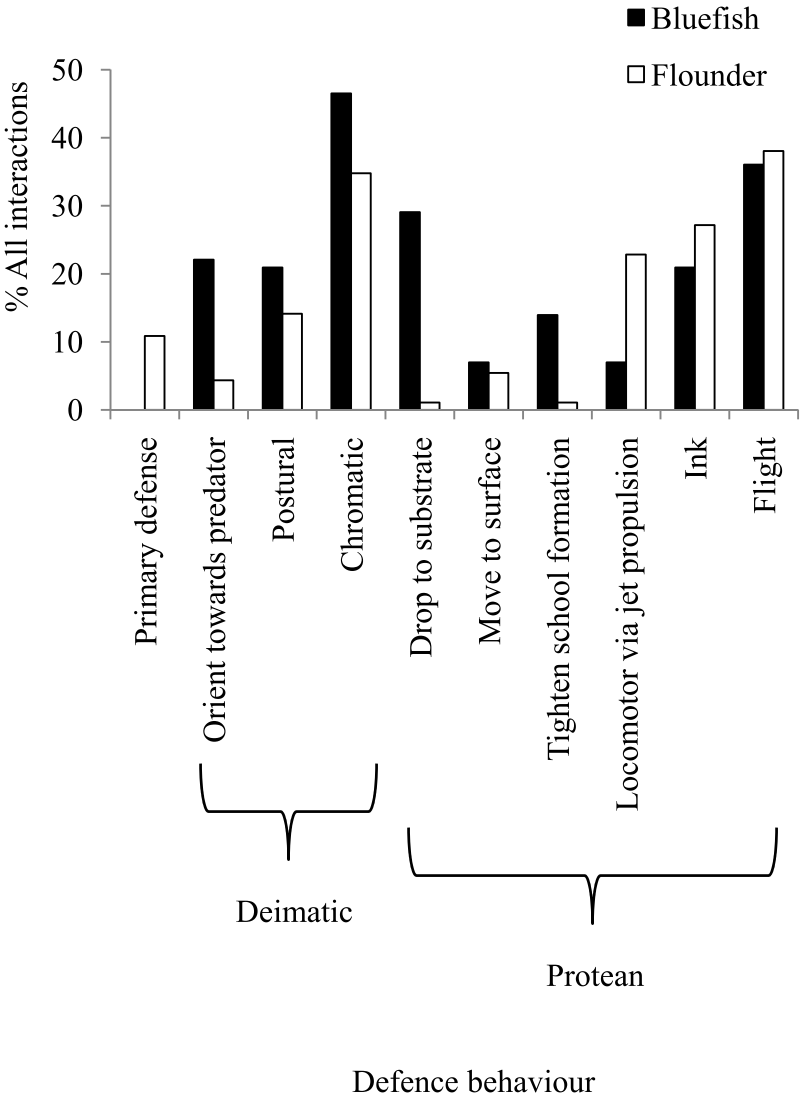


Figure 3



100
80
60
40
20
0
Frequency (%)
▨ Stay
☐ Flee
Bluefish
Flounder
Predator

Figure 4



■ Bluefish
□ Flounder
% All interactions
50
40
30
20
10
0
Primary defense
Orient towards predator
Postural
Chromatic
Drop to substrate
Move to surface
Tighten school formation
Locomotor via jet propulsion
Ink
Flight
Deimatic
Protean
Defence behaviour

Figure 5

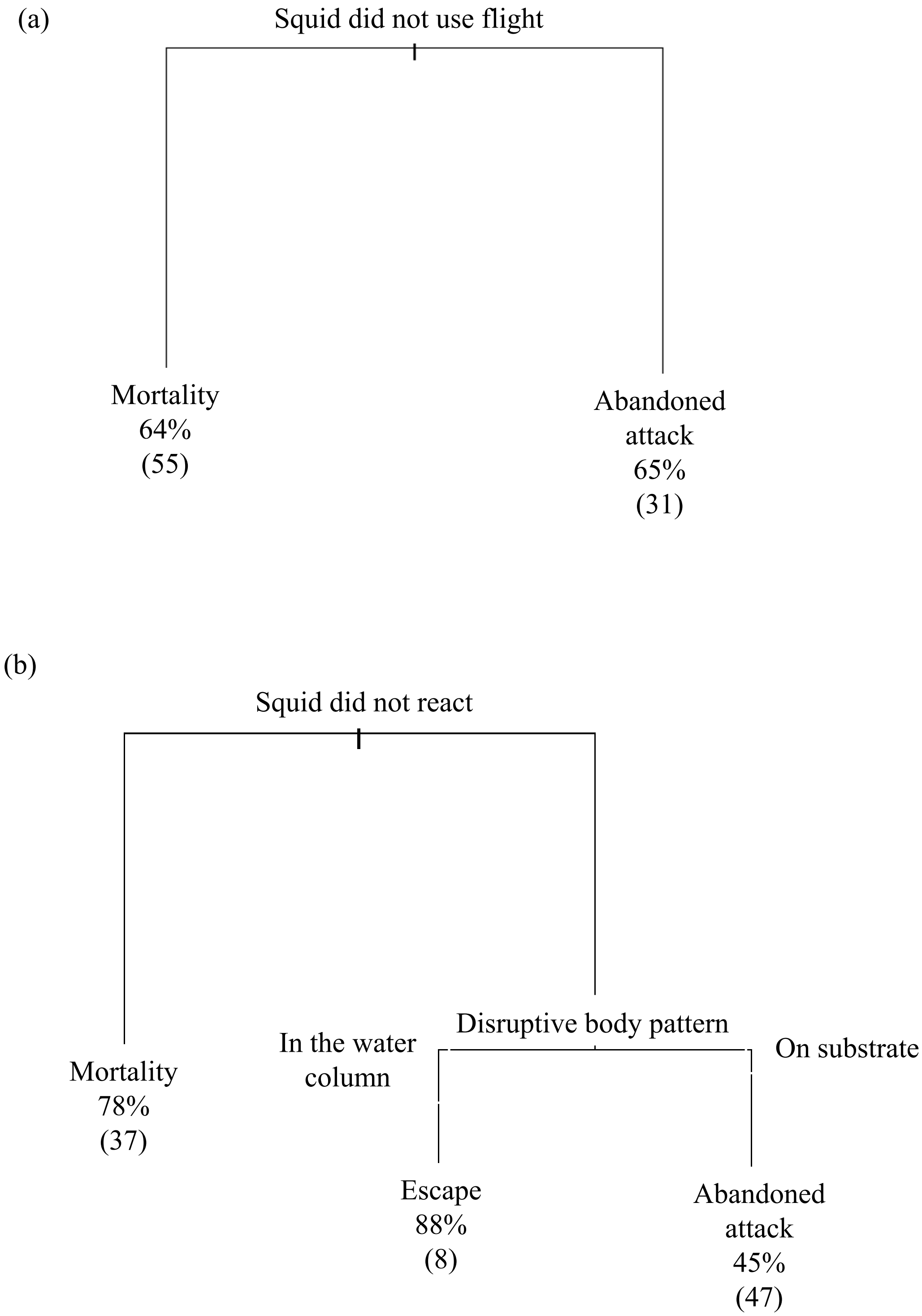
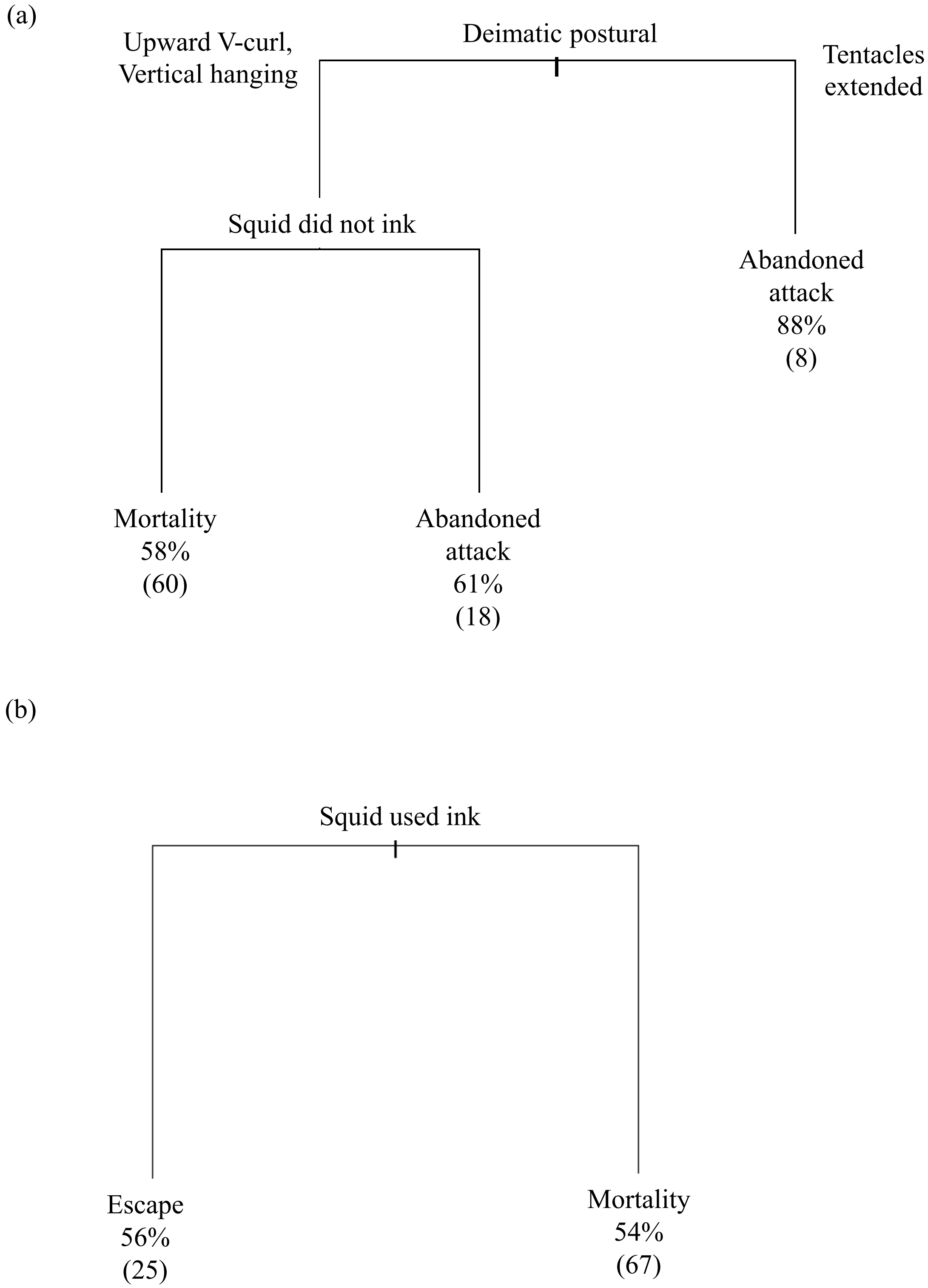
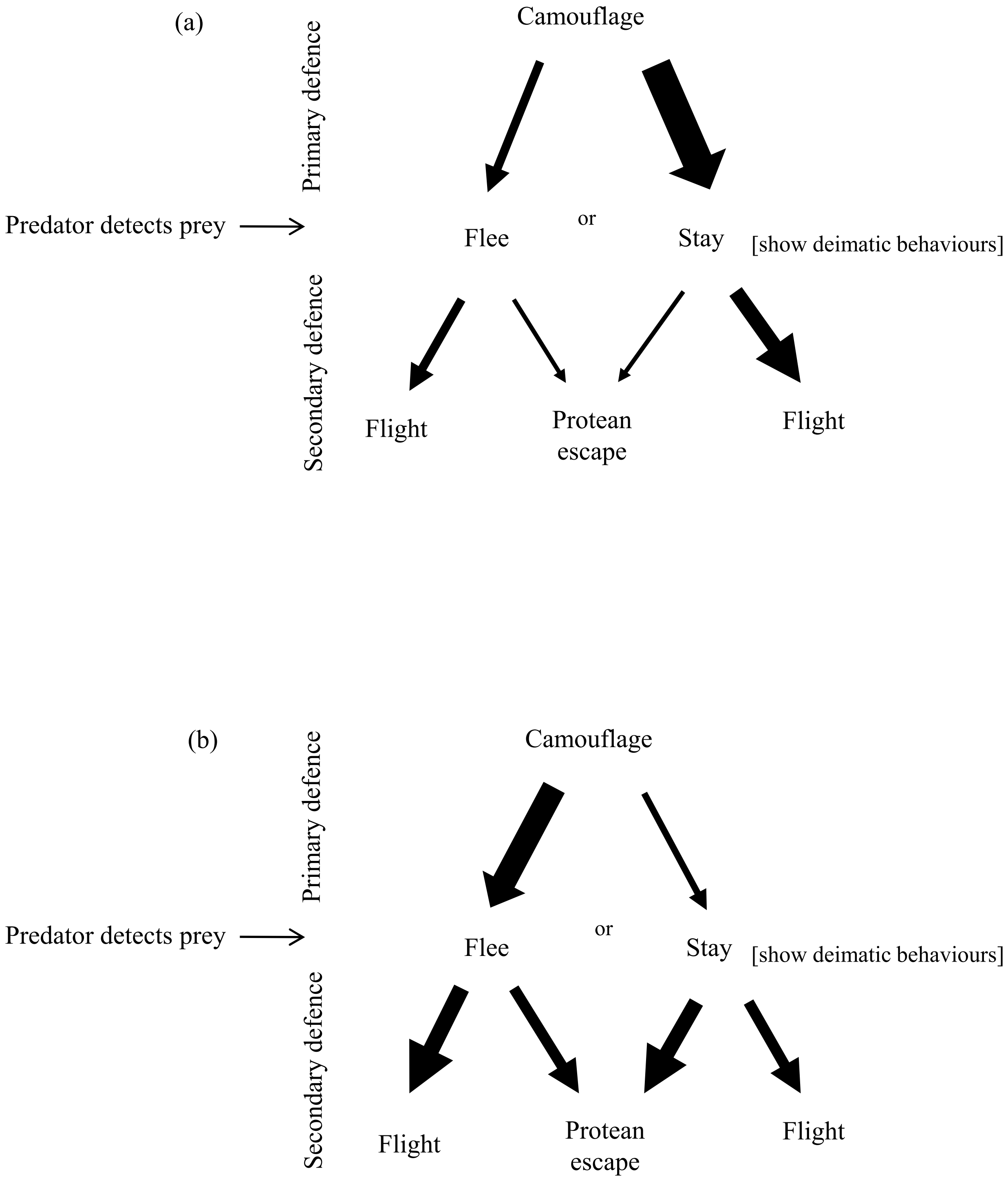

(a)
Squid did not use flight
Mortality
64%
(55)
Abandoned
attack
65%
(31)
(b)
Squid did not react
Mortality
78%
(37)
Disruptive body pattern
In the water
column
On substrate
Escape
88%
(8)
Abandoned
attack
45%
(47)

Figure 6



(a)
Upward V-curl,
Vertical hanging
Deimatic postural
Tentacles
extended
Squid did not ink
Abandoned
attack
88%
(8)
Mortality
58%
(60)
Abandoned
attack
61%
(18)
(b)
Squid used ink
Escape
56%
(25)
Mortality
54%
(67)

Figure 7


(a)
Camouflage
Primary defence
Predator detects prey
Flee
or
Stay
[show deimatic behaviours]
Secondary defence
Flight
Protean escape
Flight
(b)
Camouflage
Primary defence
Predator detects prey
Flee
or
Stay
[show deimatic behaviours]
Secondary defence
Flight
Protean escape
Flight

Appendix 1: Frequency, percent occurrence, and percent mortality of all squid defenses shown in sequence following an attack by bluefish or flounder. Behaviors selected by classification tree analyses as the best predictors of survival are shown in Figures 5 and 6.

	Bluefish			Flounder		
Behavior	N	% all interactions	% mortality	N	% all interactions	% mortality
Prey Awareness						
No reaction	0	-	-	**34**	**37.0**	**78.4**
Reaction	86	100.0	45.3	58	63.0	18.2
Primary defense						
Camouflage on substrate	0	-	-	2	2.2	50.0
Camouflage in the water column	0	-	-	7	7.6	57.1
Organized school with a countershading pattern	0	-	-	1	1.1	0.0
Secondary Defenses						
Flight	31	36.0	12.9	35	38.0	8.6
Deimatic						
Orient towards predator	19	22.1	57.9	4	4.3	0.0
Postural						
Upward v-curl	10	11.6	50.0	11	12.0	36.4
Vertical hanging	5	5.8	20.0	0	-	-
Tentacles extended	3	3.5	0.0	2	2.2	0.0
Chromatic						
Disruptive body pattern	33	38.4	51.5	15	16.3	60.0
All Dark body pattern	7	8.1	28.6	17	18.5	23.5
Protean						
Drop to substrate	25	29.1	36.0	1	1.1	0.0
Move to surface	6	7.0	83.3	5	5.4	0.0
Tighten school formation	12	14.0	66.7	1	1.1	100.0
Locomotor via jet propulsion						
Scatter	1	1.2	100.0	11	12.0	9.1
Erratic jetting	3	3.5	0.0	6	6.5	16.7
Blanch-ink-jet	0	-	-	2	2.2	100.0
Jet out of water	2	2.3	0.0	2	2.2	0.0
Ink	18	20.9	16.7	25	27.2	12.0