Research Article

Behaviour of *Robsonella fontaniana* in response to a potential predator

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ABSTRACT. Cephalopods have two main defence strategies: the first consists on reducing the odds of being detected by a predator, while the second focuses on avoiding capture. The aim of this study was to understand the basic behavioural aspects of the octopus *Robsonella fontaniana* in response to the potential predator *Schroederichthys chilensis* (catshark) in central Chile. Experiments were conducted under laboratory conditions in order to determine the effectiveness of camouflage against predators. The results show that crypsis and an increase in the inactivity period are strategies used by octopuses as protection and defence mechanisms against predators.

Keywords: *Robsonella fontaniana*, predation, camouflage, inactivity, crypsis, Chile.

Conducta de *Robsonella fontaniana* frente a un depredador potencial

RESUMEN. Los cefalópodos tienen dos estrategias de defensa principales: la primera consiste en reducir las probabilidades de ser detectados por un depredador, mientras que la segunda se centra en evitar la captura. El objetivo de este estudio fue conocer los aspectos básicos del comportamiento del pulpo *Robsonella fontaniana* frente al depredador potencial *Schroederichthys chilensis* (pintarroja). Los experimentos se realizaron bajo condiciones de laboratorio para determinar la eficacia del camuflaje para evitar al depredador. Los resultados muestran que la cripsis y un aumento del periodo de inactividad son utilizados por los pulpos como mecanismo de defensa contra los depredadores.

Palabras clave: *Robsonella fontaniana*, depredación, camuflaje, inactividad, cripsis, Chile.

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INTRODUCTION

Camouflage is a main behavioural tactic of many animals in order to avoid attack by visual predators (Mercurio *et al.*, 1985; Palma & Steneck, 2001; Cuadrado *et al.*, 2001; Hanlon, 2007; Manríquez *et al.*, 2008; Stevens & Merilaita, 2009). Crypsis is a form of camouflage, in which all traits (*e.g.*, coloration) reduce the risk of an animal being detected by a predator when it is potentially visible to an observer (Stevens & Merilaita, 2009). In cephalopods, the types of crypsis displayed ranks among the most sophisticated in the animal kingdom. In fact, their neurological control over their chromatophores allows a vast array of possible body patterns, which may even resemble poisonous organisms (Hanlon & Hixon, 1980; Hanlon *et al.*, 1994, 1999; Chiao & Hanlon, 2001; Messenger, 2001; Norman *et al.*, 2001; Hanlon, 2007). Hanlon & Messenger (1996) illustrated six mechanisms of crypsis used by cephalopods: general background resemblance, countershading/concealment of the shadow, disruptive coloration, deceptive resemblance, rarity through 'rapid, neutrally controlled polyphenism, and cryptic behaviour/vigilance. In a field study, Hanlon *et al*. (1999) hypothesized that octopuses use crypsis primarily for defence and secondarily for foraging. Notwithstanding, in Tahiti, Hanlon *et al*. (1999) showed that only 54% of octopuses

were highly cryptic, with 24% being moderately cryptic, and 22% remaining conspicuous. In a different location (Palau), 31%, 19% and 50% respectively represented each of the above-mentioned categories. These results infer that a high percentage of total individuals may adopt other types of strategies for predator avoidance, in addition to crypsis (*e.g.*, inactivity, burying itself, hiding, or escaping).

Cephalopods are primarily visual organisms, and the signals they can emit and receive are varied (Hanlon & Messenger, 1996). Various body patterns, defined as the combination of postural, textural, locomotor and chromatic components, have been described (Hanlon & Messenger, 1996; Barbato *et al.*, 2007). These patterns can be analysed as a function of their various components, which range from colour changes (chromatic components), variations in texture or shape (postural or textural components), or changes in position (locomotive components). When combined, they can generate an effective response in the event of an attack by a potential predator (Packard & Hochberg, 1977; Hanlon & Hixon, 1980; Hanlon & Messenger, 1996).

In the case of cephalopods, two general defence strategies have been described. The primary one consists of diminishing the probability of being detected by a predator, either through camouflage, or by becoming less palatable (Hanlon & Messenger, 1996; Forsythe & Hanlon, 1997; Hanlon *et al.,* 2008). The secondary strategy only functions after actual detection by a predator. This strategy involves interfering in the predator's approach or attack, or by forcing it to abandon the prey after capture or during ingestion (Hanlon & Messenger, 1996). However, development of these strategies would depend on the type of environment where octopuses live, because they regularly explore their habitat while hunting or searching dens. The importance of crypsis in diurnal species living in complex habitats determines the acquisition of more elaborate body patterns, because visual predators exert selective pressures over this group of species (Hanlon & Messenger, 1996). In contrast, nocturnal species have developed simple body patterns because of the limited selective pressure by visual predators over these species (Hanlon & Messenger, 1996). Merilaita *et al.* (1999) suggest that in homogenous habitats, just increasing the degree of crypsis against the background may diminish the probability of the predator detecting its prey. In heterogeneous habitats, the degree of crypsis of a given coloration and the probability of the predator finding its prey may vary spatially, decreasing the probability of detection (Merilaita *et al.,* 1999).

Robsonella fontaniana (D'Orbigny, 1834) is a cold water benthic octopus with a wide distribution range from northern Peru to the Golfo Nuevo in Argentina, encompassing the southern cone, including the entire Chilean coast (Ibáñez *et al.*, 2008). It inhabits the intertidal zone, up to 225 m depth, over hard substrates and caves, with lesser abundance over sandy substrates (Ibáñez *et al.,* 2008). *R. fontaniana* is a relatively small sized species (under 50 mm mantle length), it has rugged skin and a fleshy expansion over each eye, with rough areas on the mantle and arms (Ibáñez *et al.,* 2008). These attributes may provide higher structural complexity as a basis for camouflage from predators in its environment.

Using the octopus *R. fontaniana* as a case study, we tested two hypotheses: (i) the time taken for the expression of behavioural components to take effect (*i.e.* textural, postural, locomotive, and chromatic) is modified in the presence of a predator; and (ii) the duration of periods of inactivity (cessation of all activity) is greater in the presence of a predator.

MATERIALS AND METHODS

Capture and maintenance of the octopuses

Between February 2003 and April 2003, 12 individuals of *R. fontaniana* were captured by scuba divers at depths between 3 and 10 m, in Lenga, San Vicente Bay, Chile (36°45'S, 73°10'W). Mantle lengths of these octopuses range between 25-65 mm (average 44.1 ± 9.9 mm SD) and weighed 4.9-69.5 g (average 24.81 ± 3.3 g SD). After capture, the octopuses were acclimatized in glass aquaria, of 750 L capacity with constant air and seawater supplies. Artificial caves were placed within the aquaria in order to avoid territoriality and to reduce capture-related stress. The acclimatization period lasted one week and featured a crustacean diet *ad libitum* (Ibáñez *et al.*, 2009). Three specimens of the catshark *Schroederichthys chilensis* (Guichenot, 1848) (30-40 cm of total length) were chosen as predators, given their high abundance at the same sites and microhabitats where the octopuses are found. Moreover, this catshark species is maintained with relative ease under laboratory conditions.

All experiments were conducted in 60×60×30 cm aquaria with isolated octopus individuals, given that several octopus species often display solitary behaviour and are aggressive in the presence of conspecific individuals (Mather, 1980). A substrate of sand combined with white and black gravel (approximately 1-5 mm in diameter) was used for these experiments. Each aquarium was covered with a

plastic sheet in order to diminish the stress of octopuses in response to the presence of humans. The experiments were recorded with a video camera model SCM51, within a time interval of 300 s and taped in VHS. Each octopus was filmed alone, and in the presence of the catshark, for a 10 min period in both cases.

Identification of body patterning

Octopuses were filmed both in the presence and absence of the potential predator (catshark). During these treatments, researchers quantified the time the octopuses dedicated to the four behavioural components defined in Hanlon & Hixon (1980) and Hanlon & Messenger (1996): (i) chromatic changes, defined as changes in body coloration going from fair to dark; (ii) textural changes, defined as changes in body texture from smooth to rough skin; (iii) postural changes, defined as changes in body position (*e.g.*, stretching or retracting the arms); and (iv) locomotive changes, defined as the capacity to become mobile. The mean time registered for each component was expressed as a percentage of the ratio of all components, following Leite *et al.* (2009). Periods of activity were defined as periods during which the individuals of *R. fontaniana* were in motion. Periods of inactivity were considered as time periods of 30 s or more when individuals remained still.

Statistical analysis

Behavioural changes were assessed using paired permutation t-tests with 10,000 iterations to estimate parametric measurements of mean, variance and bias, and to assess the statistical significance of each behavioural component (*i.e.*, chromatic, textural, postural and locomotive changes), as well as periods of inactivity. A permutation test is a type of statistical significance test in which the distribution of the test statistic under the null hypothesis is obtained by calculating all possible values of the test statistic under rearrangements of the labels on the observed data points (Gotelli & Ellison, 2004; Good, 2006). Confidence intervals were derived from the permutation test. This test was performed because few replicates of the experiments were available, the reason for why the data did not show normal distribution and homoscedasticity (Crowley, 1992; Gotelli & Ellison, 2004). All analyses were performed using the PAST software v.2.14 (Hammer *et al.*, 2001).

RESULTS

The changes in body pattern adopted by octopuses in the presence and absence of the catshark revealed that the time spent by each individual of *R. fontaniana* in each behavioural category was different (Fig. 1, Table 1). In the absence of the catshark, octopuses exhibited an increase in movement (45% in relation to other components), as well as a coloration pattern indicating 32% of chromatic changes (Fig. 2a). Almost no textural changes were evident in the absence of the predator (0%, Fig. 1). Under the predator presence, octopuses displayed low mobility (15%), and a cryptic and pale coloration pattern (39% chromatic changes and 15% textural changes), very similar to the substrate (gravel), along with rough skin texture (Fig. 2b). All components displayed significant differences in response to the presence of the predator (Table 1, Fig. 1).

The analysis of periods of inactivity in the presence and absence of the predator revealed that *R. fontaniana* individuals maintain longer periods of inactivity in the presence of the predator (mean = 204.9 s, C.I. 95% = 157.6-252.2 s) *vs* the potential predator's absence (mean = 98.5 s, C.I. 95% = 45.1-151.9 s) (Permutation t-test, $t_{(22)}$ = 3.28, P = 0.003). On two occasions during our experiments, the shark detected and attacked the octopuses. When the predator got closer to the octopuses (*i.e.*, between 30 and 50 cm), they changed their cryptic pattern to one which was more conspicuous and dark (Fig. 2c). Upon escape, they released ink or adopted deimatic behaviour (mottle pattern Fig. 2d) (see also Hanlon & Messenger 1996), which featured extended arms with rolled up extremities, and also shifted from an intense red colour to white and spotted (Fig. 2d). The escape behaviour occurred twice (16.6%) during the predator treatment.
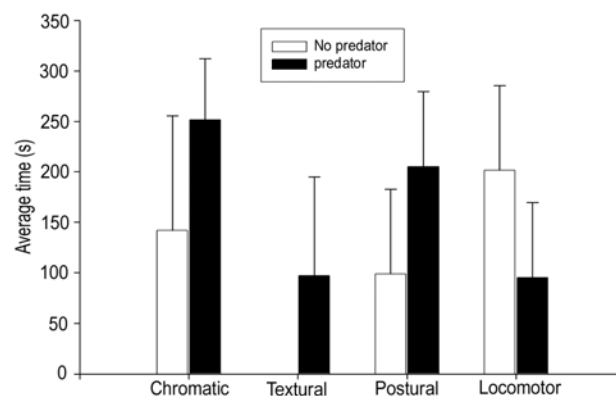

Figure 1. Average times of effectiveness (n = 12) of the behavioural components of *Robsonella fontaniana* under the presence and absence of the predatory shark *Schroederichthys chilensis*. Error bars = 1 SD.

Figura 1. Tiempo promedio (n = 12) de los componentes conductuales de *Robsonella fontaniana* en presencia y ausencia del tiburón depredador *Schroederichthys chilensis*. Barras de error = 1 SD.

Table 1. Descriptive statistics and permutation t-test of the behavioural changes of *Robsonella fontaniana* individuals in the absence (NP) and presence (P) of the predatory shark *Schroederichthys chilensis*. *Statistical significance with $P < 0.01$.

Tabla 1. Estadística descriptiva y prueba de t con permutación para los cambios conductuales de los individuos de *Robsonella fontaniana* en ausencia (NP) y presencia (P) del tiburón depredador *Schroederichthys chilensis*. *Significancia estadística con $P < 0,01$.

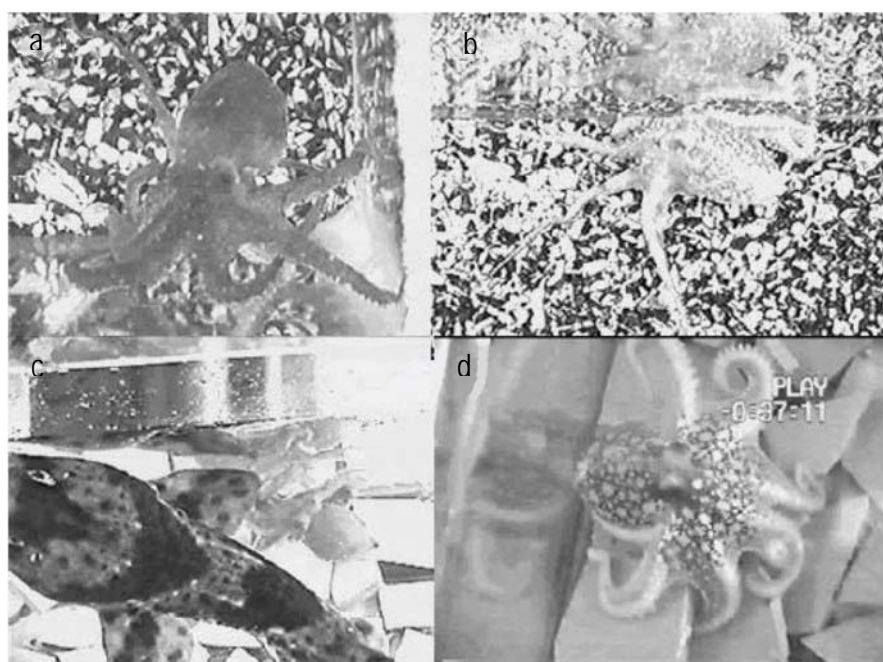|	Chromatic		Textural		Postural		Locomotive	
	NP	P	NP	P	NP	P	NP	P
Mean	141.8	251.3	0.08	96.8	98.5	204.9	201.5	95.1
C.I. (95%)	70-214	213-290	0-0.26	35-159	45-152	158-252	148-255	48-142
Permutation t-test	-2.94*		-3.42*		-3.28*		3.28*	

Figure 2. Pictures of *Robsonella fontaniana*. a) Without predator, b) in presence of predator, c) shark predator and octopus, d) deimatic behaviour.

Figura 2. Fotografías de *Robsonella fontaniana*. a) Sin depredador, b) en presencia del depredador, c) depredador y pulpo, d) conducta deimática.

DISCUSSION

This study confirms that *Robsonella fontaniana* uses camouflage (crypsis and inactivity) as its primary defence mechanism against a potential predator (*Schroederichthys chilensis*). Upon detection by the predator, octopuses displayed intense spotted coloration patterns (*i.e.* deimatic, Fig. 2d), escaped or released ink when feeling threatened. These findings are coherent with the behaviours displayed by other cephalopod species (Hanlon & Hixon, 1980; Anderson & Mather, 1996; Hanlon & Messenger, 1996; Forsythe & Hanlon, 1997; Hanlon *et al*., 1999, 2008; Caldwell, 2005). These behaviours were also observed in their natural environment at the time of their collection. For these reasons, we assume that *R. fontaniana* prefers to adopt a defensive strategy of hiding while remaining cryptic and inactive. In contrast, escaping appears to be a riskier strategy and therefore appears to be adopted only as a secondary response. Remaining cryptic may be less costly and provide advantages upon encountering a predator.

Octopuses, like *O. cyanea*, stay vigilant while foraging and usually move in a high head posture, positioning their eyes for maximum visibility (Hanlon *et al*., 1999).

On the other hand, and in addition to body patterns which help camouflage over various types of substrate, *R. fontaniana* also shows other cryptic behaviours which help them to hide and minimize the risk of detection. An inactive strategy is best applied against visual predators that are sensitive to movements occurring within their visual range (Hanlon & Messenger, 1996). In the case of the above-described experiments, the specimens displayed an intense white hue (pale), which made them almost undetectable over the gravel substrate. This type of cryptic behaviour is a common preventive strategy among the majority of cephalopods (Hanlon & Messenger, 1996).

R. fontaniana displayed a response to the presence of the predator in the form of changes in the effective times of each behavioural component. In the presence of the predator a longer time was spent in cryptic camouflage. This may be due to the fact that these organisms use camouflage mechanisms as their primary defence in response to the presence of predators, whereby the octopus resembles its surrounding environment or, perhaps, during cryptic or vigilant behaviour (Hanlon & Messenger, 1996; Hanlon *et al*., 1999). The fact that the time dedicated to display body patterns is greater in the absence of the predator could be potentially explained by exploratory behaviour of their environment in search of food or shelter (Mather & Anderson, 1999). In effect, the postures and general body patterns are more complex and varied during slow locomotion, than during quick escape manoeuvres, because individuals use several elements of crypsis (*e.g.* background matching, disruptive coloration) and polymorphisms when the probability of being found by a predator is higher (Huffard, 2006).

The inactivity of an organism may be induced by the propensity of the predator to attack, as seen in the *R. fontaniana* individual in the presence of a potential predator. On the other hand, activity was much higher while alone, under controlled conditions, in comparison to individuals under stress (*i.e.*, the presence of the predator), where the individual inactivity is key to avoiding detection. These cases of periods of inactivity are usually concurrent with cryptic behaviour, similar to those previously described by Forsythe & Hanlon (1997) and Hanlon *et al*. (1999).

We used the catshark *Schroederichthys chilensis* as a potential predator given its high abundance at the sites and microhabitats where octopuses are found. However, we found no information in the literature regarding the predators of *R. fontaniana* in Chile. Moreover, studies of the catshark diet showed a preference for crustaceans in central Chile (Fariña & Ojeda, 1993). Lastly, sharks detected and attacked octopuses twice during the experiment, which reinforces the use of this shark as a predator.

Under laboratory conditions, *R. fontaniana* uses camouflage to prevent predator attacks or to avoid detection. If the octopuses are found, they escape, release ink or adopt deimatic behaviour. Although this marked behaviour was observed at the time of capture, it should be corroborated with field studies.

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