Testing for camouflage of the Brazilian seahorse *Hippocampus reidi* (Syngnathidae) using the territorial damselfish *Stegastes fuscus* (Cuvier) (Pomacentridae)

Leonardo de Oliveira Armesto[1] and Natalie Villar Freret-Meurer[2*]

1) Gama Filho, Departamento de Biologia, Rua Manuel Vitorino, 553 – Piedade, Rio de Janeiro, Brasil – CEP: 20748-900.
2) Universidade do Estado do Rio de Janeiro, Instituto de Biologia Roberto Alcântara Gomes, Avenida São Francisco Xavier, 524 – Maracanã, Rio de Janeiro, RJ, Brasil – CEP: 20550-013.
*Corresponding Author: nataliefreret@yahoo.com.br

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Abstract

Seahorses present many characteristics such as variable colour, skin fronds and sedentary behaviour that provide camouflage as a defense mechanism. This study aims to test seahorse camouflage effectiveness in relation to a territorial species. Seahorses were translocated into the territory of the damselfish *Stegastes fuscus* (Cuvier) and its aggressive behaviour was recorded. *Stegastes fuscus* was chosen as being a good model of an aggressive fish, probably able to detect any transgressor. Seahorse size, sex, colour and mobility were recorded, as was the colour of the substrate used as holdfast. Eighty-four percent of the translocated seahorses were not attacked. There was no relation between number of attacks and size, sex or colour of seahorse and its holdfast. Seahorses that remained still during the experiment (84%) were not attacked, but those that moved were attacked (16%). It was observed in this study that neither seahorse color nor size were relevant characters in camouflage fitness, but probably all these together, including behavior, improved survival fitness.

Zusammenfassung

Eine Reihe von Merkmalen der Seepferdchen dienen der Tarnung als Verteidigungsmaßnahme, so die variable Färbung, Hautanhänge und sesshaftes Verhalten. In dieser Studie sollte die Wirksamkeit der Tarnung gegenüber einer Revier bildenden Art untersucht werden. Exemplare von Seepferdchen wurden in das Revier von Vertretern der Riffbarsch-Art *Stegastes fuscus* (Cuvier) versetzt, deren Angriffsverhalten wurde aufgezeichnet. *Stegastes fuscus* wurde als günstiges Modell für einen aggressiven Fisch ausgewählt, weil die Wahrscheinlichkeit einer Reaktion besonders hoch ist. Größe, Geschlecht, Farbe und Bewegungsverhalten der Seepferdchen wurden aufgezeichnet, außerdem die Farbe des Substrats, das zum Festhalten diente. 84 Prozent der räumlich versetzten Seepferdchen wurden gar nicht angegriffen. Zwischen der Zahl der Angriffe auf der einen und Größe, Geschlecht und Farbe von Seepferdchen und Haltesubstrat auf der anderen Seite gab es keine Korrelation. Die Seepferdchen, die sich während des Experiments ruhig verhielten (84%), wurden nicht angegriffen; alle, die sich bewegten, wurden angegriffen (16%). Nach den Ergebnissen dieser Studie sind weder Farbe noch Größe die entscheidenden Merkmale für den Tarnungserfolg, sondern es sind alle Faktoren zusammen, nicht zuletzt das Verhalten, die die Überlebensrate verbessern.

Résumé

Les hippocampes offrent une série de caractéristiques comme la variabilité de la couleur, les excroissances feuillues et le comportement sédentaire qui constituent un mécanisme de défense sous forme da camouflage. Cette étude a pour objectif de tester l'efficacité du camouflage des hippocampes en présence d'une espèce territoriale. Des hippocampes on été délocalisés dans le territoire de *Stegastes fuscus* (Cuvier) dont le comportement agressif a été observé. *Stegaster fuscus* a été choisi pour ses caractéristiques de poisson agressif, sans doute capable de détecter n'importe quel transgresseur. Taille, sexe, couleur et mobilité de l'hippocampe ont été notés, tout comme la couleur du substrat servant d'ancrage. Quatre-vingt-quatre pour cent des hippocampes délocalisés n'étaient pas attaqués. Il n'y avait pas de relation entre le nombre d'agressions et la taille, le sexe et la couleur de l'hippocampe ou de son ancrage. Les hippocampes qui n'ont pas bougé durant l'expérience (84%) n'ont pas été attaqués, mais ceux qui ont bougé ont été attaqués (16%). Cette étude a permis d'observer que ni la couleur ni la taille de l'hippocampe n'ont joué un rôle important dans l'efficacité du camouflage, mais il est probable que tous ces facteurs, y compris le comportement, améliorent ladite efficacité pour la survie.

Sommario

I cavallucci marini presentano molte caratteristiche come

colore variabile, fronde della pelle e comportamento sedentario che forniscono camuffamento come meccanismo di difesa. Questo studio mira a testare l'efficacia mimetica del cavalluccio marino in presenza di una specie territoriale. I cavallucci marini sono stati trasferiti nel territorio della castagnola *Stegastes fuscus* (Cuvier) registrandone il comportamento aggressivo. *Stegastes fuscus* è stato scelto poiché è noto essere un buon modello di pesce aggressivo, probabilmente in grado di rilevare qualsiasi intruso. Dimensioni, sesso, colore e mobilità del cavalluccio sono stati registrati, così come il colore del substrato utilizzato come sostegno. L'ottantaquattro per cento dei cavallucci marini traslocati non era stato attaccato. Non c'era alcuna relazione tra il numero di attacchi e le dimensioni, il sesso o il colore del cavalluccio marino o del suo sostegno. I cavallucci marini rimasti fermi durante l'esperimento (84%) non sono stati attaccati, ma lo sono stati quelli che si muovevano (16%). In questo studio è stato osservato che né il colore né le dimensioni erano di per sé caratteristiche rilevanti nel comportamento mimetico, ma probabilmente sono tutti questi aspetti insieme, compreso il comportamento, che contribuiscono a salvaguardare la sopravvivenza della specie.

INTRODUCTION

Among all marine ecosystems, coral reefs present the highest diversity of species. They shelter around 25% of all marine fishes known to scientists (Spalding et al. 2001). The great mosaic of habitats is one of the main reasons for such diversity (Sale 1980). This huge diversity improves ecological interactions among species, favoring evolution of defense and attack mechanisms (Williams 1991).

Camouflage is a primary defense mechanism developed by many species, which allows them to look like their environment, thereby avoiding detection by predators (Merilaita et al. 1999) and prey (Shine et al. 1998). Camouflage may be exhibited by a change in body shape and color, and is more efficient in homogeneous environments because it increases the degree of crypsis of the animal. Heterogeneous environments, such as coral and rocky reefs, tend to decrease the effectiveness of camouflage due to the presence of numerous microhabitats. However, crypsis might increase with the capacity to change color and low degree of displacement (Merilaita et al. 1999).

Seahorses present camouflage as predator avoidance and also as hunting strategy. They are teleost fish that belong to the family Syngnathidae, placed in a single genus *Hippocampus* Rafinesque (Nelson 1994; Lourie et al. 1999). Seahorses occur in many different habitats such as mangroves, seagrasses, coral and rocky reefs. They present many adaptations to benthic life, such as vertical body posture,

Fig. 1. *Hippocampus reidi* in its natural habitat. Photo by N. V. Freret-Meurer.

a prehensile tail and highly sedentary behavior (Schmid & Senn 2002). Besides these adaptations, they are also capable of changing color, from a discreet base color like black and brown to strong colors like yellow, orange and red (Foster & Vincent 2004) and to develop skin filaments (Lourie et al. 1999), allowing the animal to hide itself. All of these adaptations improve fitness in prey capture and predator defense. It is possible that the ability to change color and morphology, and similarity to its holdfast, may increase crypsis effectiveness.

Hippocampus reidi (Ginsburg) is the most abundant seahorse species along the Brazilian coast, being frequently observed in rocky reefs and mangroves (Fig. 1). They usually use sponges, seaweeds, mangrove plant roots and artificial substrates as holdfasts. The species is characterized by a long thick snout, narrow body and the absence of skin filaments in general. The color pattern consists of brown dots, many small white dots around the body and stripes in the dorsal region (Lourie et al. 1999). This species is considered "Data Deficient" on the Red List of Threatened Species of the International Union for Conservation of Nature – IUCN (IUCN 2006) and "Vulnerable" on the List of Threatened Species of Rio de Janeiro State (Mazzoni et al. 2000).

Finding seahorses is usually difficult for human divers, but it is not known if their predators have difficulty in finding them. The aim of this study is to test the crypsis effectiveness of the Brazilian seahorse *H. reidi* in its natural environment, using an aggressive territorial fish species to assess possible differences on crypsis effectiveness between size and mobility and checking if similarity between animal color and substrate color interferes with the attack behavior of the territorial fish. As there are no reported predators of seahorses in Brazil, we chose an aggressive species as a proxy for this study.

MATERIAL AND METHODS

Study sites: This study was conducted in Arraial do Cabo, Rio de Janeiro State, Brazil. A pilot study was undertaken to survey sites with the highest densities of seahorses. Six sites were chosen accordingly (Fig. 2): 1) Saco do Cardeiro; 2) Ilha de Porcos – Ponta Sul; 3) Ilha de Porcos – Saltador; 4) Ilha de Cabo Frio – Abobrinha; 5) Ilha de Cabo Frio – Anequin; 6) Forno – Ponta D'água, which are part of the Extrativist Marine Reserve of Arraial do Cabo (RESEX-MAR). The RESEX-MAR controls fisheries, promoting the sustainable use of natural resources (Lages 2003).

Experimental design: This study was carried out at six sites selected according to the presence of

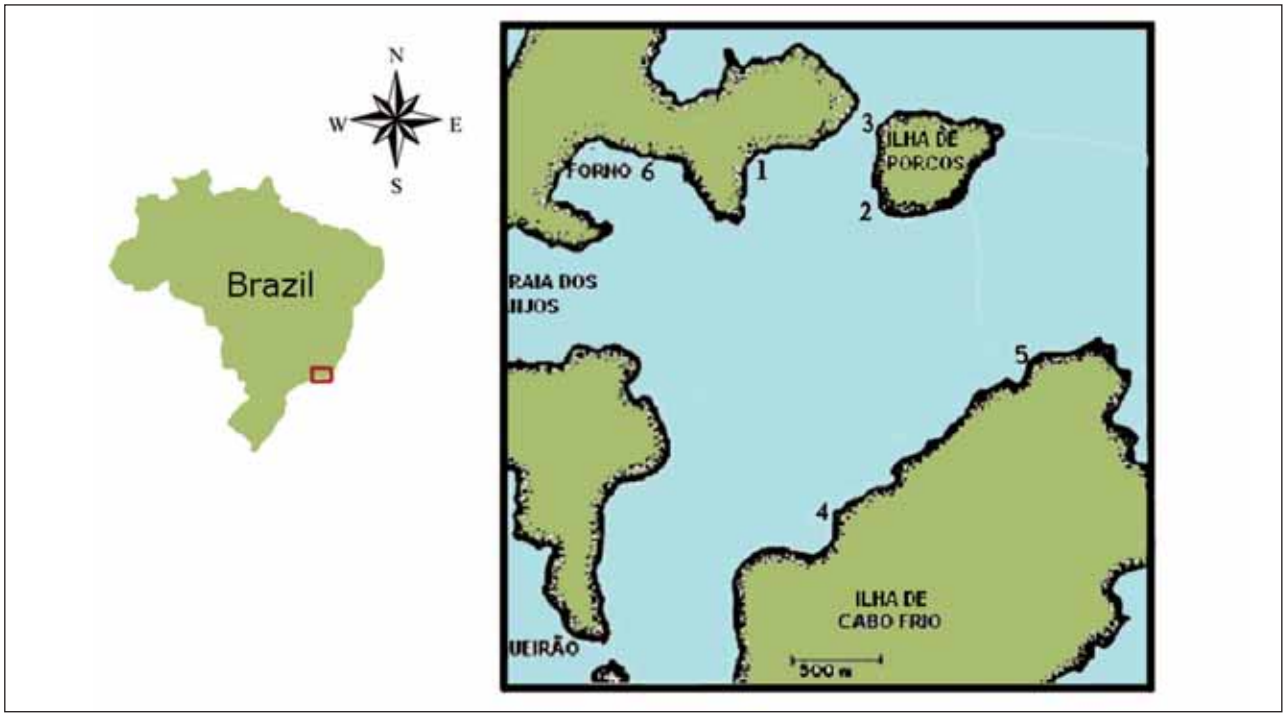

Fig. 2. Study sites in Arraial do Cabo, RJ: 1) Saco do Cardeiro; 2) Ilha de Porcos – Ponta Sul; 3) Ilha de Porcos – Saltador; 4) Ilha de Cabo Frio – Abobrinha; 5) Ilha de Cabo Frio – Anequin; 6) Forno – Ponta D'água.

H. reidi. The camouflage effectiveness of the seahorse was tested by experiments *in situ*, with SCUBA diving equipment. The damselfish *Stegastes fuscus* (Cuvier) was used to test the crypsis effectiveness of the seahorse. That species was chosen because it was considered a good model for detection of other invasive species, especially due to its extremely aggressive and territorial behavior, attacking all fish species which enter its territory (Ferreira et al. 1998; Menegatti et al. 2003). The use of an aggressive model efficient in detecting intruders is a new method developed specially to assess the effectiveness of avoiding detection.

Identification of *Stegastes fuscus* territory: The territory occupied by *Stegastes fuscus* was identified in all experiments for each individual. The most frequent area in which the damselfish occurred was characterized as the nuclear site of the territory and it was used in the experiment. These sites were established by noting specific characteristics of the benthic cover (eg. sponge, seaweed) in which the damselfish patrolled and exhibited frequent agonistic behavior by attacking other fishes. Limits of the nuclear site of the territory were observed for five minutes. The same territory was only used once in the study to avoid pseudoreplication. Dives were made at a distance from each other to prevent sampling the same territory.

Experiment of camouflage effectiveness: After identification of the *S. fuscus* territory, each specimen was collected with a hand net and removed from its territory. Afterwards, a specimen of seahorse, which had been previously captured and maintained in another hand net, was translocated inside the now-vacated *S. fuscus* territory. Seahorses were held only a few minutes in the net to avoid major stress. Distance between the original site of the seahorse and translocation was about 5 meters. After the seahorse selected the holdfast inside the territory, the damselfish was reintroduced. The holdfast was chosen by the seahorse. In general, sponges were used as holdfast as they are the most abundant substrate at the area. Aggressive behavior was observed in *S. fuscus* in relation to the seahorse for five minutes (Menegatti et al. 2003) using the animal focal method (Lehner 1979). The observation distance was 1.2 m. That distance was selected so as to not interfere with damselfish behavior. This was previously tested with several damselfish before the experiment. The number of attacks on the seahorse was quantified and presence or absence of attacks on other species during the experiment were recorded. It is important to highlight that no seahorse received any injuries during the experiments.

Control treatment: Aggressive behavior of additional specimens of *S. fuscus* was also observed in a nearby territory without any seahorse within. This was tested to verify the effect of catch and release in this environment. The same experimental procedure was used but without the seahorse introduction. The number of attacks on other fish species was recorded during a period of 5 minutes (Menegatti et al. 2003) to ensure that damselfish still presented the same aggressive behaviour. A total of 76 experiments were conducted, of which 38 were with seahorse translocations and 38 with controls.

Assessment of color and biometry: After the experiment, individuals were measured by height (HT) according to Lourie (2003), which is represented by the distance between the coronet and the straightened prehensile tail. Seahorse color and its holdfast color were also recorded. Similarity between seahorse and holdfast color was verified by a waterproof table containing a color pattern scale of colors found in available holdfasts and seahorses made specifically for this study. Similarity between seahorse and holdfast was only considered when the base color of the seahorse was the same as the holdfast. Dissimilarity was considered when the base color of the seahorse was different from the holdfast color. No gradient of similarity was considered. All experiments were conducted with different individuals. Seahorses were visually identified by schematizing and photographing their coronets according to Freret-Meurer and Andreata (2008) to avoid possible pseudoreplication (Hulbert 1984). Females were identified by absence of a brood pouch and males, by its presence (Lourie et al. 1999).

Statistical analyses: The number of attacks on individuals similar and dissimilar to the holdfast and the difference between the size of male and female were compared by the Mann-Whitney test, because data did not follow homoscedasticity. Relation between effectiveness and individual size was tested by linear regression (Zar 1999). Mean values were expressed by mean ± standard deviation.

RESULTS

Seahorse translocation experiments showed that 15.7% (n = 6) were attacked by *S. fuscus* and

84.3% (n = 32) were not attacked. In the control group all observed *S. fuscus* (n = 38) attacked other fish species, as well as in the treatment group.

There was no significant difference between attacks on seahorses that were similar or different to their holdfasts (*p* = 0.531; U = 126; gl = 37). Specimens that had similar color to the holdfast (n = 18) were attacked by 13% of damselfish in all experiments and individuals that had a different color (n = 20) were attacked by 20%.

The study seahorses had a mean height of 11.93 ± 2.94 cm. Males were longer than females, with a mean height of 12.72 ± 3.69 cm and 11.15 ± 1.69 cm respectively, although there was no significant difference between the sexes (*p* = 0.09; t = 1.69; n =). There was no relation between the number of attacks and the size of the seahorse (*p* = 0,406; r = 0,147) and no statistical significance among the size of attacked and not attacked seahorses (p = 0,805; U = 142).

Seahorse mobility was an important factor to *S. fuscus* attack. Individuals that did not move were not attacked, but all individuals that swam during the experiment were attacked. *Stegastes fuscus* presented regular aggressive behavior, attacking other fish species in all 76 translocation experiments, including the control treatment. A total of 123 attacks were recorded (3.26 ± 0.714 attacks/ experiment) during the control treatment.

DISCUSSION

Seahorses were rarely attacked by the damselfish in our experiments. The results did not show any relation between attacks and other physical features such as seahorse size, sex and color similarity/dissimilarity to holdfast. Size was expected to have some relevance as large animals were supposed to be easier to detect (Kiltie 2000). But in the experiments, the difference between maximum and minimum size was not significant, which could explain the absence of relation between both groups and the attacks. Color is considered to be an important feature in the camouflage of several species and they usually choose backgrounds upon which they will be better concealed (Mercurio et al. 1985; Moey 1990; Steen et al. 1992). However, the experiments showed that it did not matter if seahorse color matched the holdfast color. This fact is probably related to Merilaita et al.'s (1999) hypothesis on cryptic coloration in heterogeneous habitats. They proposed that heterogeneous habitats tend to shelter species that present color patterns adapted to several types of environment. In that way the species might conceal itself along many types of habitat without being detected, optimizing its color. The rocky reef is a very heterogeneous habitat full of different colors provided by many benthic and pelagic species. The ability of the seahorses to change their base color combined with the habitat structure, turn these animals into cryptic organisms even when they do not match their holdfast. In fact, crypsis might be the correct classification of the seahorse's method of avoiding detection. Stevens and Merilaita (2009) define crypsis as "features of physical appearance (e.g. coloration), but also behavioral traits, or both, to prevent detection". This definition also fits perfectly with our results in relation to the importance of movement. Our results demonstrate the relevance of the absence of movement. In general, cryptic animals avoid detection by decreasing their movement rates (Edmunds 1974; Sih 1987). These data are consistent with the experiment of Morey (1990) who compared vulnerability of the treefrogs *Pseudacris regilla* on matching and non-matching backgrounds. The matching background treefrog appeared to be as vulnerable as the non-matching treefrog when moving. No specific studies have been accomplished about seahorse crypsis, but several authors report the relevance of seahorse movement, color and body shape, such as Lourie et al. (1999) who highlight the small number of seahorse predators due to the seahorse camouflage, low mobility and bony body shape; Perante et al. (2002) reports that *Hippocampus comes* Cantor develops sedentary behavior as a strategy to make its camouflage more efficient; and Foster & Vincent. (2004) state that seahorses present efficient camouflage that improves fitness in prey capture and escape from predators and that they do not show territorial defense against other fish species, indicating that color might be a crypsis strategy and also a mechanism of communication between individuals of the same species. These data are consistent with the present study, assigning relevance to displacement behavior.

Loss of habitat is one of the major stresses suffered by the seahorses, decreasing their populations over time (Foster & Vincent 2004). This habitat change may lead individuals to die or forces them to migrate to other sites (Dulvy et al. 2003). Forced displacement implies vulnerability in seahorses, making loss of habitat even more dangerous to the species. Migration has been pointed out as a way

for maintaining stable stocks, but particular attention should be given to this. More effective management of coastal areas should be applied to avoid loss of habitat, such as restriction of construction near rocky reefs and mangroves and reduction of bottom trawling near the coast.

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