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Abstract Individual identification is particularly important for ethological studies and sampling design. Previous studies have developed various types of marking by tags and chemical marks, but these methods involve significant manipulation of the individuals. Other studies have reported natural marks as an efficient method for individual recognition. Our study aims to elucidate a new method for natural mark identification of seahorses, which we tested with the endangered Brazilian seahorse *Hippocampus reidi*. We avoid pseudoreplication by recognizing individuals. Seahorses have a hard bony structure on the top of their heads called the coronet, which has a different shape for each individual, corresponding to a fingerprint. We tagged seahorses in the field with collar tags and photographed their coronets. After two days, we identified seahorses by their photographs and verified their identification with the collar tags. We correctly identified all individuals by fingerprint identification. Although this method was only tested with adults, we suggest that it applies to seahorses in general, as all species possess the coronet structure.

Keywords Syngnathidae · New method · Natural marks

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Introduction

Individual identification in a population is a particularly important tool for ecological studies and sampling design. It allows us to estimate population size (Block et al. 2005), movement patterns over both small (Freret-Meurer and Andreata 2008) and large scales (Hussey et al. 2009), and reproductive and mortality rates (Iversen 1962), in addition to detailed behavioral studies (Freret-Meurer et al. 2009; Teo et al. 2005). Previous studies have often used individuals as a parameter to assess population dynamics. There are many techniques for tagging or marking animals, which many researchers frequently use for different taxa (Hutchings and Gerber 2002; Arntzen et al. 2004; McGregor et al. 2008; Fiedler 2009). Researchers often use artificial tags; however, this usually involves handling the animal, which can cause stress and lead to injuries (DeRoche 1963; Jensen 1967; Eames and Hino 1983; Titus and Vanicek 1988). Several studies have reported natural marks as an effective technique for individual recognition. For example, Petersen

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(1972) recognized different black and white stripe patterns for zebras *Equus burchelli* Gray 1824, and Glockner and Venus (1983) reported different individual patterns for whale dorsal fins. Several studies have also used dorsal fin tagging for other marine mammal species (Payne et al. 1983; Friday and Smith 2000; Giklinson et al. 2007; Calambokidis et al. 2009). Studies on fish species of the family Syngnathidae (e.g. leafy seadragon *Phyllopteryx teniolatus* Martin-Smith 2011) have also used natural tags by identifying patterns of yellow spots and blotches.

The family Syngnathidae includes leafy seadragons, pipehorses, pipefishes, and seahorses. Seahorses belong to only one genus: *Hippocampus* Rafinesque, 1810 (Nelson 1994; Lourie et al. 1999), and the number of described species is frequently updated (Horne 2001; Kuiter 2001, 2003; Lourie et al. 2004). Several seahorse species have significantly reduced populations due to frequent capture for aquariums or as souvenir, trawl by-catch, and habitat destruction (Vincent et al. 2011). This has turned these animals into threatened species, and many are now included on the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN), as well as on equivalent local or national lists. The development of methods with reduced manipulation is important to decrease animal stress during scientific studies. Higher levels of stress may have several negative effects on biological functions such as reproduction or depression of the immune system (Dobson and Smith 2000).

The Brazilian seahorse *Hippocampus reidi* Ginsburg, 1933 is considered Data Deficient by the IUCN Red List. This seahorse presents much phenotypic variation, such as the presence or absence of skin filaments and different base colors, but these characteristics are not efficient natural tags because seahorses may change color or develop filaments during their lives (Lourie et al. 1999). However, seahorses have a structure on the top of their heads called the coronet. Measurements of the coronet such as height, type of inclination, number of spines, and overall shape (e.g.: rounded, triangular) can help to classify a species. The top of the coronet is relatively flat, except for those species that possess spiny coronets (*H. histrix*, *H. jayakari*, *H. spinosissimus*, *H barbouri*, *H. angustus* — Lourie et al. 1999), and each individual coronet is shaped differently with unique patterns of cavities and elevations. The aim of the present study was to test the flat area of the top of the coronet of the Brazilian seahorse *Hippocampus reidi* as a natural tag to identify different individuals of the same population.

Material and methods

The present study was conducted in rocky reefs and *Sargassum* sp. branches of Araçatiba Cove, located in Ilha Grande (23° 09′S and 044° 20′W), Rio de Janeiro state, Brazil. We chose this site because of the reliable presence of the seahorse *H. reidi*, which is a threatened species in the state of Rio de Janeiro (Mazzoni et al. 2000), and its crystal clear water. We surveyed seahorses in the cove in eight belt transects (20× 5 m) made by plastic measuring tapes laid down on the substrate. Twenty different individuals were tagged by underwater photographs from May to December 2006. We captured all sighted seahorses and took photographs of individuals and the flat area of the top of the coronet (Fig. 1) with a Sony Cyber-Shot DSC-W210 12.1 mp camera. It was essential to be sure that the flat area of the top of the coronet was turned to the lens of the camera, otherwise the shape photographed could appear different. We selected the best images of each animal for subsequent analyses. Photographic quality was based on the angle of the flat area of the top of the coronet to the camera, focus, lighting, and contrast (Giklinson et al. 2007). Photograph identification consisted of comparing the design of the cavities and lifts of the center of the top of the coronet. We used the software I^{3}S Classic v.2.0 to test matches between each coronet (Martin-Smith 2011) (Fig. 2). We included all digital images in a digital catalog. The flat area of the top of the coronet of each animal photographed was schematized by hand in the laboratory and scanned to be included in the catalog. All individuals had a digital image and a hand-drawn scheme of the flat area of the top of the coronet. We also drew the schemes in a Polyvinyl chloride (PVC) slate that was used underwater for identification during diving. The 20 naturally tagged individuals were also artificially tagged as a control of this method. Artificial tagging consisted of a collar tag composed of a polyvinyl chloride disc of 5.5 mm× 3 mm with a three-digit code on one side, and was attached to the seahorse's neck with a cotton thread (Vincent and Sadler 1995). All tested individuals were adults (>10 cm), according to Silveira (2005); 13 of which were females and seven were males (Table 1).

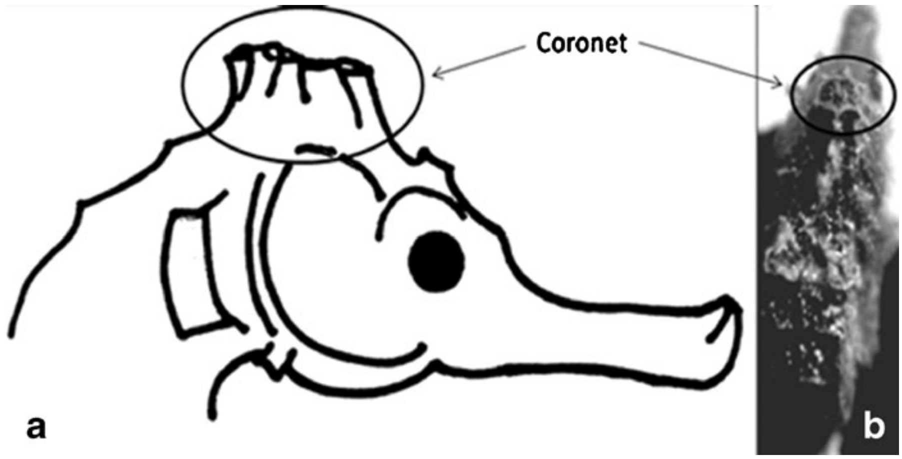

Fig. 1 Seahorse, *Hippocampus reidi*, head: **a** lateral view of the coronet; **b** superior view of the coronet

We identified sexual dimorphism only by the presence (male) or absence (female) of the brood pouch. Body size (height) and color are individually different, and no sexual pattern has ever been found or described.

After 2 days, the same diver repeated the survey procedure. Upon finding the seahorses, they were first identified by the coronet according to hand drawings (the coronet was once again photographed), and then the collar tag was checked to verify if they matched. The risk of seeing the identity code on the collar tag before the coronet recognition was minimal; although the collar tag was visible, the code was written in small lettering and was covered by the thumb. We repeated this identification procedure monthly for 8 months to assure that identification could be long lasting. This study did not evaluate the capacity of

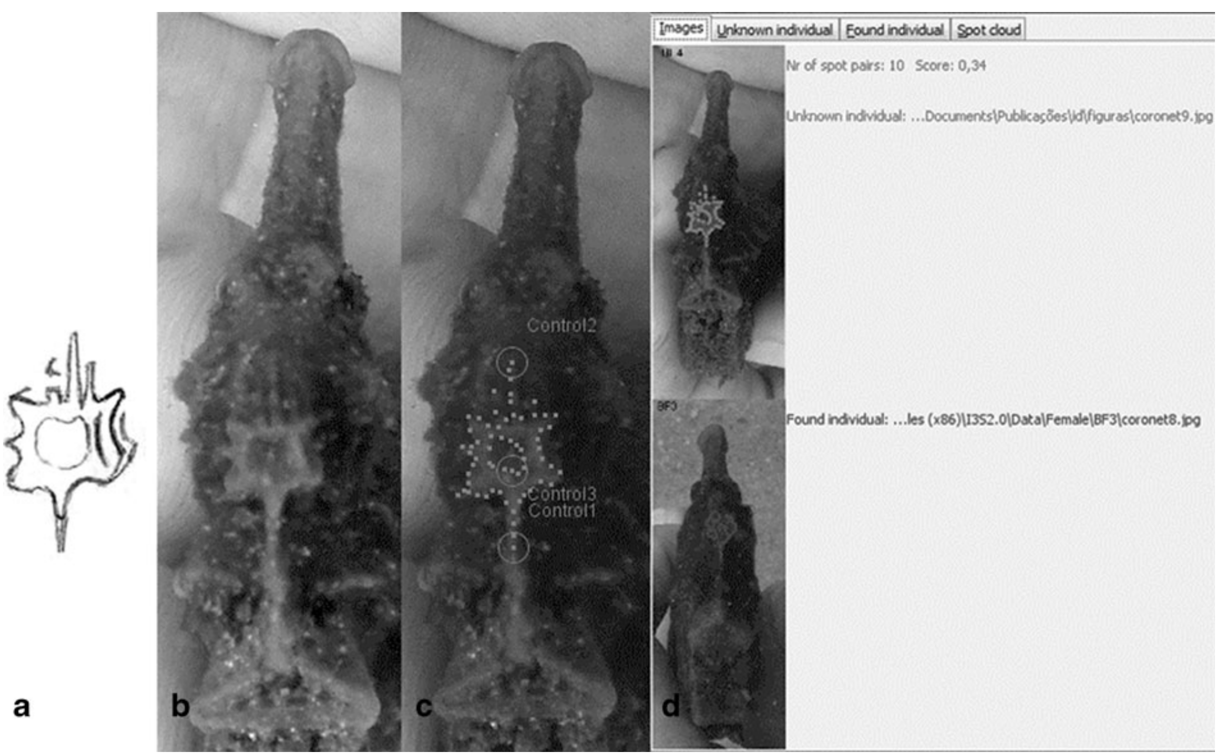

Fig. 2 Procedures for individual identification of *Hippocampus reidi*: **a** Hand drawing of F16 coronet; **b** F16 photograph of the coronet; **c** F16 cavities and lifts marked by spots (The software uses Control 1, 2, and 3 as a reference for the other spots); **d** Program compared spots of F16 with F3. F means female

Table 1 General characteristics described for the tagged seahorses, *Hippocampus reidi*, in Araçatiba beach, Ilha Grande, Rio de Janeiro state, Brazil: code (M- male; F- female), size, stage, and color

Code	Size (cm)	Stage	Color
F12	12.7	Adult	Black
F13	14.1	Adult	Red
F14	12.2	Adult	Pale red
F15	12.4	Adult	Yellow
F16	13.2	Adult	Pale yellow
F17	12.6	Adult	Pale red
F18	13.7	Adult	Pale yellow
F19	13.4	Adult	Pale yellow
F20	13.4	Adult	Pale yellow
F21	12.9	Adult	Pale red
F22	11.6	Adult	Pale red
F23	14.1	Adult	Pale red
F24	10.9	Adult	Orange
M9	14.3	Adult	Pale yellow
M10	12.9	Adult	Pale red
M11	12.4	Adult	Pale yellow
M12	13.7	Adult	Pale red
M13	13.4	Adult	Pale red
M14	12.7	Adult	Pale yellow
M15	13.2	Adult	Black

different researchers to identify seahorses by this method in the field, but we recognized the seahorses by comparing their photographs in the laboratory.

Results and discussion

Between May and August 2006, we tagged 20 seahorses and recaptured them 2 days later. We compared the flat area of the top of the coronet to the scheme made in field and photographed each (Fig. 3). After recognition, we verified the identification using the collar tag and the code of each recaptured animal. We compared the digital images to the catalog in the laboratory. We verified the recognition by scheme, digital photograph, and collar tag. All of the animals sampled (n=20) were positively identified by both the scheme in the field and the digital images in the laboratory.

Of all 20 tagged seahorses, we recaptured only five at the site two more times after the initial recapture described in the methods. The first additional recapture was 1 month after tagging and the second was 2 months after tagging (Fig. 4). Furthermore, three of these individuals were recaptured a third time 3 months after tagging. All of them were still wearing the collar tag. Afterwards, none of them returned to the study site. We applied the same identification procedure as before. Coronets did not change their shape or develop skin filaments during the study, but epiphytes algae have frequently been registered and we removed them from the top of the coronet for identification. Skin filaments usually grow on the bodies of seahorses and this has already led to errors in the species identification of *H. guttulatus* and *H. hippocampus* (Curtis 2006). Many precautions should be taken when such skin filaments are present. Seahorse populations in Rio de Janeiro state rarely present skin filaments, but when they appear, they are usually found under the snout or on the back and head flanks (personal observation).

Algae may use the coronet as a substrate, but that is usually seasonal and does not harm the seahorse or change the coronet structure, as it is a bony structure covered by epidermis. The epiphytes could visually prejudice identification of the coronet, but in most cases they can be removed gently with the finger. We have not registered other organisms using the coronet as a substrate.

Seahorses are usually tagged with collar tags or Visible Implant Elastomer Marking. Collar tagging is the most commonly used technique to assess individuals in a seahorse population (Vincent and Sadler 1995; Perante et al. 2002; Bell et al. 2003; Vincent and Giles 2003; Moreau and Vincent 2004; Martin-Smith and Vincent 2005; Vincent et al. 2005) and it is also the least costly. This tag should be monitored for a short time because it may have longer-term consequences for seahorses, such as attracting predators or impacting behavior and swimming ability of younger seahorses. This method is recommended for adults of species that present site fidelity and for short-term studies. The Visible Implant Elastomer Marking is a permanent tag technique that consists of injecting a plastic polymer under the skin of the seahorse. This method uses four different fluorescent colors (red, orange, green, and yellow) and identification of individuals is according to color and body mark location. Tag loss is usually low (Morgan and Martin-Smith 2004), and individual recognition is immediate, but it depends on compatibility between the polymer

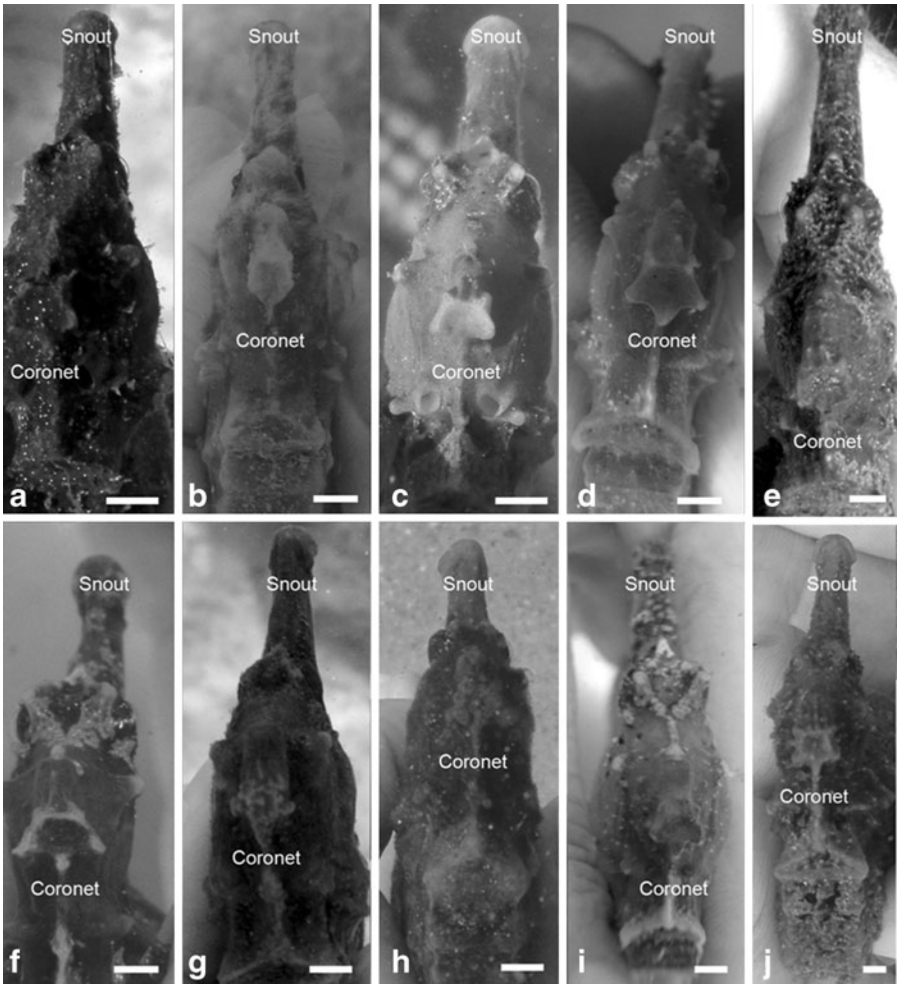

Fig. 3 Seahorse, *Hippocampus reidi*, coronet photographed in the field: **a** F12; **b** F13; **c** F14; **d** F15; **e** F16; **f** M9; **g** M10; **h** M11; **i** M12; **j** M13. The *white bar* scale represents 0.3 cm. F and M means female and male, respectively

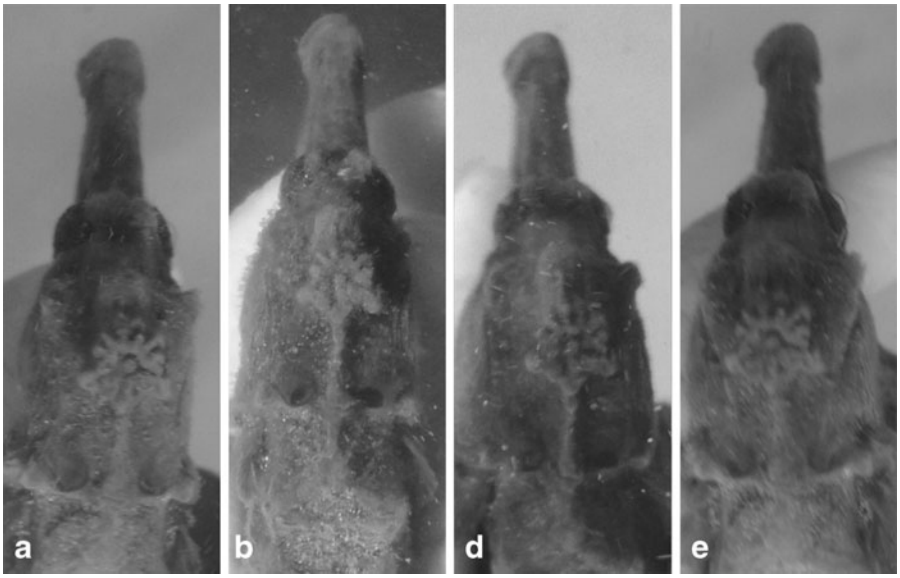

Fig. 4 The female F12 was recaptured three times after the first tagging: **a** first tagging (May 2006); **b** first recapture (the day after); **c** second recapture (April 2006); **d** third recapture (March 2006)

and the species. This marking method has not yet been tested in *Hippocampus reidi* and is expensive compared to other methods (Le Cheminant 2000; Woods and Martin-Smith 2004). Nevertheless, it has proven effective with seahorse species due to their segmented bodies.

Dummy tags have recently been tested in the seahorse species *H. guttulatus* (Caldwell et al. 2011). Although they weighed up to 6 % of the animal's body mass, the authors did not observe any behavioral change (e.g., movement pattern, holdfast use), except by vertical orientation. Instead, animals tagged by acoustic tags increased their body mass, which should carefully be interpreted. This could be an excellent tool to understand movement pattern and habitat use, but still requires further studies.

The natural tagging seemed to be an effective method to recognize individuals of the same population. It is a relatively cheap method, does not harm the animal, and does not hinder its natural behavior. It is a method that can be applied even in long-term studies, unlike the collar tagging. We tried to draw the coronet on the slate without manipulating the animal; however, this was not possible, as the important details of the coronet were not clearly visible from far away and the seahorses hid behind the holdfast if we approached too close. This method was successful in studies of population structure (Freret-Meurer and Andreata 2008) and ethological studies (Freret-Meurer et al. 2012), and has been tested in small populations (mean density=0.18 ind.m^{-2}). This is the standard method used in the state of Rio de Janeiro and the most common method reported for most studies of the seahorse species (Foster and Vincent 2004). Natural tagging is perfectly suitable for small adults (10 cm) but it has not been tested in juveniles (<8 cm). Therefore, we recommend it only for adults, or that it be tested first in juveniles before being applied in further studies. The disadvantage of natural tagging is that it demands more time and work for each identification. Individual recognition depends on checking the archive of photographs and hand-drawn images.

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