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Personality in the longsnout seahorse, *Hippocampus reidi* Ginsburg, 1933: Are males shyer than females?

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ABSTRACT

Individual responses, particularly based on personality, can have important consequences for individual fitness, based upon success in exploring new habitats, feeding on novel foods, and aggressiveness in competitive interactions. We conducted laboratory experiments to analyze individual responses to different artificial conditions that could suit specific male and female personalities in the endangered seahorse, *Hippocampus reidi.* Our experiments with *H. reidi* evaluated individual responses to a new habitat, novel objects, level of inactivity and social interactions. We demonstrate that approximately half of the seahorses have a bold personality, readily inspecting new habitats and objects and sporadically presenting social approaches. The remaining shy individuals had high levels of inactivity and did not check novelties in their habitats. Although we expected that males would have shyer personalities when compared with females, due to their ecological role in the provision of parental care, we found no statistical difference between the sexes in terms of these aspects of personality. The similar frequency of both types of personality in males and females suggests that these features may be balanced and evolutionarily stable in the sampled population.

1. Introduction

Many animal behaviour studies are based on behaviour of a specific population or group, but fail to consider the variability among individuals. However, the function of multiple behaviours in a population might be crucial for ecological fitness (Carter et al., 2013; Ogden, 2012). Behavioural diversification in a population is analogous to the concept of ecological niche, in which multiple behaviours can reflect individual specializations that diversify the use of resources, thus reducing inter-individual conflict (Bergmüller and Taborsky, 2010). The consistent behavioural differences between individuals across time and contexts is called personality (Dingemanse et al., 2010).

Variations in personality among individuals of a population are common and can be assessed by their reaction when faced with novelties, social interactions, breeding opportunities and habitat exploration, and may vary according to each stimulus (Corr et al., 2013; Dingemanse et al., 2012; Sih et al., 2004a). Understanding individual personality traits allows us to determine how animals may respond to environmental and ecological challenges (Reale et al., 2007; Sih et al., 2004b). Variations in personality may reduce conflicts among individuals and species, because distinct personalities result in different uses of resources (e.g. habitat, food), thus reducing competition and enhancing productivity (Bolnick et al., 2011). Populational variability in personality also leads to a wider exploration of resources, reducing density fluctuations in extreme situations (McCann, 2000). Intraspecific conflicts may instigate the occupation of different habitats and can lead to several dispersal strategies (Cote and Clobert, 2007). Depending upon each personality's ability to face new environments, individuals may or may not be able to tolerate high density populations. Therefore, the diversification of personality is an important feature for population health (Castanheira et al., 2013).

Recent studies show that the shy-bold axis of behaviour may have important consequences for an individual's lifespan and fitness, based upon success in exploring new habitats, feeding and mating opportunities and in coping with competition (Adriaenssens and Johnsson, 2010; Dall, 2004; Frost et al., 2007; Sinn et al., 2008). The bold-shy

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continuum has a strong effect in decision making, especially in unpredictable environments. Degree of boldness is usually assessed across several tests wherein bold individuals take more risks, learn faster, are more active and more explorative, while shy individuals present the opposite characteristics (Martins et al., 2012; Sneddon, 2003).

Male-female differences in personality also have been recorded in nature and are basically explained by their differences in ecological niche (Harris et al., 2010; Hedrick and Kortet, 2012; Vilela et al., 2017). Their ecological niche provides them with different experiences, driving the development of different traits (Piyapong et al., 2009; Schuett and Dall (2009)). There are few studies about sexual differences in personality types (Titulaeur et al., 2012; Zwoinska et al., 2013), but theory leads us to expect such differences. The risk-reward hypothesis suggests that females should be shyer than males due to reproductive traits (Jolles et al., 2015). For example, female guppies (*Poecilia reticulata*) have evolved to store sperm and thus avoid risky searching for mating partners (Laland and Reader, 1999; Harris et al., 2010). Males usually take more risks during breeding due to the possibility to improve reproductive success (Zwoinska et al., 2013). On the other hand, parental care appears to be associated with shy behaviours, as reported for the rhesus macaque *Macaca mulata* (Boissy, 1995; Maestripieri, 1993) and for the cichlid *Chiclasoma nigrofasciatum* (Budaev et al., 1999). Therefore, differences in male versus female ecological function may lead to variations in personality.

Seahorses are a type of fish distributed worldwide, inhabiting shallow waters of marine and estuarine ecosystems, such as mangroves, seagrass beds, rocky and coral reefs (Lourie et al., 1999). They have a unique division of reproductive roles, wherein courtship may take a couple of days and then females put their eggs inside the brood pouch of the males. Males are the ones that carry the eggs and provide protection, osmorregulation and nutrition to the fry (Vincent and Sadler, 1995). Seahorses have fascinated people for centuries due to their unique equine shape and unusual breeding habits. However, many species are currently threatened due to over-exploitation. They have been overharvested for use in traditional Chinese medicine, aquarium market and to be dried and sold as souvenir (Vincent, 1996).

In the present study we used seahorse *Hippocampus reidi* Ginsburg, 1933 as a model species, which is the most abundant seahorse in the Brazilian coast. Although this species is categorized as *Near threatened* by the IUCN Red List of Threatened Species (IUCN, 2017), regionally it is considered as *Vulnerable* (Mazzoni et al., 2000). The population decrease of this species in Rio de Janeiro occurs specially due to by catch and seahorse trading (Rosa et al., 2007). Seahorses are captured in nature for the aquarium trade, because it is difficult to develop an efficient protocol for rearing seahorse massively. Understanding how animals tend to react to captive conditions, it is possible to adapt a better condition for rearing the juveniles with the proper environmental enrichment and suitable aquarium partners, reducing capture of wild seahorses. Our objective was to examine the potential sex differences in personality, based upon the hypothesis that the ecological responsibility of carrying the fry might have led to shyer behaviour in males, while females would be bolder. This study examined differences in personality could potentially contribute towards management and conservation of seahorses in captivity, as a way to reduce pressure over natural populations.

2. Material and methods

This study was conducted in captivity and is part of a larger study of seahorse communication, authorized by the Brazilian Government, license number 25663-2 (Instituto Chico Mendes - ICMBio) and followed Animal Behaviour Society's Animal Care protocol (2012) and the Ethics Committee for Animal Care and Use in Experiments from Rio de Janeiro State University (number: 006/2015).

2.1. Animal sampling

Seahorses were manually collected at Urca beach (22° 56′ S and 043° 09′ O) using snorkeling equipment from November 2013 to September 2014. They were placed in plastic bags containing seawater and taken to the laboratory within 20–30 min. A maximum of five animals were collected per dive to prevent negative effects on the local threatened population, and our final sample size was limited due to the small populations that currently exist in the state of Rio de Janeiro. All animals were returned to the same sampling location after the experiments were conducted within a maximum period of one month. Before returning them to their natural habitat we took measure to determine that they were in good physical condition. These included assessments of skin and eye membrane color, behaviour pattern during feeding, and absence of sores, skin injuries and edemas. Pregnant males were not used for the experiments to prevent abortion. To avoid pseudoreplication during experiments, animals were individually identified and catalogued by photo-identification of the coronet, according to Freret-Meurer et al. (2013). No seahorses were recaptured during the study. Juveniles were not considered in trials, because we did not have a reasonable number for statistical analyses.

2.2. Behavioural assays

In the laboratory, seahorses were separated by gender and maintained in two 1.00 × 0.35 × 0.45 m aquariums. The salinity, temperature and pH were maintained at 34–35, 23–25 °C, 8.1–8.2, respectively. Nitrite was kept at 0–0.25 ppm and ammonia at 0 ppm. Photoperiod was kept at 10 day (0730 h–1730 h) and 14 night (1730 h-0730 h). The seahorses were fed twice a day (0800 h and 1600 h) with live *Artemia salina* enriched with higly unsaturated fatty acids for 24 h. The assays were conducted individually in a 0.80 × 0.30 × 0.40 m aquarium with two plastic plants used as substrate and holdfasts for the seahorses 35 cm apart from each other. All the behavioural trials were recorded with a Nikon Coolpix AW130 and later scored. Assays were divided into trials that evaluated exploratory behaviour, neophilia, anti-predatory response and sociability, detailed below.

2.2.1. Exploratory behaviour

Each seahorse was introduced individually into the experiment aquarium. After a period of 3 min acclimation restricted in a corner of the aquarium, the seahorse was released for the duration of the 20 min trial. The following behaviours were recorded: a) *inspection of the new habitat*: the animal swam throughout the aquarium; b) *protection:* the animal grasped the holdfast without exhibiting any other active behaviour, except for ocular movement. We scored the time elapsed until the animal grasped the holdfast.

2.2.2. Neophilia

After about 30 min of the end of the exploratory behaviour, we tested for neophilia/neophobia response. We introduced a red tube filled with live shrimp (*Artemia salina*) inside the aquarium in front of the seahorse. We scored for neophilia when seahorses approached the tube and for neophobia, when they moved away or remained motionless. Time was scored for 5 min.

2.2.3. Anti-predatory response

The anti-predatory behaviour was observed in the next 24 h of captivity. Seahorses usually remain attached to a holdfast using its tail to grasp the substrate. We simulated attempt of predation by using our hands to capture and retain the seahorse for 30 s, after which it was released. The *resistance to handler* was divided into four specific behaviours of the seahorse: a) grasping harder to aquarium plants while we try to capture it; b) swimming away from holdfast while we approach; c) grasping our hand hard trying to break free; d) wrapping our hand softly with its tale. We measured the time the seahorse took to get quiet