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Short Communication

Bird-like complex nesting behaviour by the Brazilian-endemic reef fish *Gramma brasiliensis*

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Abstract. Nesting is a common behaviour associated with reproduction in several taxa. Nevertheless, this important parental care behaviour is rarely reported for reef-associated fish. The present study provides the first description of bird-like complex nesting behaviour of the endangered basslet (*Gramma brasiliensis*). Males were observed building nests using macroalgae thalli around holes and depressions, in coralline substrate. Algae were used to camouflage the nest entrance and to form a cushioned bed for egg laying. Nesting is a critical aspect of the reproductive strategy of *G. brasiliensis*, so, this behaviour is a key factor for the management measures implications on the conservation of the species.

Additional keywords: fish nest, habitat manipulation, reproductive behaviour.

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Introduction

Nesting is a common type of parental care in several vertebrate taxa (Pough *et al.* 2005), associated with the enhanced offspring quality by improving the physical and biological conditions for developing eggs and early life stages (Gross 2005). However, other than in birds and reptiles, nest building is rare among vertebrates (Brown 2015; Refsnider 2016), and implies in several trade-offs in the life history of these nest builders, as this strategy is under natural selection from predation and local conditions (Barber 2013). Indeed, nests are often viewed as extraorganismal extended phenotypes (Dawkins 1982), that is traits that arise from gene expression and have direct effects on the environment.

At least 9000 fish species are nest or burrow builders (Brown 2015), mostly in fresh water (Pough *et al.* 2005). Because of the ubiquity of pelagic spawning among marine fish, nesting is much less studied in the ocean and is frequently confused with burrowing, which is associated with predator and parasite avoidance (Nanami and Nishihira 1999; Deloach and Humann 1999).

Nesting occurs across a broad taxonomic spectrum of ray-finned fish (Actinopterygii), with considerable behavioural heterogeneity and a diverse repertoire of structures (Reebs 2011). Despite such widespread taxonomic distribution, studies are biased towards a small number of species, including sticklebacks (Gasterosteidae), gobies (Gobiidae) and damselfish (Pomacentridae; Östlund-Nilsson and Holmlund 2003; Barber 2013; Navarrete-Fernández *et al.* 2014). Fish nests can be classified into four categories: (1) mouth- or fanning-cleaned areas or hard surfaces, with or without farmed algae (Deloach and Humann 1999); (2) excavations or burrows in gravel, sand or mud (Kawase *et al.* 2013); (3) simple walls or piles of sand, pebbles, shells or artificial substrata (Colin 1973); and (4) cemented nests built with secreted chemical adhesives that

aggregate algae, detritus or sand (Rushbrook *et al.* 2008). Despite the strong dependence of reef fish on the substrate (Sale 2002), reports of nesting and its ecological role are still scarce for reef ecosystems.

The West Atlantic reef fish family Grammatidae includes 13 species, but only *Gramma loreto* (Poey, 1868) is known to build nests (Asoh and Yoshikawa 1996). The Brazilian basslet (*Gramma brasiliensis* Sazima, Gasparini & Moura 1998) is the only species that occurs outside the Caribbean, being recorded from shallow coralline and rocky reefs from Maranhão (01°01′S, 41°48′W) south to Rio de Janeiro (22°54′S, 41°59′W) in Brazil, inhabiting vertical walls and shaded areas (Sazima *et al.* 1998). Akin to its Caribbean sister species *Gramma loreto*, the Brazilian basslet spawns in couples or harems, but is a protogynous hermaphrodite (Leite *et al.* 2016).

The present study describes the bird-like complex nesting behaviour of *G. brasiliensis*, discussing its ecological implications. Specifically, we describe nest preparation and nesting behaviour. The present data are part of a broader project aiming to support conservation measures and best practices for the aquarium trade of this Brazilian-endemic reef fish (Leite 2013; Leite *et al.* 2016). From 1995 to 2004, *G. brasiliensis* was one of Brazil's five most exported ornamental reef fish, reaching US, European and Asian markets (Monteiro-Neto *et al.* 2003; Gasparini *et al.* 2005). There are no previous official statistics, and the *G. brasiliensis* harvest was halted in 2004. Despite its importance as a fisheries resource, the biology of *G. brasiliensis* remains poorly studied.

Materials and methods

Sampling was conducted between April 2011 and February 2012 in the Taipu de Fora Reef (13°56′20″S, 38°55′32″W), Bahia State, Brazil. The study site is a tide pool ~400 m long and ~50 m wide, with depth ranging from 0.5 to 8 m and a channel opening to the ocean on its north side. This large tidal pool located in the back reef is isolated only during low spring tides. The structure of the reef walls is made up of carbonate framework, crustose coralline algae and corals (e.g. *Mussismilia* sp., *Montrastrea* sp. and *Siderastrea* sp.). Worm tubes, octocorals (e.g. Carijoa) and encrusting sponges such as *Spirastrella* sp. dominate the benthic cover in this habitat.

In the present study we conducted a total of 120 h of direct observations using SCUBA, with 30h of focal animal sampling (Lehner 1996; Martin and Bateson 2009), covering all seasons (summer: 21 December–19 March; autumn: 20 March–20 June; winter: 21 June–21 September; spring: 22 September–20 December). Because this species is not sexually dimorphic, after 25 min of observation couples or harems ($n = 64$ individuals) were collected with hand nets to determine sex and gonadal maturation stage (National Permit number 22123-1, SISBIO, Sistema de Autorização e Informação em Biodiversidade). Fish were anaesthetised with eugenol (50 mg L^{-1}), measured, weighed, fixed in formalin (10%) and then transferred to ethanol (70%) after 48 h. Gonads were subsequently removed, dehydrated in an increasing ethanol series, cleared in xylene and embedded in histological paraffin (Beçak and Paulete 1976). Transverse and longitudinal histological sections (~5 μm) were stained with Harris haematoxylin and eosin. Sexual development was classified according to Brown-Peterson *et al.* (2011) and Leite *et al.* (2016), encompassing five phases: immature (never spawned); developing (ovaries or testes beginning to develop, but not ready to spawn); spawning capable (developmentally and physiologically able to spawn in this cycle); regressing (cessation of spawning); and regenerating (sexually mature, reproductively inactive).

All specimens collected were also used in an integrated study of reproductive biology, diet, age and growth, as part of a broader research project.

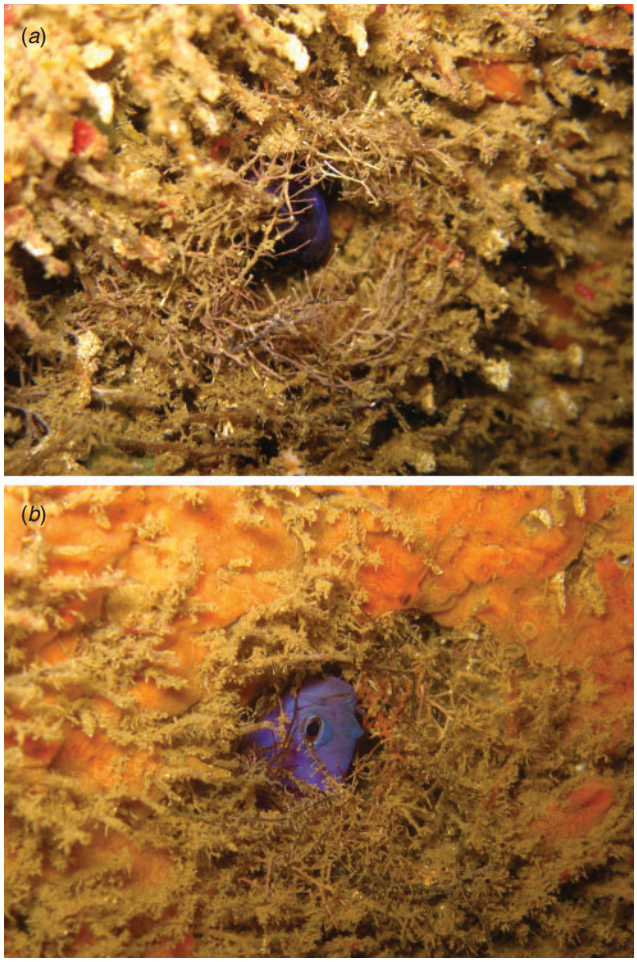

Fig. 1. Nests built by the Brazilian-endemic and endangered reef fish *Gramma brasiliensis*. The male either used calcareous algae (*Jania* spp. and *Gelidiopsis* spp.) to camouflage the entrance (*a*) or interlaced the algae to build an outside wall, leaving a small entrance (*b*). (Photographs taken by J. R. Leite.)

Results and discussion

All sampled specimens were adults, with total lengths (TL) ranging from 31 to 84 mm for females ($n = 33$), and from 44 to 96 mm ($n = 31$) for males. In all, 15 nests were observed. Only males were directly involved in nest building. These males were spawning capable and, in most cases ($n = 12$), represented the largest individuals of pairs or harems. In all, 12 pairs and 8 harems (3–6 individuals) were recorded.

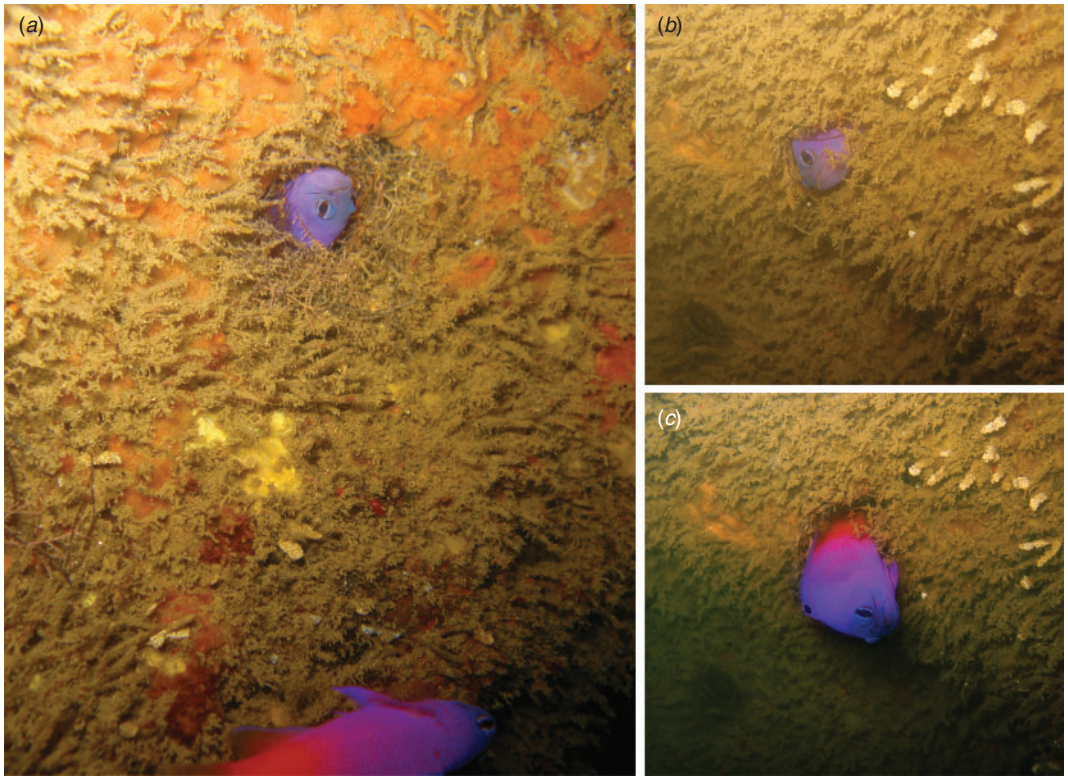

Fig. 2. Nest preparation and behaviour of the Brazilian-endemic and endangered reef fish *Gramma brasiliensis*. (*a*) Male guarding the nest entrance with a female around. (*b*) Nesting male. (*c*) An individual moving out of the nest during nesting preparation. (Photographs taken by J. R. Leite.)

Nest building started once the largest male selected holes or depressions in the lower surfaces of ledges and ceilings of small caves and started aggregating the thalli of calcareous algae (*Gelidiopsis* spp. and *Jania* spp.) harvested floating in the water column (see Video S1, available as Supplementary material to this paper). Algae were also used to form tangles in the deepest section inside the nest, resembling a cushion for egg laying. Remarkably, *G. brasiliensis* was not observed picking algae that was adhered to the substratum. Indeed, the algae used in nests were absent from the vicinity of the nests, suggesting active selection.

All nests were positioned in the ceilings of caves, and *G. brasiliensis* was always observed upside down beneath ledges. The selected crevices had a single narrow entrance surrounded by algae thalli interlaced with worm tubes (Fig. 1*a*). When the nest site was just a depression, males interlaced algae until a front wall was created for the nest. After nest preparation, sponges and other organisms grew among the algae thalli, consolidating the wall and contributing to nest camouflage (Fig. 1*b*).

Nest maintenance and protection was only undertaken by the largest dominant male (Fig. 2). Functional females (in all gonadal stages), small males and juveniles were observed entering crevices and burrows for refuge, but never manipulated the algae or undertook nest maintenance. These non-active male individuals did not exhibit nest fidelity, hiding in any available burrow when threatened, unlike the largest males, which exhibited nest fidelity.

Most reef fish are pelagic spawners and most benthic-egg layers tend to be abundant (e.g. pomacentrids; Sale 2002), in contrast with grammatids, which are never abundant. The bird-like nests and nesting behaviour described herein for *G. brasiliensis* are similar to those of *G. loreto*, the Caribbean sister species of *G. brasiliensis* (Asoh and Yoshikawa 1996). Given that most grammatids are deep-water dwellers, observational studies to determine whether other species also build nests are limited. Nesting is a critical aspect of the reproductive strategy of *G. brasiliensis*, which is a protogynous hermaphrodite that spawns few asynchronously developed egg masses (<100) and exhibits male parental care (Leite *et al.* 2016). Although protogynous hermaphroditism is expected in other grammatids (de Mitcheson and Liu 2008), it has not been thoroughly assessed across the family, not even in shallow-water dwellers such as *G. loreto* (Asoh and Shapiro 1997).

Compared with other protogynous hermaphrodite fish, the sex ratio of the studied population (1 : 1) can be considered largely biased towards males (Sale 2002). Although females and juveniles frequently use burrows and crevices as protective retreats, only the larger dominant males in pairs or harems displayed territoriality and were engaged in nest building. The social structure and sex ratio of *G. brasiliensis* can be related to its protogynous hermaphroditism, combined with male parental care and nest building. This pattern contrasts with that of other protogynous hermaphrodites that are generally pelagic spawners, non-territorial and larger sized. Although

larger males compete agonistically for the dominant status within pairs or groups (J. R. Leite, pers. obs.), the capable but non-dominant male may rapidly take over the dominant role following the death of the largest male.

Geniculate calcareous algae and macroalgae were harvested in the water column and used as construction materials in *G. brasiliensis* nests. Such selectivity and harvesting mode are related to the absence of algae suitable for nest building along the dark surfaces of the ledges where nests are built, as well as the species' small home range and feeding tactics. *G. brasiliensis* visually selects food items in the water column in the vicinity of its territory, rapidly retreating to burrows and crevices when threatened. Picking prey in algae attached to the bottom was only eventually recorded. Therefore, the harvesting of building materials in the water column is similar and compatible with the feeding tactics of *G. brasiliensis*, as longer-ranging harvesting of suitable materials in well-lit habitats would greatly increase predation risk. Interestingly, instead of choosing the most abundant building materials, nest-building reef fish tend to select specific algae for nest construction. For example, the Mediterranean wrasse *Symphodus ocellatus* selects *Jania rubens* as the main building material for its nests (Sinopoli *et al.* 2015), similar to *G. brasiliensis*, which also selected *Jania* spp. for nest building in tropical coralline reefs

Gramma brasiliensis was heavily exploited by the ornamental trade until 2004, and its populations were significantly reduced (Monteiro-Neto *et al.* 2003; Gasparini *et al.* 2005). Habitat loss has also escalated, and this species is now considered 'endangered' and its trade has been banned (Instrução Normativa N0 5. Diário Oficial da União 28/05/2004, 136-142; Sampaio and Notthingan 2007). In recent decades, the ornamental trade of marine fish has grown extensively, both worldwide (Andrews 1990; Fujita *et al.* 2014) and in Brazil (Monteiro-Neto *et al.* 2003). Andrews (1990) suggested that almost all marine fish in the ornamental trade are wild caught under uncontrolled regimes, resulting in several cases of marked population declines. In Brazil, at least one case of local collapse after aquarium trade exploitation of *G. brasiliensis* has been reported (Ferreira *et al.* 2005).

The complex bird-like nesting behaviour of the Brazilian basslet, combined with its territorialism, reduced number of eggs and male parental care, suggests that its populations are highly vulnerable to selective exploitation by the ornamental trade. Therefore, this Brazilian-endemic and endangered species requires specific conservation and management strategies. In addition to the establishment and enforcement of marine protected areas and captive breeding programs, restrictions on effort and establishment of a total allowable catch must be implemented if the fishing ban is halted.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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