

# Breeding pattern and nest guarding in *Sicyopterus lagocephalus*, a widespread amphidromous Gobiidae

N. Teichert · P. Keith · P. Valade · M. Richarson ·  
M. Metzger · P. Gaudin

Received: 18 December 2012 / Accepted: 4 April 2013  
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**Abstract** Amphidromous gobies are usually nest spawners. Females lay a large number of small eggs under stones or onto plant stems, leaves or roots while males take care of the clutch until hatching. This study investigates the breeding pattern and paternal investment of *Sicyopterus lagocephalus* in a stream on Reunion Island. In February 2007 and January 2010, a total of 170 nests were found and the presence of a goby was recorded at 61 of them. The number of eggs in the nests ranged from 5,424 to 112,000 with an average number of 28,629. We showed that males accepted a single female spawning in the nest and cared for the eggs until hatching. The probability for a nest to be guarded increased with the number of eggs within it, suggesting that paternal investment depends on a trade-off between the reproductive value of the current reproduction and future nesting events. We showed that large nest stones were occupied by large males (TL >80 mm), whereas smaller males (TL <50 mm) were found under smaller cobbles,

probably because of male–male competition for available nests. Our results suggest that the male's choice relies upon a similarity to the female size, while the female's choice was based on both body and nest stone sizes.

**Keywords** Mating system · Nest guarding · Sexual selection · Nest size · Nest choice

## Introduction

Freshwater gobies are usually nest spawners (Manacop 1953; Daoulas et al. 1993; Fitzsimons et al. 1993; Keith 2003; Yamasaki and Tachihara 2006; Tamada 2008). Males delimit a territory and build a nest on or under various supporting structures, such as rocks and pebbles or submerged plants (Manacop 1953; Daoulas et al. 1993; Fitzsimons et al. 1993; Takahashi and Kohda 2002;

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N. Teichert (✉) · M. Metzger  
Association Réunionnaise de Développement de l'Aquaculture  
(ARDA), Les Sables, BP 16, 97427 Etang Salé, Reunion  
e-mail: teichert.arda@orange.fr

N. Teichert · P. Gaudin  
UMR 1224 Ecobiop, UFR Sciences et Techniques Côte Basque,  
Université Pau & Pays Adour, Anglet, France

N. Teichert · P. Gaudin  
UMR 1224 Ecobiop, Aquapôle, INRA, St Pée sur Nivelles,  
France

P. Keith  
Département Milieux et Peuplements Aquatiques, Biologie des  
Organismes Marins et Ecosystèmes (BOREA UMR CNRS-  
MNHN 7208), Muséum National d'Histoire Naturelle, CP-026,  
43 rue Cuvier, 75231 Paris, France

P. Valade  
Centre Régional d'Application Aquacole, O.C.E.A. Consult'-  
Organisme Consultant en Environnement Aquatique, BP 22, 97  
427 Etang Salé, La Reunion

M. Richarson  
Fédération Départementale de Pêche et de Protection du Milieu  
Aquatique de la Réunion, 208, route de la Passerelle, 97480  
Saint Joseph, Reunion

M. Metzger  
UFR Sciences Fondamentales et Appliquées, Université de  
Lorraine, Campus Bridoux, 57070 Metz, France

M. Metzger  
AQUAGO, ZA des Chemins Croisés, 16 rue René Cassin, 62223  
Saint Laurent Blangy, France

reviewed in Keith 2003). For example, nests of *Sicyopterus stimpsoni* are composed of clean cavities under rocks (Fitzsimons et al. 1993), whereas males of *Pomatoschistus minutus* (Pallas, 1770) build nests under mussel shells, which they excavate and cover with sand (Lindström et al. 2006). After egg deposition, the female generally leaves the nest and the male takes care of the eggs until hatching (e.g. Takahashi and Yanagisawa 1999). In few species, the female also guards the nest and shows a territorial behaviour around the nest (Ha and Kinzie 1996). Parental care enhances development and survivorship of progeny, mainly thanks to nest defence against intruders and a better egg oxygenation through fanning (Lissaker and Kvarnemo 2006; Meunier et al. 2009). Furthermore, it has been shown that uncared for egg clutches are more prone to clogging and microbial development (e.g. Daoulas et al. 1993; Giacomello et al. 2008).

Several studies have shown that females preferentially mate with larger males, which are more likely to defend large nests (e.g. Forsgren et al. 1996a; Ito and Yanagisawa 2000; Takahashi et al. 2001; Takahashi and Kohda 2002). In several goby species, the male is able to simultaneously handle several females laying eggs in one nest, as has been observed in *Rhinogobius* spp. DA and CB (Takahashi and Yanagisawa 1999; Tamada 2008) or *Pomatoschistus minutus* (Singer et al. 2006). In this case, the females often prefer to lay in large nests where other females already have spawned (e.g. Forsgren et al. 1996b; Mazzoldi and Rasotto 2002; Takahashi and Kohda 2002), since nest cover behaviour can be considered as an honest signal of male condition and parental care ability (Lindström et al. 2006; Olsson et al. 2009). The males may also discriminate between females by preferentially choosing large ones, considering their higher body size-related fecundity (Côte and Hunte 1989; Ito and Yanagisawa 2000).

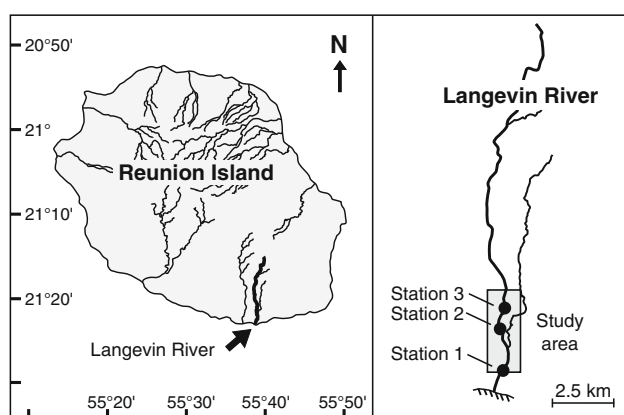
*Sicyopterus lagocephalus* (Pallas, 1767) is an amphidromous goby widely distributed throughout the Indo-Pacific region, from the Western Indian Ocean to the Eastern Pacific (Berrebi et al. 2005; Keith et al. 2005; Lord et al. 2010, 2012). In Reunion Island (southwest Indian Ocean), this goby dominates the fish assemblages and is subjected to a heavy fishing pressure (e.g. Delacroix 1987; Delacroix and Champeau 1992; Bell 1999). Adults measure between 4 and 12 cm and have a benthic life-style, feeding on micro-algae that cover the river substratum (Bielsa et al. 2003). The spawning season extends almost all year, with a short pause in reproduction activity during the cooler months as observed on Reunion Island (Delacroix 1987; Teichert et al., in preparation) and the Philippines (Manacop 1953). Females lay a large number of small eggs onto the underside of pebbles and rocks (Delacroix and Champeau 1992) and males take care of the clutches, as observed in other Sicydiinae (e.g. Fitzsimons et al. 1993; Bell 1994; reviewed in Keith 2003). The fecundity is related to the female size and supposedly linked to the clutch size (Delacroix 1987; Teichert 2012), as observed in *Sicyopterus japonicus* (Iida et al. 2011; Yamasaki et al. 2011). Females of 50 mm length lay about 17,000 eggs, whereas fecundity is about 142,000 eggs when they reach 100 mm length (Teichert 2012). The eggs hatch around 48 h after fertilisation; free embryos passively drift downstream to the sea immediately after hatching (Valade et al. 2009).

Mating system and sexual selection are known to influence the fish spatial distribution and demographic process (e.g. Takahashi 2008; Tamada 2011), highlighting an interest for population management. Even if mating systems and sexual preferences have begun to be well known in some gobies, the mate choice of the Sicydiinae sub-family remains fragmented and poorly documented (Fitzsimons et al. 1993; Keith 2003; Iida et al. 2011; Yamasaki et al. 2011). Our study aims to elucidate the breeding pattern, nest size selection and possible nest guarding of *Sicyopterus lagocephalus* (Gobiidae, Sicydiinae) on Reunion Island. Our analyses were based on field-searches for egg clutches, associated with a description of clutch size, nest places and nest guarding.

## Materials and methods

### Fish assemblage

The study was conducted in Langevin River, located in the southwest of Reunion (river mouth coordinates: 23.13°S, 38.64°E). In February 2007, three sites located throughout the river (Table 1; Fig. 1) were sampled a week after the egg clutch collection to describe fish assemblage. This



**Fig. 1** Localization of study area and distribution of the 3 fishing stations (filled circles) in Langevin River of Reunion Island, Mascarene archipelago

sampling was performed to estimate the densities of Sicydiinae in the studied areas and to quantify the bias induced by the non-identification of the species of the clutches. Indeed, *Cotylopus acutipinnis*, a sympatric endemic species, lays a similar type of clutches that cannot be differentiated from those of *S. lagocephalus* in the field. The identification of eggs to species requires genetic analyses, which would involve destroying an excessive number of egg clutches. Fishes were caught using electrofishing (portable DEKA 3000; Germany) in about 30 m<sup>2</sup> per station. Individuals were identified at species level and then were released in the river.

#### Search for clutches and nest description

Egg clutches were searched for in February 2007 and January 2010 in random locations from 800 to 3,300 m from the river mouth (Fig. 1). On the underside of rocks, the egg clutches form white (recently laid, <24 h) or grey spots (advanced ripening: larvae can be seen inside the egg) depending on the developmental stage (Delacroix and Champeau 1992; Ellien et al. 2011). The random sample procedure used an observation quadrat (1 m<sup>2</sup>) placed on the river bottom. The longitudinal distance between each quadrat was randomly determined and ranged from 1 to 25 m. The transversal position of the quadrat was randomly determined among 5 modalities: 1, right edge; 2, right side; 3, river center; 4, left side; 5, left edge). The search was conducted by two snorkelers working in the same way, moving upstream and each using a different quadrat, in water 0.1–1.2 m depth. In each quadrat, all the pebbles and rocks were delicately turned, one after another, to find the egg clutches laid onto their underside. The large boulders (>600 mm) were too large to be upturned. To check whether the nest was guarded, first the observer carefully watched whether there was a goby under the upturned stone. The downstream side of each stone was lifted upstream to facilitate the observations of gobies. Then the observer checked whether there was an egg clutch. In 2010, we compared the frequency of guarded nests between the two snorkelers using a Fisher's exact test, which did not show significant differences ( $n = 40$ ,  $P = 0.349$ ). This result suggests that observation of goby under stone was not affected by the identity of observer. When a clutch was found, the absence or presence of a goby was recorded and three features were noted: species (*S. lagocephalus*/*C. acutipinnis*/unidentified), sex (male/female/unidentified) and body size (total length <50 mm/50–80 mm/>80 mm/unidentified). We considered that observation of individuals under the nest was a good indicator of species identification, as suggested by Yamasaki et al. (2011). The fish size was visually estimated, after a previous training with

an underwater ruler. Small body size (<50 mm) corresponded to newly matured fishes (Delacroix 1987); 50–80 mm represented the main part of the population; and >80 mm were the largest gobies within the stream. The water depth ( $\pm 1$  cm) was measured from the centre of the quadrat. Later on, the clutch and its stone were moved on the river bank. The clutch was photographed adjacent to a scale (1 cm) and the development stage was recorded (white or grey). The size of the stone was recorded according to four classes (Cailleux 1954): small cobble (20–100 mm), large cobble (100–200 mm), small boulder (200–600 mm), and large boulder (>600 mm). Finally, the nest was carefully replaced onto the river bottom.

#### Clutch area and egg number

As the clutches formed regular, circular spots with homogeneous egg development stages, we considered that one nest contained a single female's clutch, as observed for other Sicydiinae (e.g. Kinzie 1993; Yamasaki et al. 2011). To investigate the number of eggs in a clutch, a complementary sample of 48 white clutches (newly laid eggs) was collected without any description of nest parameters, in March 2008. The clutches were photographed and both nest stones and clutches were transferred in buckets filled with river water for transportation to the laboratory. The areas of egg clutches attached to stones were measured ( $\pm 0.01$  cm<sup>2</sup>) using the ImageJ software (v.1.45s; Rasband 2012), after picture scaling. A gravimetric method was used to estimate the numbers of eggs. Each clutch was carefully separated from its stone and two sub-samples of about 900–1,000 eggs were removed. The eggs from both sub-samples were manually segregated in a Petri dish and photographed under a binocular microscope. The number of eggs was counted for each of them, using the image processing software. Then, the samples were placed in a stove (60 °C, 24 h), then a desiccator (24 h), before precision weighing ( $\pm 10^{-5}$  g). The number of eggs per clutch was calculated as follows:

$$N = (n_1 + n_2)/(w_1 + w_2) \times (W_t),$$

where  $N$  is the number of eggs per clutch,  $n_1$  and  $n_2$  the number of eggs in each sub-sample,  $w_1$  and  $w_2$  the weights of each sub-sample and  $W_t$  the total clutch weight.

#### Statistical analysis

Statistical analyses were performed using the open source R software (v.2.14.1; R Development Core Team 2011). A linear regression was performed to investigate the relationship between area of clutch and number of eggs, using the clutches collected in March 2008. This linear

relationship was used afterwards to estimate the number of eggs for the other clutches collected in 2007 and 2010 (described in “Results”), as has been performed in the freshwater goby *Tridentiger brevispinis* (Takahashi 2008). A Fisher’s exact test was used to compare the guarded and non-guarded nests according to the nest stone size, in order to verify the effect of the stone size on the guarding behaviour or the possibility of a bias in observing guarding individuals depending on the nest’s size. Similarly, a Mann–Whitney *U* test was performed to compare the water depth between guarded and unguarded nest to verify whether the observation of gobies was not reduced in shallow areas.

A logistic regression was used with presence or absence of guarding male as dependent variable and number of eggs in the nest as independent variable. Significance of tests was based on deviance reduction compared to the null model (Chi-square test). Differences in clutches’ development stage (white–grey) between guarded and unguarded nests were tested using Fisher’s exact test. A ratio of two development stages of clutches guarded by *S. lagocephalus* was examined by a binomial test. Rejecting the null hypothesis would indicate that both egg development stages were not equally guarded.

The studied parameters were non-normally distributed, and the number of samples between the analyzed groups was not equal, hence we performed non-parametric statistic tests. Differences in number of eggs in the nests according to nest stone size (small cobble–large cobble–small boulder–large boulder) were analyzed using a Kruskal–Wallis rank sum test. If the latter test was significant, it was completed by a post hoc Wilcoxon rank sum test with a holm correction. Differences in number of eggs between unguarded and *S. lagocephalus* guarded nests were tested for each nest stone size class using a Mann–Whitney *U* test. A Kruskal–Wallis rank sum test was performed to compare the number of eggs in the nests among size classes of nest-guarding *S. lagocephalus*. Differences in nest stone sizes between size classes of nest-guarding *S. lagocephalus* were tested using Fisher’s exact test.

**Table 1** Number of gobies caught in the three stations located throughout Langevin River in February 2007, using electrofishing (number of fish 497)

Site	Area (m <sup>2</sup> )	DFS (m)	Number of fish	
			<i>S. lagocephalus</i>	<i>C. acutipinnis</i>
Station 1	65.34	810	199	0
Station 2	33.81	2080	124	0
Station 3	72.26	2670	172	2

The sampled area (m<sup>2</sup>) and the distance from the sea (DFS, m) of the stations are shown

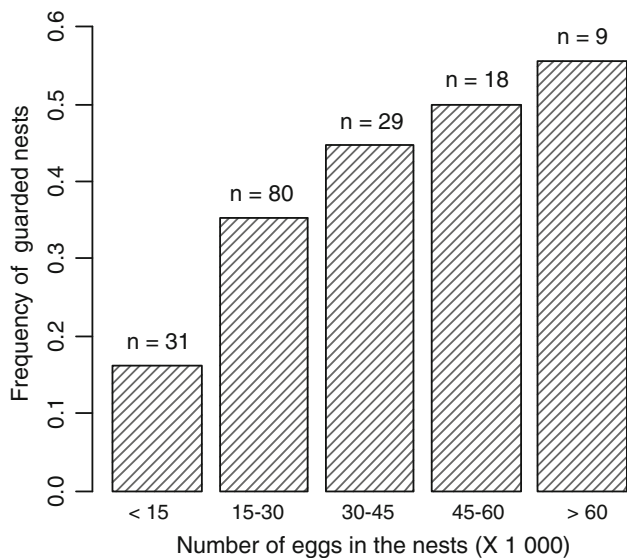
## Results

Four hundred and ninety-seven fishes were caught in the three stations sampled for study of fish assemblage in February 2007 (Table 1). All fishes were *S. lagocephalus*, except two *C. acutipinnis* caught in the station 3. The latter species is common in some rivers of Reunion Island but scarce in Langevin River (in this study: 0.4 % for the three sampled stations and 1.2 % for station 3).

The number of eggs estimated in the 48 clutches sampled in March 2008 ranged from 9,109 to 85,718 with an average number of 35,710 (SE 1,425). We found a linear relationship between the clutch area and the number of eggs ( $N_{\text{eggs}} = 3,238 \times A_{\text{clutch}} + 376.91$ ,  $n = 48$ ,  $R^2 = 0.85$ ,  $P < 0.001$ ). This relationship explained 85 % of total variability, showing that the clutch area was a good proxy of the number of eggs. We used this relationship to estimate the number of eggs of each clutch observed in February 2007 and January 2010.

In February 2007 and January 2010, a total of 170 nests with egg clutches were found. The number of eggs in the nests estimated by a linear regression ranged from 5,424 to 112,000 with an average number of 28,629. The presence of a goby was recorded under 61 nest stones. Among them, *S. lagocephalus* males were the most commonly observed ( $n = 51$ ), while 6 nests were occupied by both genders, two by sex unidentified individuals and two by *C. acutipinnis* (one male and one sex unidentified individual). There was no significant difference in the frequency of observation of guarding gobies according to the nest stone size (Fisher’s exact test,  $P = 0.567$ ), suggesting that observation success of guards by snorkelers did not depend on the nest’s size (not reduced for large stones). Likewise, the water depth was not significantly different between the guarded and unguarded nests (Mann–Whitney *U* test,  $n = 170$ ,  $W = 3,553.5$ ,  $P = 0.45$ ), reflecting that the observation of gobies was not reduced in shallow areas.

For further data analysis, we did not consider the two nests occupied by *C. acutipinnis* in all analysis (59 guarded nests and 168 egg clutches). For the analysis of guarded nests, we considered only the male body size when both genders were present and we assumed that the 2 gender-unidentified individuals were males. Although a large majority of guarded nests were occupied by *S. lagocephalus*, the observation of two *C. acutipinnis* nests suggested that few clutches of this species may have been included in the non-identified nests data set (109 nests). But, considering the low numbers of *C. acutipinnis* nests identified and the species’ very low density (i.e. <1 % fishes caught by electrofishing), we considered that the possible presence of clutches of this species was negligible. Finally, every analysis that took into account the number of eggs were

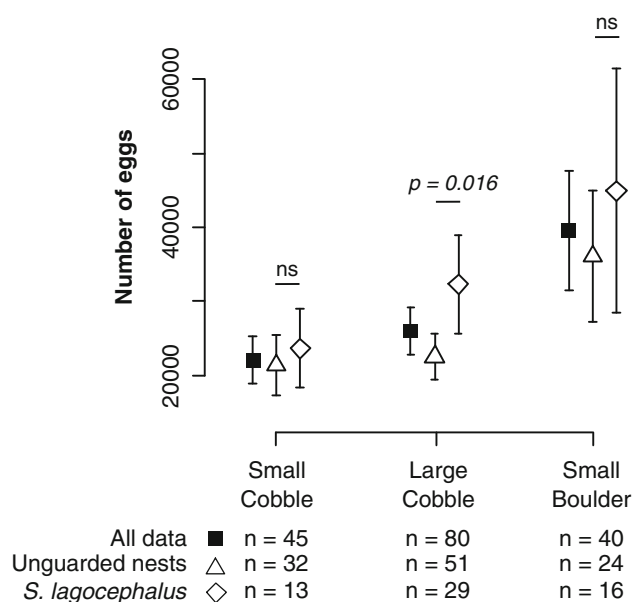


**Fig. 2** Frequency of observation of *Sicyopterus lagocephalus* guarded nests according to the number of eggs in the nests observed in February 2007 and January 2010 in Langevin River. *n* = sample size

performed for only 167 egg clutches, because one clutch was damaged in the field.

The probability of finding a guarded nest significantly increased with the number of eggs in the nest (logistic regression,  $\text{Logit}(p_{\text{guard}}) = N_{\text{eggs}} \times 2.352 \times 10^{-5} (\pm 9.071 \times 10^{-6}) - 1.296 (\pm 0.315)$ ,  $n = 167$ ,  $\chi^2 = 7.25$ ,  $df = 1$ ,  $P = 0.007$ ). Only 16 % of the nests with small egg clutches (<15,000 eggs) were found with a guarding goby, whereas over 50 % nests containing a large number of eggs (>45,000 eggs) were guarded (Fig. 2). The ratios of egg development stages (white or grey) were not significantly different between guarded and unguarded nests (Fisher's exact test,  $n = 168$ ,  $P = 0.237$ ). Similarly, the ratio of clutches between two developmental stages guarded by *S. lagocephalus* was not significantly different from 1:1 (Binomial test,  $n_{\text{total}} = 59$ ,  $n_{\text{white}} = 25$ ,  $P = 0.297$ ).

Only two clutches were found under large boulders. They were excluded from the current analysis because this low number might have resulted from the low occurrence of large stones (>600 mm) that were possible to upturn. As a result, the following analysis used 165 clutches. The number of eggs in the nest significantly differed and increased with the size of the stone, for all data (Kruskal–Wallis test,  $n = 165$ ,  $K = 14.60$ ,  $df = 2$ ,  $P < 0.001$ ) and unguarded nests (Fig. 3) (Kruskal–Wallis test,  $n = 107$ ,  $K = 10.18$ ,  $df = 2$ ,  $P = 0.006$ ) (Wilcoxon post hoc test, based on  $P < 0.05$ : small cobble was not significantly different with large cobble, small cobble and large cobble were significantly different with small boulder). There were no significant differences between these three groups in sizes of stones for guarded nest (Kruskal–Wallis test,



**Fig. 3** Average number of eggs in the nest according to the size of the nest stone from *Sicyopterus lagocephalus* clutches observed in February 2007 and January 2010 in Langevin River, using all data (black squares), unguarded nests (open triangles), and guarded nests (open diamonds). The bars represent the confidence interval at 95 %. Significant differences between unguarded and *S. lagocephalus* guarded nests were presented, based on Mann–Whitney *U* tests. *n* = sample size

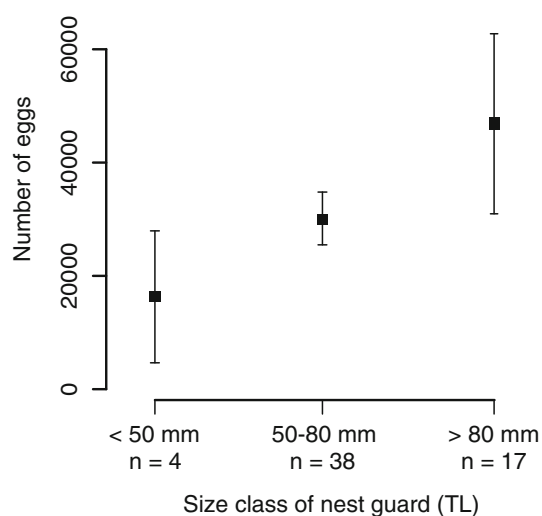
$n = 58$ ,  $K = 4.14$ ,  $df = 2$ ,  $P = 0.125$ ). The number of eggs under both small cobbles and boulders was not significantly different between unguarded nests and *S. lagocephalus*-guarded nests (Fig. 3) (Mann–Whitney *U* tests,  $W = 263$ ,  $P = 0.174$  and  $W = 214$ ,  $P = 0.557$ , respectively, for small cobbles and small boulders), whereas the number of eggs under large cobbles was greater for guarded than unguarded nests (Mann–Whitney *U* test,  $W = 979$ ,  $P = 0.016$ ).

Among the guarded nests, the number of eggs increased with the body size of guarding males (Fig. 4) (Kruskal–Wallis test,  $n = 59$ ,  $K = 7.35$ ,  $df = 2$ ,  $P = 0.025$ ). The distribution of the nest stone sizes significantly differed among the body size classes of male guards (Fig. 5) (Fisher's exact test,  $n = 59$ ,  $P = 0.004$ ). Bigger fish used larger nest sizes than small ones.

**Discussion**

Spawning pattern

Amphidromous gobies show various patterns of reproduction; some are semelparous, some annual spawners, and some repetitive spawners (Daoulas et al. 1993; Ha and Kinzie 1996; Keith 2003; Tamada and Iwata 2005; Shimizu et al. 2008). For *S. lagocephalus*, Lord et al. (2011)

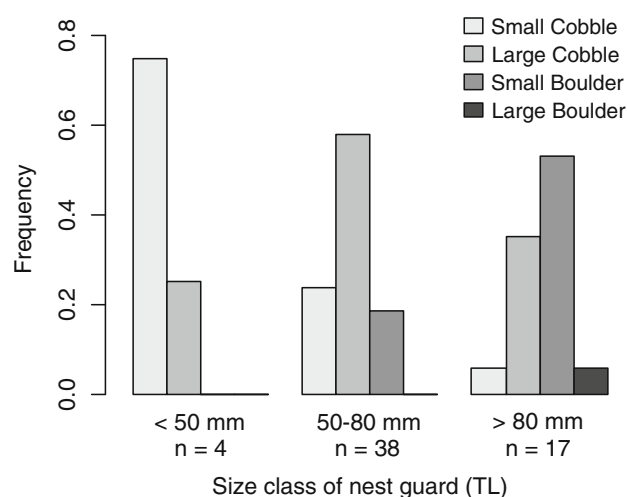


**Fig. 4** Mean number of eggs in the nest according to the body size of the nest guard for *Sicyopterus lagocephalus* from clutches observed in February 2007 and January 2010 in Langevin River. Bars confidence interval at 95 %.  $n$  = sample size

showed an adult up-and-down migration which can occur several times during the fish's life cycle and which is probably linked to reproduction purposes. This species has an extended breeding period and females probably lay eggs several times throughout the season (Manacop 1953; Hoareau et al. 2007; Teichert 2012), as observed among other tropical gobies (Daoulas et al. 1993; Kinzie 1993; Bell 1994; Ha and Kinzie 1996). In several goby species, a nest may simultaneously contain more than one female's egg mass (Daoulas et al. 1993; Ito and Yanagisawa 2000; Takahashi and Kohda 2002; Tamada 2008), suggesting that polygamy is a common mating pattern (reviewed in Keith 2003). However, among Sicydiines, Kinzie (1993) showed that *Lentipes concolor* (Gill, 1860) was not polygamous; our own observations seem to confirm this for *S. lagocephalus*, as has been suggested by Delacroix (1987). The clutches of this species showed homogeneous egg development stages, but this sole criterion was not always an accurate indicator of the number of females spawning in the nests, as observed in the goby *Rhinogobius* sp. (e.g. Takahashi and Ohara 2006). Nevertheless, clutches form regular and uniform egg masses, and we showed that the range of numbers of eggs found in nests was realistic according to the batch fecundity of females (i.e. from 14,000–230,000 eggs depending on the female size; Teichert 2012) which would be consistent with a single spawning event per nest.

#### Nests guarding

In the present study, no nest was observed with a lone female, suggesting that they did not take part to parental care, while males seemed to guard the clutch after egg



**Fig. 5** Distributions of nest stone sizes for *Sicyopterus lagocephalus* clutches observed in February 2007 and January 2010 in Langevin River, according to the size of nest guard.  $n$  = sample size

deposition, as observed in other gobies (Daoulas et al. 1993; Lindstrom and brown 1994; Takahashi and Kohda 2002; reviewed in Keith 2003). We did not find any significant difference between the number of newly laid clutches and advanced ones between guarded and unguarded nests, which confirms the assumption that eggs are guarded until hatching (Valade et al. 2009). This result also showed that the maturation stage of the eggs—freshly laid or advanced—did not influence the guarding behaviour of male gobies. However, a large number of nests were found without any guard. We showed that the probability to find a nest guard was positively correlated to the number of eggs, suggesting that male investment for both care and defence of its nest was different according to the number of eggs contained in the nest. Absence of males in the nests could result from an unguarded nest since egg fertilization or from a goby which had fled prior to or because of the observation. In the first case, it is possible that males which mated with small females did not care for the clutch in order to enhance their chances to find other females and increase their reproductive success. For instance, the male of the amphidromous species *Awaous guamensis* (Valenciennes, 1837) probably do not take care of clutches in order to improve their chances of multiple spawning events (Ha and Kinzie 1996). On the other hand, the snorkelers might have scared and led the male guards to flee, suggesting a lesser defence investment for small egg clutches (Royle et al. 2012). In the goby *Rhinogobius brunneus* (Temminck and Schlegel, 1845), the parental investment of males increased with the brood size, because of a greater reproductive success associated with the larger clutches (Suk and Choe 2002). As for most species, the male investment of *S. lagocephalus* in parental care probably

depends on a trade-off between reproductive value of the current reproduction and their expectation for future nesting events (Gross 2005).

#### Mating system and sexual preferences

In gobies, the spawning site is often chosen by the male which builds the nest and displays in courtship to females (Daoulas et al. 1993; Fitzsimons et al. 1993; Ito and Yanagisawa 2000; Mazzoldi and Rasotto 2002; Takahashi and Kohda 2002; Keith 2003; Takahashi et al. 2004; Olsson et al. 2009; Yamasaki et al. 2011). The selection of spawning sites and nest building is closely linked to the reproductive success of males because it strongly influences female preferences (e.g. Takahashi and Kohda 2002; Tamada 2011). Several studies have shown that goby females prefer larger males and/or larger nest sizes when laying their eggs (Ito and Yanagisawa 2000; Takahashi et al. 2001; Takahashi and Kohda 2002; Tamada 2011). In the stream goby, *Rhinogobius brunneus*, it has been shown that courtship displays in fast currents are also used by females as honest indicators of parental quality (Takahashi and Kohda 2004). During the reproductive period of *Sicyopterus stimpsoni* (Gill, 1860), the male exhibits a distinctive blue coloration, as observed in *S. lagocephalus*, and spends most time occupying elevated “perches” above rocks close to the nest (Fitzsimons et al. 1993). In *S. lagocephalus*, we showed that larger males preferentially selected boulders or large cobbles as nests, whereas smaller ones were found under cobbles. This pattern of nest distribution could result from male–male competition for nest sites (Takahashi et al. 2001; Takahashi 2008). Male gobies are mainly territorial and the success of agonistic behavior is often correlated to the body size (Fitzsimons et al. 1993; Kroon et al. 2000; Takahashi et al. 2001). Similarly, the coloration intensity of the *S. stimpsoni* male is a main factor of hierarchical dominance and is likely to influence the male attractiveness for spawning (Fitzsimons et al. 1993; Keith 2003). It can be expected that large males have greater abilities to defend large nests, which are better perches to display a courtship and might increase their reproductive success (Fitzsimons et al. 1993; Mazzoldi and Rasotto 2002; Takahashi and Kohda 2002; Takahashi 2008).

In *S. lagocephalus*, the number of eggs in the nest was significantly correlated with both nest stone size and body size of the nest-guarding male, highlighting the interest of large nest stones for male reproductive success (e.g. Takahashi and Kohda 2002; Takahashi 2008). Nest size preference could lead a strong selection for spawning sites based on substratum composition. As nests presumably contain the clutch of a single female, and since fecundity is correlated with the female size (Delacroix and Champeau 1992), the number of eggs in the nest may be considered as a proxy of

the female size. This observation reflected that large females preferentially select large both males and nests, as verified in other gobies (Fitzsimons et al. 1993; Takahashi et al. 2001), and also suggests a selection by males based on the female size or an avoidance of larger males by small females, as has been discussed in other fish (Labonne et al. 2009). Male mate choice for similar-sized females has already been reported in *Rhinogobius* spp. and plays an important role in the formation of mating pairs (Ito and Yanagisawa 2000; Tamada 2011). The mating pattern probably results from a compromise between success of mating due to female choice and the male preference for large females exhibiting high batch fecundity (Delacroix and Champeau 1992). The guarding behaviour is costly because often associated with a restricted (Takahashi and Yanagisawa 1999) or non-feeding period (Daoulas et al. 1993). We could expect that large males more frequently refuse to mate with small females than males of species simultaneously guarding the eggs of multiple females. As observed in this study, acceptance of small females’ eggs has often been associated with less parental care from the male. We showed that the number of eggs under large cobbles was significantly lower for unguarded than *S. lagocephalus*-guarded nests, suggesting that medium size males might accept to mate with smaller females but probably do not attend the clutch or tend to escape when an observer disturbed their nests.

To conclude, this study was a first approach of breeding pattern for *S. lagocephalus*. Field observations were both a simple and a satisfactory method to access the main traits of mating, and the results may be considered as a reference base without the potential bias of a laboratory approach. Nevertheless, controlled experiments could be a complementary approach to confirm that nests contain the clutch of a single female and to investigate male choice in *S. lagocephalus*. Similarly, the small number of *C. acutipinnis* in the field highlighted the interest of laboratory experiments for sexual selection investigations of this endemic species.

**Acknowledgments** We are grateful to the ARDA staff for their help and commitment: Pierre Bosc (director) and Henri Grondin (Technician). We are grateful to reviewers for critical comments and helpful suggestions. The present study was conducted with the financial support of Electricité De France (22-30 avenue de Wagram, 75382 Paris Cedex 8), Office de l’Eau Réunion (49 rue Mazagran, 97 400 Saint Denis), Région Réunion (Avenue René Cassin, BP 7190, 97719 Saint Denis cedex 9), Parc National de La Réunion (112 rue Sainte Marie, 97400 St Denis) and the European Union (European Social Fund).

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