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APOGONIDAE
EGG
PARENTAL CARE
ACTIVITY PATTERN

ABSTRACT. – The cardinal fish *Apogon imberbis* (Linnaeus, 1758) is a common species in the Mediterranean sea but its reproductive biology is poorly known. Here we present information on the duration of the breeding season, the daily activity pattern and the mating behaviour of *A. imberbis* in the field, recorded in an Italian Marine Protected Area (Catania, Sicily). Mouthbrooding males were observed from July to September. Courtship behaviour was recorded throughout the day, however its highest frequency, as well as spawning, occurred at sunset. The abundance of cardinal fish varied during the study period, reaching a peak during the breeding season, when individuals exhibit site fidelity. Observations of mouthbrooding males and collection of egg masses allowed to estimate that parental cares last for 5 to 7 days, and brood size is around 4000-6000 eggs.

INTRODUCTION

The cardinal fish of the family Apogonidae comprise more than 200 species, most of which are abundant in the shallow waters of temperate and tropical seas (Nelson 1994). Information on their reproduction is limited but, in all species, males appear to perform parental care bearing the eggs in the mouth at least until hatching (Breder & Rosen 1966, Kuwamura 1985, Thresher 1984). However, other traits of cardinal fish reproductive biology, such as the size of the brood and the duration of paternal care, appear to vary significantly among species. Indeed in species, such as *Pterapogon kauderni*, broods are composed of less than one hundred large eggs and male cares are performed both on developing eggs and newly hatched young (Kolm & Berglund 2004, Vagelli 1999), while in others, such as *Apogon lineatus* and *A. doederleini*, each brood contains several thousand tiny eggs that are mouthbrooded only until hatching (Kume *et al*. 2000b, Kuwamura 1985, Okuda *et al*. 1998). The modalities of pair formation are very diverse too, varying from living apart from the group several days before spawning to briefly pairing for mating with the male remaining in its location (Kolm & Berglund 2004, Kuwamura 1985, Vagelli 1999).

The cardinalfish *Apogon imberbis* (L., 1758) is the only native apogonid in the Mediterranean, and is widespread along the Mediterranean as well as the Eastern Atlantic coasts (Froese & Pauly 2007, Whitehead *et al*. 1986). Despite its abundance (Bussotti *et al*. 2002, 2003), only few studies have dealt with the reproductive biology of this species (Garnaud 1950a, b, Lahnsteiner 2003) which thus remains largely unknown. This study investigated the duration of the breeding season and the activity pattern in *A. imberbis*, and collected information on its mating behaviour, paternal care duration and brood size, in terms of both egg number and egg size.

MATERIALS AND METHODS

The study was conducted on the north-east coast of Lachea Island, the Marine Protected Area of "Isole Ciclopi" (Catania, Italy), from April to October 2005. The cardinalfish abundance, vertical distribution and size were visually estimated by three trained SCUBA divers in one grid of 30 m x 20 m, ranging in depth from 5 to 18 m. The grid, remained on site through the study period, and was divided, by floating buoys, in three 10 m large corridors, helping to delimit sampling areas and to avoid re-sampling. Rocky substrates with small sandy strips characterized the grid and the three corridors presented similar substrates. No large caves were present in the considered area. The number of cardinalfish in the grid was quantified while swimming in each corridor, starting from the deepest part, carefully searching inside crevices and cavities, and recording all observed individuals. For each individual, the size (total length, TL), estimated to the nearest 0.5 cm with the aid of a ruler drawn on a PVC slate (De Girolamo & Mazzoldi 2001), and the depth, using a dive computer, were recorded. Preliminary dives, dedicated to set up data collection, were carried out simultaneously by the three divers together in order to insure the collection of comparable data. A total of 13 abundance estimates were carried out during the whole study period; all samplings were performed in daytime and with a visibility > 5 m. The duration of the breeding season was assessed recording the presence of mouthbrooding males, in fortnightly dives up to the end of October. The sampling method was chosen in order to assess variability in abundances within the same area used for the activity pattern study.

The activity pattern, in terms of any individual's action, during the breeding season, was investigated in July. The daily time was split in three periods: "morning" (8.00-10.00), "midday" (12.00-14.00), "evening" (18.00-20.00). During the last sampling period some light was still available, with sunset taking place at ca. 20.30. In eight sites inside the grid, chosen at the beginning of the study for the different numbers of individuals recorded (from 1 to 10) and kept fixed for the whole study period, three 30 min observations per time slot per site were performed. During the observations, the number and size of individuals, the presence of mouthbrooding males, the occurrence and duration of three behaviours: a) the parallel circling, a typical courtship display (see Garnaud 1950a, Kuwamura 1985), b), the aggressive chase away actions, and c) the occurrence of mating were recorded. To analyze the frequency of the aggressive behaviours and parallel circling, only observations in which more than one individual was present were considered.

On 2nd of August, two brooding males (TL = 8 cm) were collected with a hand net, placed in a plastic bag and, after a gentle shaking, their egg masses were collected and brought to the laboratory. Males were released at the site of collection. Egg masses were measured and fixed in 7% formalin in sea water. Total egg number was counted and a sample of 20 eggs per batch was measured under a stereomicroscope, to the nearest 0.01 μm with the image analyser MicroImage 3.4.

All data are reported as mean ± standard deviation. Activity pattern data were analysed with non-parametric tests for dependent data, comparing fish number or behaviour within each site. Sizes of individuals within a pair involved in chasing or courtship behaviours were compared with the Wilcoxon test for paired data.

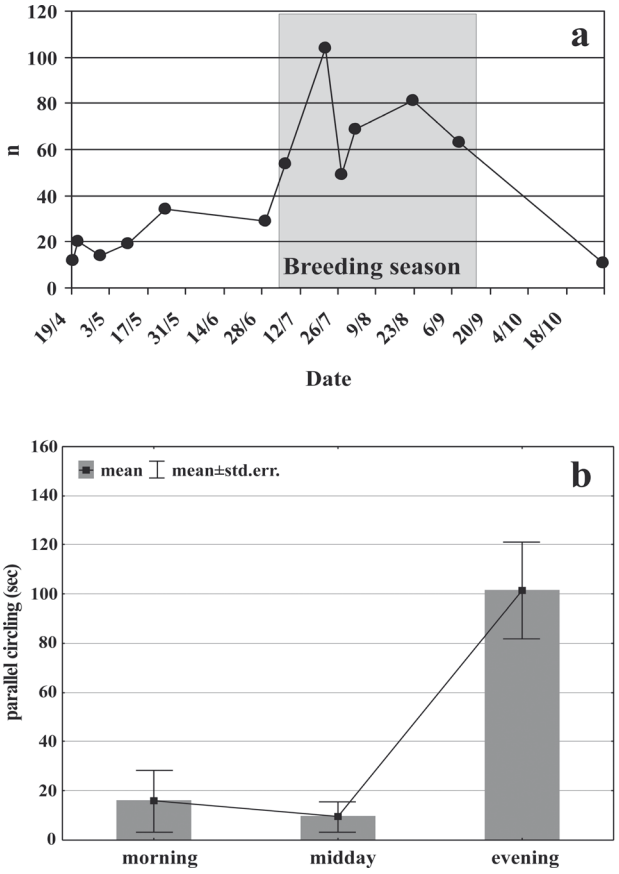

Fig. 1. – a, Number (n) of *Apogon imberbis* recorded for each sampling date in the grid (30 m x 20 m). b, Duration of circling behaviour (mean ± standard error) in the different time periods of the day.

RESULTS

Males with eggs were observed from 6th of July to 8th of September. Cardinalfish abundance within the grid varied during the sampling period (Fig. 1a), all the values collected during the breeding season were higher (70.0 ± 20.1; range: 49-104) than before (21.3 ± 8.6; range: 12-34) and after it (11). Most of the individuals were observed at depths > 8 m. Using the data of the day with the maximum recorded density, the 21st of July, no correlation between the number of cardinalfish and the depth was found (rS = + 0.08; n = 9; P = 0.84); rather fish occurrence can be attributed to the availability of shelters. Indeed, all individuals were observed close to crevices and small cavities. The individual body size ranged between 5 and 8 cm and only 2 individuals of 4 cm TL were recorded during the first sampling, the 19th of April. No juveniles, even at the end of the breeding season, were observed.

The number of individuals present in each site varied among observations (coefficient of variation: CV = 39.15 ± 18.76). However, considering the mean number of individuals in the three time periods of observation ("morning", "midday" and "evening"), a high and significant correlation between counts in every site was found (for all correlations: rS > + 0.92; n = 8; P < 0.01, corrected for multiple comparisons with the Bonferroni's method). The number of individuals per site did not vary significantly throughout the day (non-parametric Friedman's method: $Chi^2 = 4.87$; d.f. = 2; P = 0.088), even if more individuals were recorded during the morning (6.0 ± 4.2) and evening (5.7 ± 4.6) observations, than at midday (4.9 ± 4.1). The aggressive interactions involved two individuals, with the chasing animal (TL: 7.33 ± 0.46, range: 6.5-8 cm) significantly larger than that chased away (TL: 6.36 ± 0.79, range: 5-8 cm; Wilcoxon test: Z = 3.94; n = 30; P < 0.001). The frequency of aggressive behaviours did not vary with the time period (non-parametric Friedman's method: $Chi^2 = 3.71$; d.f. = 2; P = 0.156; mean number of aggressive behaviours in 30 min: 2.61 ± 1.68; n = 7), whereas the parallel circling behaviour was performed significantly more during "evening" than during the other time slots (non-parametric Friedman's method: $Chi^2 = 12.33$; d.f. = 2; P = 0.002; Fig. 1b). Mouthbrooding males were never observed performing the parallel circling behaviour.

During the evening observations, in four different episodes, the circling behaviour ended in a spawning act. Sex

in the pair was recognized from the presence of a swollen belly in females. The egg masses were rapidly released and immediately taken into the mouth by the males that started the typical churning behaviour, as described by Kume *et al*. (2002). Male and female in the pair did not differ in size (male TL: 7.42 ± 0.45; range: 7-8 cm; female TL: 7.61 ± 0.36; range: 7-8 cm; Wilcoxon test for paired data: Z = 1.07; n = 13; P = 0.286). All males brooding eggs were larger than 7 cm (7.65 ± 0.47 cm TL; n = 13). In two cases, it was possible, on the basis of individual size and location, to follow the paternal care, from the spawning to the disappearance of eggs in the male mouth. The duration of egg care was respectively estimated as 5 and 7 days (at a mean water temperature of 20° C).

Egg masses were constituted by 4066 and 6208 eggs. Eggs were spherical and their diameters measured 663.43 ± 27.59 and 639.81 ± 22.61 μm, respectively. Eggs, bound together by threads raising from one egg pole, were at the same developmental stage (pigmented eyes).

DISCUSSION

The cardinalfish *Apogon imberbis* is nocturnally active, as other cardinal fish (Thresher 1984), while during the day it is known to mainly remain in caves, where it can form large groups (Bussotti *et al*. 2003). Our surveys did not include large caves and we recorded only groups smaller than 18 individuals, usually associated with crevices and small cavities. The observed increase in the number of individuals during the breeding season could be due to a temporal change in location or, alternatively, in behaviour, with fishes spending less time hidden. During the breeding season, the correlation among numbers of individuals in each marked site suggests that groups may remain fairly constant and individuals may exhibit site fidelity during the day, as reported for other species (Marnane 2000, Kolm *et al*. 2005). Our observations were performed during daylight, consequently the number of individuals occurring in the area may be underestimated, and we missed any feeding behaviour or interactions between individuals occurring during feeding activities. However, also in other species courtship and spawning have been reported to occur during the day (Okuda & Yanagisawa 1996a, Vagelli 1999), consequently the daytime appears to be the appropriate time to collect data on reproductive behaviours.

Comparing the behaviour of *A. imberbis* and other cardinal fish species during the breeding season (Kuwamura 1985, Vagelli 1999, Vagelli & Erdmann 2002) it can be inferred that the former adopts the strategy of 'transient pairs' (Kuwamura 1985). Indeed, courtship and spawning occur within the group and the mating pair does not isolate or exhibit territorial behaviour, as observed in species forming long-lasting pairs (Kolm & Berglund 2004, Kuwamura 1985, Vagelli 1999, Vagelli & Erdmann 2002). The similarity in body size of the pairing individuals suggests that in this species, as in other cardinal fishes, a size assortative mating occurs (Kolm 2002, Okuda *et al*. 1998).

The parallel circling courtship behaviour occurred mainly at dusk, the only period when spawning was observed. The behaviour of spawning pairs is similar to that described for other cardinal fish species, with the female releasing the egg clutch after parallel circling and the male immediately taking the eggs into his mouth (Kuwamura 1985, Vagelli 1999). The possible presence of internal fertilization in *A. imberbis* has been hypothesized in relation to the close proximity of partners during spawning (Garnaud 1950a, b) and has not been excluded by the analysis of sperm morphology (Lahnsteiner 2003). However, the similarities in spawning behaviour with other cardinal fish species where internal fertilization has never been documented, suggests external fertilization as the more likely reproductive modality for this species.

Similarly to other cardinal fish species, in which the occurrence of males simultaneously taking care of multiple clutches has never been reported, mouthbrooding *A. imberbis* males were never observed to perform the circling behaviour or to spawn. Consequently, the egg mass collected from males in the field, likely represents the egg clutch of a single female. The number of eggs per mass that we recorded is consistently lower than that of over 22.000 reported from an aquarium spawning (Garnaud 1950a). A comparison between these observations is difficult given the different mating conditions, the overall limited number of samples and the lack of size of parental individuals of the previously analyzed egg batch (Garnaud 1950a). Considering that filial cannibalism has been observed in cardinal fish species (Kume *et al*. 2000a, Okuda & Yanagisawa 1996a, b), the number of eggs we recorded could be an underestimation of the number of eggs released by a female. However, since filial cannibalism in cardinal fishes has been estimated to affect on average 30% of a clutch (Kume *et al*. 2000a), the herein reported number of eggs per clutch anyhow is lower than what was previously observed. The recorded egg size is slightly larger than the one reported by Lahnsteiner (2003), where measurements, however, were taken from ovaries, but is consistent with the measures reported for the other *Apogon* species where females lay clutches containing thousands of eggs (Kume *et al*. 2000b, Kuwamura 1985, Okuda *et al*. 1998).

In conclusion, this study provides the first data on the reproduction of *A. imberbis* in the field. The comparison of the observations with the mating behaviour of other cardinal fish species strongly suggests that *A. imberbis* forms 'transient pairs' with size assortative mating. In addition, even if the sample size is extremely small, results give a first indication on clutch fecundity and duration of parental care.

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