Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
www.hcmr.gr
DOI: https://doi.org/10.12681/mms.41540

In between coral branches: describing the courtship behavior of the Mediterranean cardinalfish *Apogon imberbis* (Linnaeus, 1758)

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Contributing Editor: Stelios SOMARAKIS

Received: 20 May 2025; Accepted: 14 June 2025; Published online: 02 September 2025

Abstract

The behavior of a pair of cardinalfish *Apogon imberbis* (Linnaeus, 1758) (Apogonidae) was investigated through the acquisition of time-lapse images at three-min-intervals over five consecutive days, encompassing both diurnal and nocturnal hours. We characterized the phases of courtship, quantified the frequency and alternation of activity patterns, and assessed their duration across the diel cycle, highlighting potential differences between sexes. The habitat preferences by *A. imberbis* associated to the red octocoral *Paramuricea clavata* (Risso, 1827) (Cnidaria, Anthozoa) were documented for the first time in the Mediterranean Sea. The pair exhibited pronounced diel behavioral patterns, with increased nocturnal activity likely linked to zooplankton foraging, and diurnal rest in shelters provided by the coral branches. The courtship consisted in three activities that were cyclically repeated throughout the day, with a total duration of 10.2 hours. The novel behavior named "patrolling" was played by the female after the mating event, presumably linked to the egg-laying site defense. The time-lapse technique offers the possibility to collect continuous, non-invasive observations at great depths and for prolonged periods, allowing for detailed surveys of the organisms, their interactions and the use of the habitat. The advantages and potentialities of this methodology could be further improved by machine learning programs for the recognition of animal activity patterns, allowing for the processing of massive dataset, reducing human workload and improving accuracy and time-efficiency.

Keywords: Reproductive behavior; underwater photography; marine animal forests; mesophotic; habitat use.

Introduction

The cardinalfishes (family Apogonidae) comprise more than 300 species that predominantly inhabit coral and rocky reefs in tropical and temperate latitudes (Barnett & Bellwood, 2005). Within the Mediterranean Sea, *Apogon imberbis* (Linnaeus, 1758) is the only representative of this genus, exhibiting a wide and homogeneous distribution from shallow coastal waters down to depths of approximately 200 m (Bussotti *et al.*, 2003). *A. imberbis* is a sciaphilic species closely associated with hard substrata, where it may shelter alone in crevices and cavities or aggregate in large schools of up to 18 individuals per square meter (Marnane, 2000; Bussotti *et al.*, 2003; Raventos, 2007; Mazzoldi *et al.*, 2008; Bussotti & Guidetti, 2009). As with many other cardinalfishes – such as *Ostorhinchus doederleini* (Jordan & Snyder, 1901), *Zoramia fragilis* (Smith, 1961), *Nectamia fusca* (Quoy & Gaimard, 1825), *Taeniamia fucata* (Cantor, 1849), and *Cheilodipterus quinquelineatus* Cuvier, 1828 - *A. imberbis* is oviparous and a paternal mouthbrooder, a reproductive strategy that results in subtle sexual dimorphism. Specifically, males develop a slightly larger and deeper buccal cavity to accommodate fertilized eggs (Kuwamura, 1983; Barnett & Bellwood, 2005; Vagelli, 2011), a feature that enables sex identification in species where external morphology otherwise shows little difference between males and females. Another notable aspect of apogonid biology is their distinct courtship behavior, which involves coordinated displays and movements performed by both partners before spawning (Kuwamura, 1983). This type of behavior is common to several nekto-benthonic species

within the family, including *O. doederleini*, *O. notatus* (Houttuyn, 1782), *O. quadrifasciatus* Cuvier, 1828, *Jaydia lineata* Temminck & Schlegel, 1843, *Apogonichthyoides niger* Döderlein, 1883, and *Pterapogon kauderni* Koumans, 1933 (Usuki, 1977; Kuwamura, 1983; 1985; Vagelli, 1999; Okuda, 2001; Vagelli & Volpedo, 2004; Mazzoldi *et al.*, 2008; Saravanan *et al.*, 2013). Typically, the pair swims in parallel, describing repeated circles of approximately 20 cm diameter, interspersed with trembling, physical contact, and crossing of the caudal fins. This alternation of movements can last from minutes to hours, with the partners separating and rejoining multiple times. Notably, both individuals exhibit a temporary lightening of body coloration during courtship, returning to their normal appearance after spawning (Kuwamura, 1983; 1985). For *A. imberbis*, reproduction occurs in the summer months, from July to October (Raventos, 2007). During this period, once the eggs are fertilized and laid on the substrate, the male collects them in its mouth and broods them until hatching (Mazzoldi *et al.*, 2008). The species is known to form transient pairs that exist only for the duration of the spawning event, which typically takes place during daylight hours (Mazzoldi *et al.*, 2008). Despite these insights, detailed behavioral observations of *A. imberbis* in natural habitats – especially regarding the timing, phases, and duration of courtship and brooding behaviors in relation to habitat complexity – remain limited. Here, we document the behavior of two *A. imberbis* individuals using the instantaneous sampling method for ethological studies (Lehner 1992) combined with time-lapse image acquisition. The observations were conducted near an assemblage of *Paramuricea clavata* (Risso, 1827) – an octocoral species endemic to the Mediterranean and widely distributed in the Tyrrhenian Sea from shallow (-15 m) to mesophotic (-200 m) depths (Poliseno *et al.*, 2017). In association with other gorgonians such as *Eunicella singularis* (Esper 1794), *E. verrucosa* (Pallas, 1766), *E. cavolini* (Koch, 1887), and *Leptogorgia sarmentosa* (Linnaeus 1758), *P. clavata* forms complex animal forests that significantly increase habitat heterogeneity and attract diverse faunal assemblages (Cerrano *et al.*, 2010; Gori *et al.*, 2017; Rossi *et al.*, 2017). Individuals were continuously monitored, day and night, over five consecutive days. We characterized their individual and pair behavior, described courtship phases, quantified activity pattern frequencies and durations, and compared daytime and night time behavior with attention to differences between male and female time budgets. We also discuss potential interactions with other fauna and the specific use of the habitat provided by the gorgonian forest. Finally, we evaluate the advantages and limitations of using time-lapse imaging for ethological studies in deep underwater environments, where direct observation is challenging. Although limited to two individuals, this study aims at describing the natural courtship behavior and diel activity budgets of *Apogon imberbis* in a structurally complex mesophotic habitat, highlighting the potential of the instantaneous sampling method for fish behavior through time-lapse photography.

Materials and Methods

Behavior of *Apogon imberbis* was recorded at 40 m depth at "Punta di Torre Ciana" (42.21580° N, 11.9414° E), off coast of Argentario Promontory, located in Tuscany (Italy), northern Tyrrhenian Sea (Western Mediterranean Sea). The camera was a GoPro 8 Custom inserted in a rigid steel structure (AISI 316L) and POM C compound cases connected to an external interchangeable battery (25 Ah) with waterproof cables, allowing an operational duration of ca. 150 hours before needing to be recharged. A flashlight was connected to the rigid structure through a mobile arm and operated for the only duration of the shutter click. To control the whole system, a motherboard with microcontroller Atmel® ATmega 480 was used to synchronize image caption, lightning system and datalogger to record photo date and time. The device was anchored to the substratum for the whole observation period. All photos were taken at constant distance, angle and position.

The Instantaneous Sampling method was adopted to record fish behavior, using photos as distinct observations. This method is commonly used in ethological studies to record the behavior of an individual (or group) at sequential and preselected sampling points in time, with a fixed interval in between (Lehner, 1992). The time-lapse device was deployed on July 18th 2024. The camera stopped recording on the fifth day, so records lasted from 15:10 on July 18th to 22:49 on July 22nd 2024. A total of 2,074 photos (representing the sampling points) were shot at three-minute intervals, covering a period of 102.5 hours. Days were divided in six timeslots according to Enrichetti *et al.* (2022): Late Night (LN) from 00:00 to 03:59, Dawn (Da) from 04:00 to 07:59, Morning (Mo) from 08:00 to 11:59, Afternoon (Af) from 12:00 to 15:59, Evening (Ev) from 16:00 to 19:59, and Early Night (EN) from 20:00 to 23:59. Each photo was included in a determined timeslot basing on the time of shooting. To describe behavior of *A. imberbis*, an ethogram of activity patterns was developed both for "single" behavior (performed by one single individual) and "pair" behavior (by two interacting individuals).

Sexes were identified using buccal cavity morphology (Fig. 1). If the mouth was not visible or sex could not be determined, the individual was categorized as "unknown". The activity patterns of solitary individuals and pair were discerned and categorized after a first image analysis as follows: the activities of solitary individuals were sheltering, wandering, mating, patrolling, and bottom interactions (Table 1). The activities of the pair, related to the courtship, were: parallel circling, crossing and bottom interactions (Table 2). The activity patterns were expressed as a frequency (= number of photos, n) or as percentage of occurrence of a determined activity. The percentage values of the occurrence were obtained by referring to the total number of photos portraying the female (n_{female} = 217) or male (n_{male} = 158), added to the photos of the pair activity (n_{pair} = 199) in which both were active (see Fig. S1a and b in Supplementary materials).

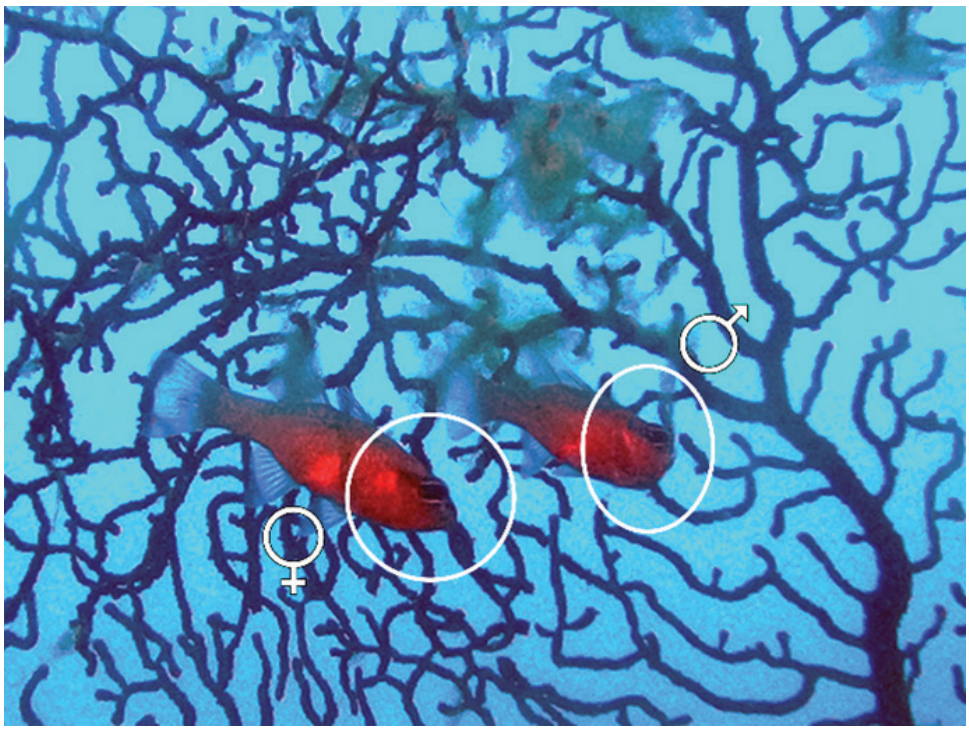

Fig. 1: The sexual dimorphism of *Apogon imberbis* is determined by the different volume of the buccal cavity. The male shows slightly deeper and larger mouth in comparison to the mouth of the female, that appears straighter. The photo has been enlarged to have a clearer vision of the mouth characteristics of *A. imberbis.*

Table 1. Behavioral categories of the single *Apogon imberbis* and their description.

Category	Description
Sheltering	*Apogon imberbis* uses the branches of the coral colony as shelter to hide, it keeps a still position for numerous subsequent photos.
Wandering	*A. imberbis* swims around the colony, being visible in different position and with motion (out of focus).
Mating	*A. imberbis* is together with the partner for mating purposes (see table 2 for mating activity patterns)
Bottom interactions	*A. imberbis* interacts with the substrate, it is oriented with the head downwards and very close to the bottom
Patrolling	*A. imberbis* swims around the colony by keeping the sight and head oriented toward the same spot, keeping the same inclination of the body, with the head slightly downwards

Table 2. Behavioral categories of pair (male and female) of *Apogon imberbis*, constituting courtship for mating and its description.

Category	Description
Parallel circling	The two fish move slowly forming a restricted circle by staying parallel to each other, the female at the external side; fish are observed while changing orientation but maintaining the parallel formation, either in the same or opposite direction.
Crossing	The two fish assumed a "X" shape, crossing their caudal fins slightly oriented upwards, while the heads diverged and were oriented downwards (photo E6), in a sort of "tail-to-tail" position
Bottom interactions	The two partners interact with the substratum, either one or both together, by swimming in its vicinity or by directly acting on it. In case only one of the two fish interacts, the other is in its proximity.

Results

General observations

During the 5-day data collection, the encounter and courtship of two individuals of *Apogon imberbis* occurred between the branches of the framed colony. No more than two individuals of *A. imberbis* were simultaneously recorded.

The total number of photos portraying either one or two *A. imberbis* was 698, corresponding to 33.7% out of the total number of acquired photos (n_{tot} = 2074), and approximately to 34.9 hours (Fig. S1 A Supplementary materials). A single *A. imberbis* was reported in 489 out of 698 photos (70%), corresponding to an estimated duration of 24.4 hours. Of these, 217 photos portrayed the female (44.5%, ca. 11 hours), 158 portrayed the male (32.4%, ca. 8 hours) and in 114 photos it was not possible to discern the fish sex (23%, 5.7 hours). The remaining time (n = 209, 30%), two individuals were simultaneously present in the photo frame, for 96% of the time displaying courtship behavior (n_{pair} = 199, ca. 10.2 hours) and in the rest of the photos (4%, ca. 0.4 hours) performing other non-interacting activities such as wandering around (Fig. S1 B Supplementary materials).

The female was observed hiding between the coral branches during sunset hours of day 1. At dawn of day 2, the male approached the colony and hid between the branches, and the female departed. The female was active during the night and visible around the colony, while the male was more present during diurnal hours, hidden in his shelter. During day 3, a first brief courtship occurred, and the two fish were simultaneously present in proximity to the colony. The real courtship event occurred during the diurnal timeslots of day 4, in two rounds separated by the departure of the male and the hiding of the female, which waited for her partner to come back few hours later. During the nocturnal hours of day 4 (after mating) and during day 5, the pair separated, and the two individuals were visible alone, either hiding or performing other behaviors. The daily cycle of the two fishes is simplified in Figure 2.

Behavioral patterns of single individuals

The activity patterns of individuals are reported by sex in the graphs in Fig. 3 A-D and are described as follows: sheltering (Fig. 4) occurred when *A. imberbis* hid between the branches of the coral colony for numerous consecutive photos without consistently changing position. The male used the shelter mostly in twilight hours (timeslots Da and Ev, 43.5% of n_{male}, ca. 7.7 hours), (Fig. 3 B). Female sheltering was observed in the evening of the first day (timeslot Ev) (17.4% of n_{female}, ca. 3.5 hours), and between the two mating sessions (timeslot Af) corresponding to the time in which the male departed, to come back few hours later (Fig. 3 A). The most repeated activity throughout the days was wandering, described as individuals that swim around the colony with no clear direction, and visible in a different position in subsequent photos, often out of focus due to rapid movements (Fig. 5). This

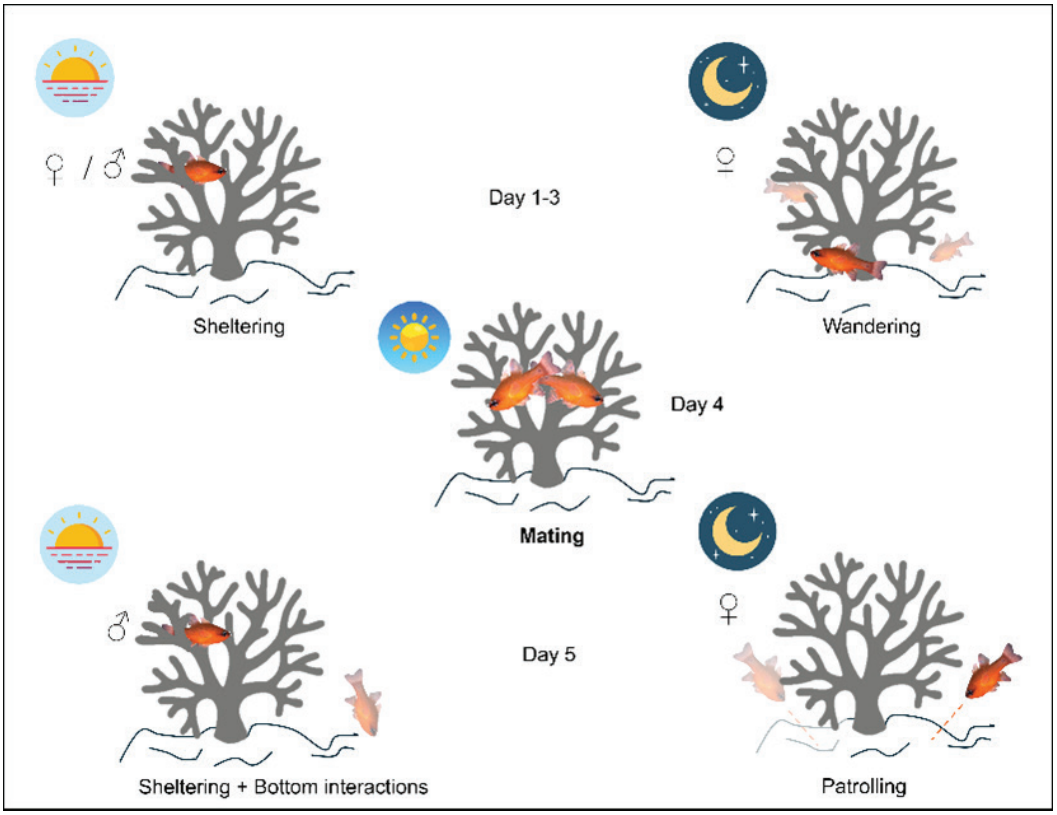

Fig. 2: Graphical representation of the daily cycle of *Apogon imberbis*. Behaviors are performed by the female or the male predominantly on twilight (half-sun icon), day (full-sun icon) or night hours (moon icon). Patrolling and single bottom interactions are visible predominantly during the night and the dawn following mating event (day 5), respectively.

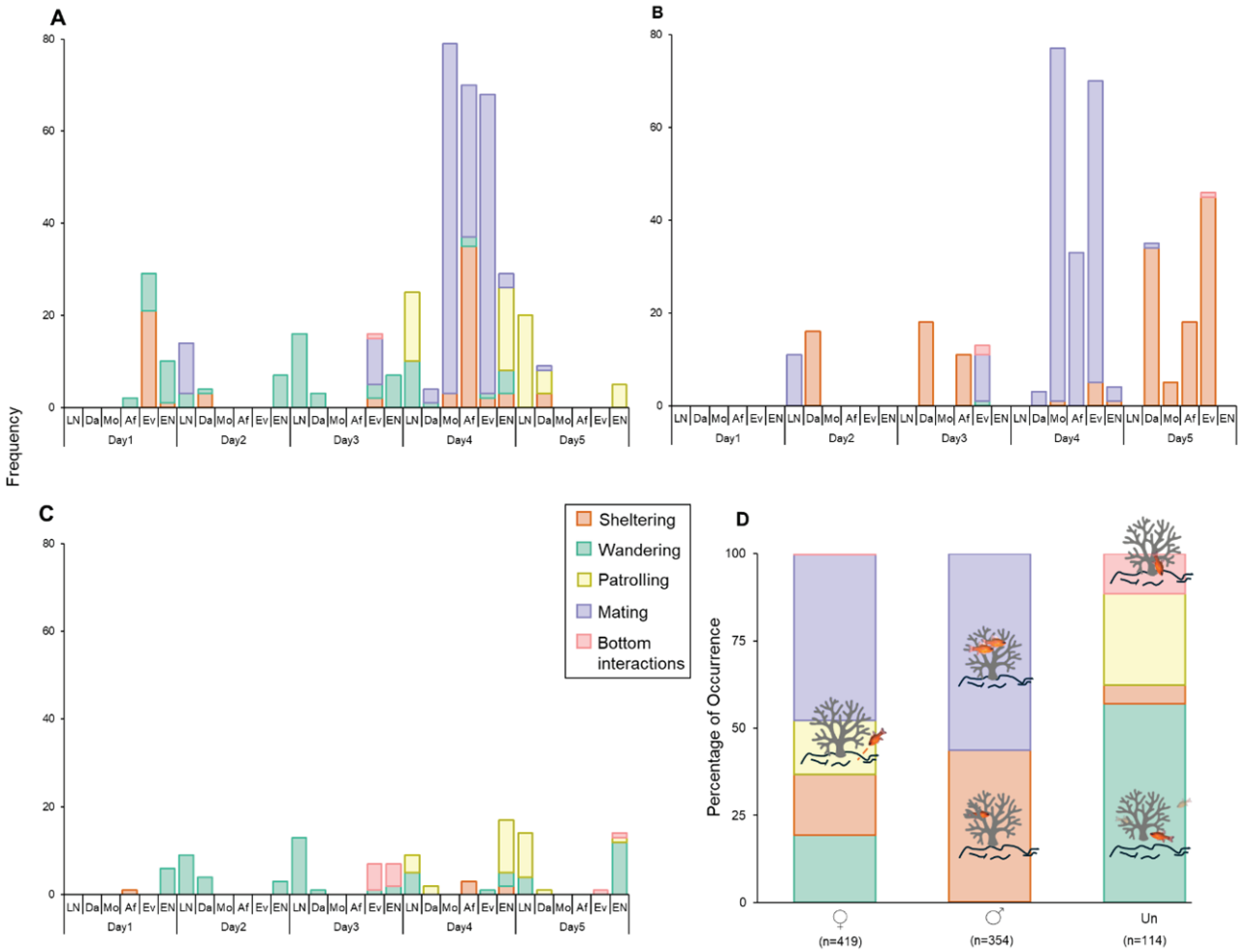

Fig. 3: Activity budget of **A)** the female (♀), **B)** the male (♂) and **C)** unknown sex (Un) *Apogon imberbis* in proximity of the coral colony, divided per timeslots and per days. On y-axis, the number of photos portraying a specific behavior (frequency) of female/male/unknown. On x-axis, the timeslots: LN = late night; Da = dawn; Mo = morning; Af = afternoon; Ev = Evening; EN = early night, repeated each day (from 1 to 5). The legend represents the categorized behaviors reported in Table 1. In D), the percentage of the total occurrence of the activity patterns of the single individual throughout the days are reported for the female (♀ n = 419), the male (♂, n = 354) and unknown gender (Un, n = 114). The asterisk refers to Fig. 8 which provides details on the phases of the courtship activity.

Fig. 4: Example of sheltering behavior, the fish *A. imberbis* uses the branches of *P. clavata* colony to hide. The fish stay in this position for several consecutive photos. Photos may be enlarged to improve the clarity of the subjects.

behavior was predominantly performed by the female during nocturnal hours (timeslots EN and LN), sometimes protracting till dawn or starting little earlier (Timeslot Ev) (19.3% of n_{female}, ca. 4 hours), (Fig. 3 A; Fig. S2 Supplementary materials). However, in 57% of the photos, this behavior was observed but the sex was not determined

Fig. 5: Example of wandering behavior, the fish *A. imberbis* moves around the colony, appearing in different positions in consecutive photos. Photos have been enlarged to improve the clarity of the subjects.

due to the orientation of the fish relative to the camera (Fig. 3 C). Bottom interactions (Fig. 6) were considered when one individual (or two, during courtship) interacted with the substratum, either swimming in close contact to it or oriented with the head down (Fig. 3 D, 8). Bottom interactions were rare (11%), and it was often not possible to discern the sex of the fish due to low brightness of the photo or to the position of the fish. This activity seemed to be related to courtship and mating, since it occurred among other courtship activities. Patrolling described fish that swam in circles with the head and sight directed toward the substratum (Fig. 7), and was performed by the female (16% of n_{female}, ca. 3.3 hours) or unknown fish (26% of $n_{unknown}$, ca. 1.5 hours) during the night after mating (Fig. 3 A, C) (Video V1, Supplementary material). However, since the photos with unidentified fish occurred within the period of female patrolling, it is likely that all photos with the unidentified individual were portraying the female. Also, male patrolling was never observed (Fig. 3 B). Mating was considered a distinct individual behavior performed both by female and male, visible in 199 photos for both (56.2% of n_{female} and 47.5% of n_{male}).

Behavioral patterns of the pair

After the previous brief encounters of the two partners on days 2 and day 3, the real mating event occurred on day 4, in proximity to *P. clavata*, both between branches and close to the base of the colony on the substratum. Mating was characterized by a long courtship that lasted approximately 10.2 hours, during daytime (timeslots Mo, Af and Ev; Fig. 3 D, Fig. 8, Fig. S2 Supplementary materials). The courtship was divided in two rounds of 4h 22m and 5h 57m, respectively, separated by an interval of ca. 2 hours in which the male left the scene, while the female hid between the coral branches and waited for the male to come back and resume the courtship. These three activities were performed by the two partners in a cyclic and alternate way. Parallel circling was the most performed activity (72.4% of n_{pair}, ca. 7 hours), followed by crossing (20.6%, ca. 2.5 hours) and bottom interactions of the pair (7.1%, ca. 40 minutes) (Fig. 8 A; Video V2 Supplementary materials). A simplified representation of the three activities constituting courtship is reported in Fig. 8 B and fish are showed displaying courtship in Fig. 9. After

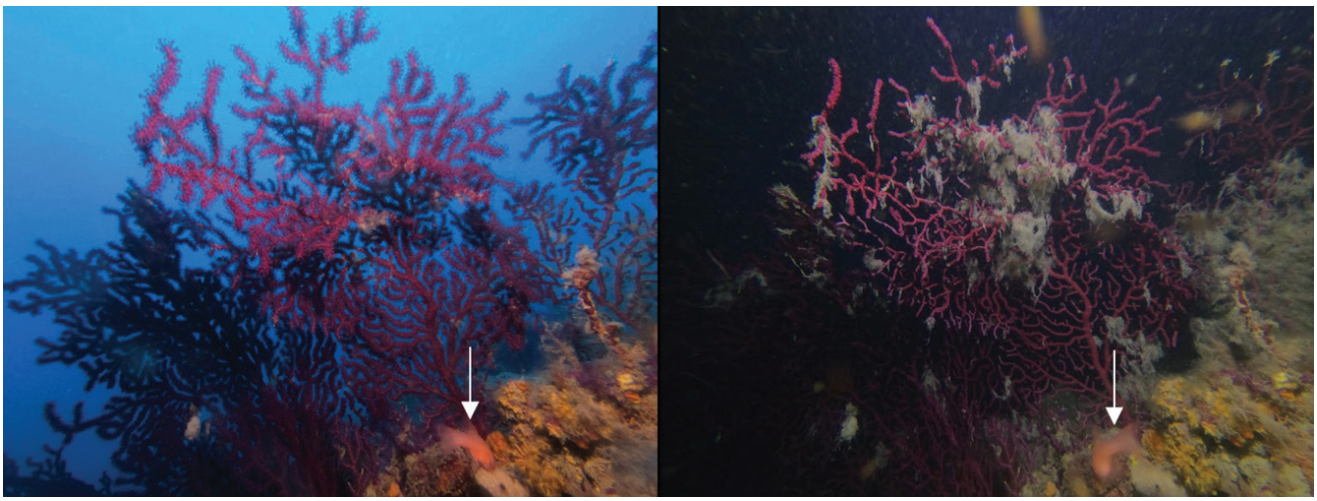

Fig. 6: Example of bottom interactions by the fish *A. imberbis*, that is oriented with the head down towards the substratum. Photos have been enlarged to improve the clarity of the subjects.

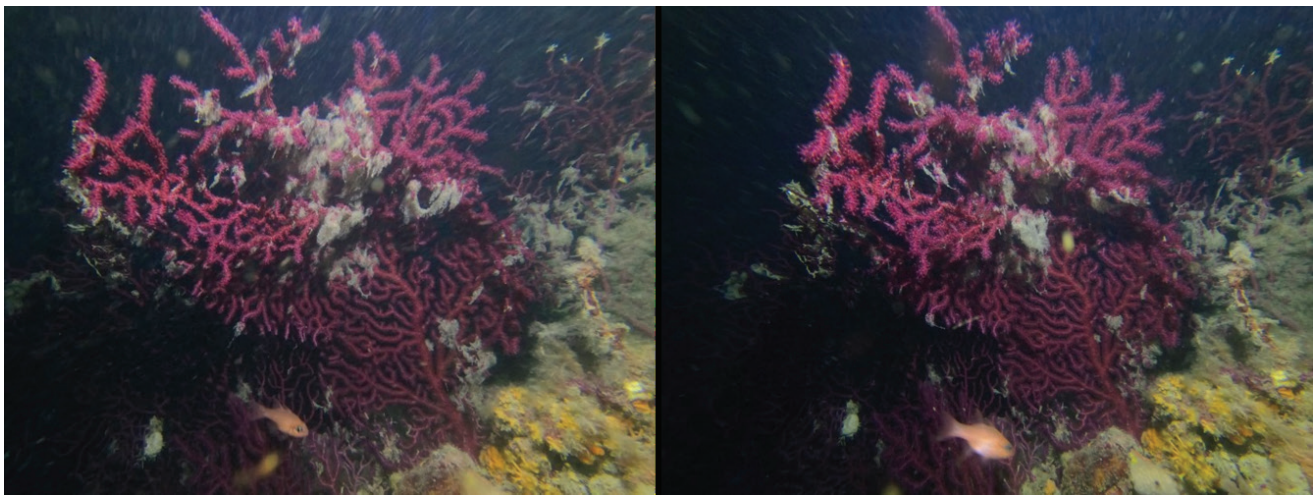

Fig. 7: Example of patrolling behavior performed by the female during the night after the mating event occurred. Photos have been enlarged to improve the clarity of the subjects.

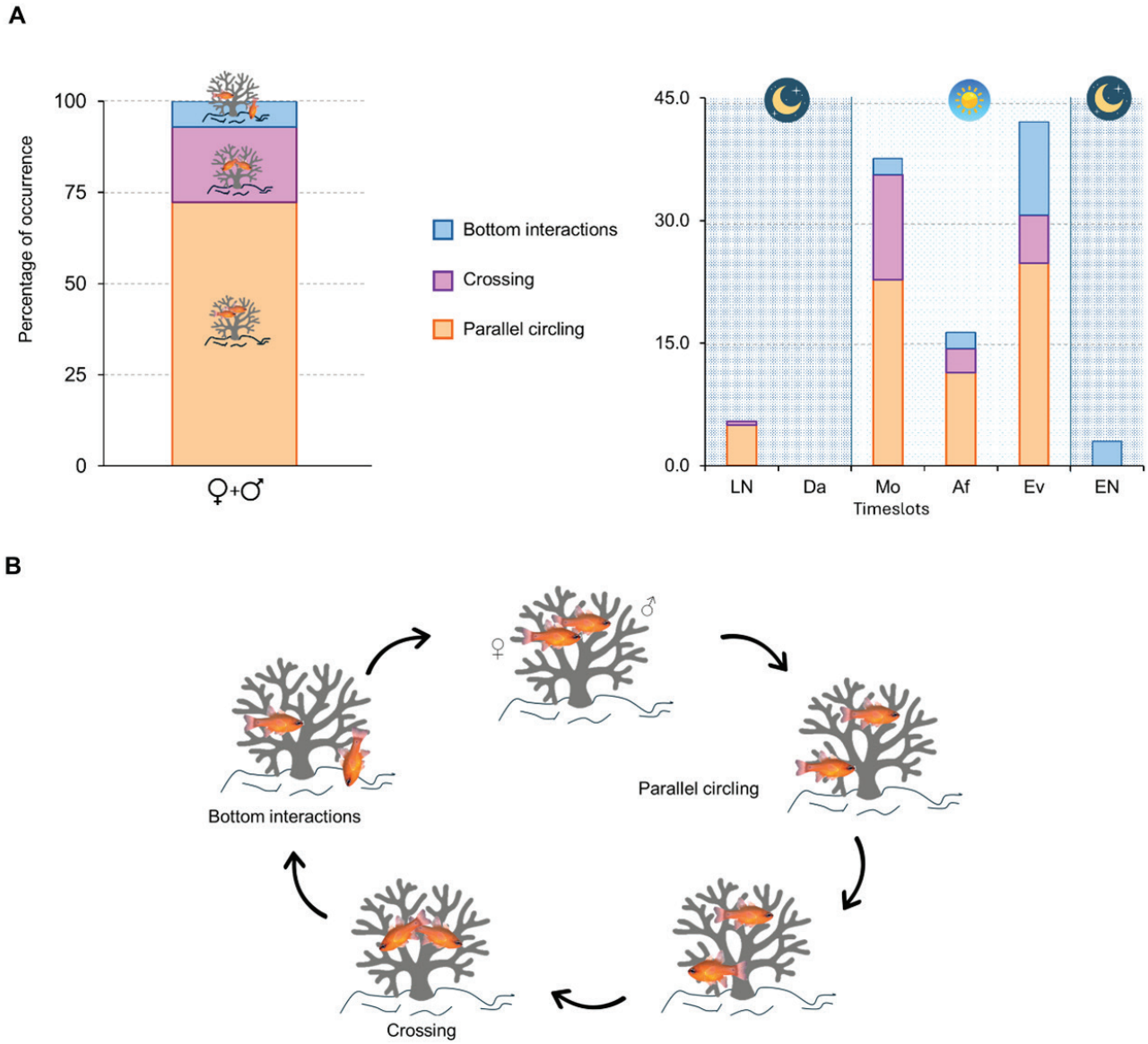

Fig. 8: **A)** Activity budget of a pair (♀ + ♂) of *Apogon imberbis* during mating. The percentage of occurrence of the three categorized behaviors (reported in the legend) are calculated based on the number of photos with that behavior out of the total number of "pair" photos (n_{pair} = 199); **B)** Graphical representation of the courtship behavior of a pair of *Apogon imberbis*. The whole courtship lasted 10.2 hours in two rounds and occurred entirely in proximity to the coral colony.

Fig. 9: Example of parallel circling, crossing and bottom interactions occurred during courtship. Photos have been enlarged to improve the clarity of the subjects.

the courtship and mating, the pair left, the male and the female were alternatively seen in the frame, hiding and patrolling, respectively. Further images with more details on males, females and pair behaviors are available as Supplementary materials: Table of Photos from Fig. E1 to Fig. E7, and Videos V1 and V2.

Interspecific association

Other fish species that frequented the location and were commonly observed near the *Paramuricea clavata* colony were: *Anthias anthias* (Linnaeus, 1758), *Spicara maena* (Linnaeus, 1758), *Coris julis* (Linnaeus, 1758), *Chromis chromis* (Linnaeus, 1758), *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), *D. sargus* (Linnaeus, 1758), *Symphodus rostratus* (Bloch, 1791), and *Serranus cabrilla* (Linnaeus, 1758). Some individuals were observed alone (e.g., *Diplodus* spp. and *S. cabrilla*) while other were present in small groups of few individuals (up to 7-10) (*e.g.*, *C. chromis*, *C. julis*). Even though these fishes frequenting the habitat, no interactions were noticed among the two individuals of *A. imberbis* and other fish species. The colony was not utilized by other species for close associations (e.g., hiding, foraging).

Discussion

Behavioral patterns

The provided description of both single and pair behaviors of *Apogon imberbis* allowed us to recognize patterns exhibited by other Apogonids as well as to confirm previous indications on this species. The increased nighttime activity could be likely associated with zooplankton foraging, in agreement with previous findings (Okuda, 2001; Gardiner & Jones, 2005; Mazzoldi *et al.*, 2008; Luehrmann *et al.*, 2020). Although, the fish was not observed while actively feeding, and the nocturnal wandering occurred near the colony as previously described (Chave, 1978; Marnane, 2000). Sheltering was mostly observed during daylight hours, primarily by the male. The consistent proximity of a male and a female, the occurrence of a mating event, and the absence of conspecifics in surrounding areas suggest that the pair was already established at the time of observation. The pair remained at the site from day 2 to day 5. Even though the participation of other males to the mating event has been described in Apogonids (Kuwamura, 1985), the prolonged observation in consecutive photo frames of the two partners and the absence of other males in the surroundings suggest that individuals were not mating promiscuously. After the mating event they separated, confirming the transient nature of the pair bond (Vagelli, 1999; Mazzoldi *et al.*, 2008). Courtship behaviors, including parallel circling, head-down crossing, and constrained circular swimming, resemble those reported in other species of the family (Kuwamura, 1983; 1985; Okuda, 2001; Lu *et al.*, 2024). Although behaviors such as 'crossing' with fin contact and 'trembling' were documented in other Apogonids (Vagelli, 1999; Saravanan *et al.*, 2013), they were not observed here, likely due to the brief duration and non-continuous nature of the recording. Analogously, the spawning and egg deposition were not perceived, presumably because of their limited duration (few seconds; Vagelli, 1999). Likely, these two events occurred in sampling-intervals. Continuous sampling methods, such as videos and direct observation, would be more accurate to describe such aspects, although characterized by time constraints. Following the reproductive event, the female engaged in prolonged 'patrolling' behavior, staring at the presumed egg-laying site. This behavior, which may represent territorial defense, resembles that reported in other cardinalfish species (Vagelli, 1999; Okuda, 2001; Fukumori *et al.*, 2009), and it may indicate continued investment in reproductive success, despite the male's role in mouthbrooding. The hypothesis that the egg-mass remained on the substratum cannot be excluded, although this would contrast with reports of rapid collection by the male (Vagelli, 1999). As spawning-related activity, the lack of aggressive chasing of intruders was not seen, might because of the lack of intruders or of observational limitations. No lightening of body coloration was observed, which has been noticed in other Apogonids (Kuwamura, 1985).

Habitat use

Apogon imberbis was absent from camera shot for 67% of the recorded time, suggesting that the spot was not used permanently. Since the camera framed only the frontal *P. clavata* colonies, we could not observe other

behaviors performed by the fish away from the corals. However, we noticed the fidelity of both female and male to their hiding spot, between two branches in the upper part of the coral colony. Once selected, the fish seemed attached to their spot and return there to accomplish their activities. This observation is in line to that by Mazzoldi *et al.* (2008) which showed that during the breading season, *A. imberbis* exhibited site fidelity.

The presence of corals with different morphologies attracts several species with wide range of body sizes and shapes. The use of branched corals by demersal fish families (e.g., Pomacentridae, Apogonidae, Serranidae) is a common trait of their lifestyle (Ménard *et al.*, 2012; Kerry & Bellwood, 2012; Bo *et al.*, 2024). The differences among microhabitats created by branches offer a wide set of opportunities for cardinalfishes, that could be either specialized showing strong affection to determined characteristics of the shelter, or more generalist (Luehrmann *et al.*, 2020). Some species are found predominantly 1-2 meters away from the reef, e.g., *Rhabdamia gracilis* (Bleeker, 1856), while others prefer exposed locations in proximity to the reef, *e.g.*, *Fibramia thermalis* (Cuvier, 1829). Other apogonids, such as *Ostorhinchus cookii* (MacLeay, 1881), *O. compressus* (Smith & Radcliffe, 1911), *O. doederleini* (Jordan & Snyder, 1901), and *O. nigrofasciatus* (Lachner, 1953), are found hidden between coral branches or close to the substratum for most of the time (Luehrmann *et al.*, 2020). Presumably, the Mediterranean *A. imberbis* is a generalist hider that uses several types of shelters, although the distribution pattern of this species are mostly restricted to caves and crevices, used during diurnal rest (Bussotti *et al.*, 2003; 2017). Even so, individuals of this species have been observed associated with several anthozoan species, whose branches are used by the fish as spots to rest and shelter (Gardiner & Jones, 2010). Our observation, in line with that observed for other species of the family, indicate that, when available, *A. imberbis* select coral branches rather than complexities of the rocky substratum, further confirming the role of habitat formers in attracting organisms and increasing biodiversity. Yet, these ecosystems, especially at mesophotic depths, are particularly vulnerable to anthropogenic pressures (*e.g*. fishing activities, pollution, increase in sea water temperature, sedimentation, mucilage outbreaks) (Montalbetti *et al.*, 2023; Jacobsen *et al.*, 2024), that can lead to mass mortality events with rising frequency (Linares *et al.*, 2008; Azzola *et al.*, 2023; Pierdomenico *et al.*, 2024).

Advantages and limitations of underwater time-lapse imaging

The most used methodologies to observe fish behaviors and activity patterns and to survey fish assemblages and biodiversity include direct visual censuses by SCUBA diving and snorkeling, thanks also to the possibility to engage local communities and citizen scientists (Harmelin-Vivien *et al.*, 1985; Bussotti *et al.*, 2003; Del Rio *et al.*, 2013). The activity patterns of cardinalfish were observed by divers in several locations of Southeast Asia, Australia and Japan where they are most abundant (Usuki, 1977; Kuwamura, 1983; 1985; Okuda & Yanagisawa, 1996; Kolm & Berglund, 2004; Takeyama *et al.*, 2007; Marnane, 2010; Gardiner & Jones, 2005; 2010). All these descriptions, however, only included diurnal hours and/or short time-intervals in shallow waters. Indeed, direct observation involving human efforts precludes continuous data collection over multiple days (and hours), since deep, prolonged and nocturnal dives are logistically inconvenient. Time-lapse techniques have been increasingly employed for the survey of underwater subjects, allowing to overcome these constraints (Salvati *et al.*, 2023; Bo *et al.*, 2024). However, although its great potential data collection through time-lapse approaches has been seldom performed, mostly keeping the depth limit of recreational diving (40 meters) (Boero *et al.*, 1991; Dunbrack & Zielinski 2003; Chabanet *et al.*, 2012; Aguzzi *et al.*, 2018; Francescangeli *et al.*, 2023; Fanelli *et al.*, 2024). The great advantage of time-lapse camera deployment, if accurately designed, is represented by its autonomy over multiple days and considerable depths and allows for continuous diurnal and nocturnal data collection. A possible disturbance is represented by the flashlight that could interfere with fish behavior. An appropriate selection of the photo-intervals could be determinant for reliable ethological descriptions. According to the surveys conducted by De Brauwer *et al.* (2019) on several demersal fish species, flash strobes at frequencies of 1 photo every 30 seconds awas not a disturbing factor, since it did not elicit changes in behavior compared to thecontrol groups. Doya *et al.* (2014) described the behavior of *Anoplopoma fimbria* and other benthic species over one month by collecting 50-sec-videos at 1-hour intervals at 900 meters depth, by using land-cabled cameras connected to a platform. Other studies focused on the feeding behavior and attraction of deep-sea macrourid fish to a bait by using baited cameras for a maximum of 66 hours and 45 minutes, recording 15-sec-videos every 30 minutes at a depth range from 1175 to 3000 meters depth (Jeffreys *et al.*, 2010; 2011). The only studies performed in the Mediterranean Sea using continuous monitoring over long-periods by time-lapse records aimed at describing the diel rhythms of the scleractinian coral *Dendrophyllia ramea* (Linnaeus, 1758) (Salvati *et al.*, 2023), and the diel rhythms and sleeping behavior of *Anthias anthias* associated to a forest of the black coral *Antipathella subpinnata* (Ellis & Solander, 1786) (Bo *et al.*, 2024) at mesophotic depths (60 – 75 m). In conclusion, even though our observations were limited to two individuals and could be considered mainly descriptive, our findings suggests that *Apogon imberbis* presents the common activity patterns described for the species and for other species of the same genus, both singularly and in pair. To further confirm these results and provide more detailed description of the reproductive behavior, more studies should be carried out implementing the sample size and the observational sessions, with the possibility to tag fishes in order to discern them when present in numerous schools. The time-lapse photography revealed to

be a promising cost-efficient methodology for ethological studies of ichthyofauna at considerable depths, where standard methods (involving direct observations by operators) are not feasible or logistically challenging. The disturbance is reduced to its minimum, being limited to the deployment and the retrieval of the tool. However, if on the one hand, the time-lapse cameras increase the feasibility and the magnitude of data collection, on the other hand the workload needed to post-process thousands of photos is substantial (Elhorst *et al.*, 2025). Recently, machine learning approaches have been implemented to extract animal activity patterns from video records (Valletta *et al.*, 2017), and have the potential to support the analysis of underwater photos and videos. The development and greater accessibility of Artificial Intelligence programs could assist in the training and recognition of animal behaviors, leading to promising perspectives in the methodologies to decrease human workload and simultaneously increase time-efficiency and accuracy of analyses (Elhorst *et al.*, 2025).

Acknowledgements

We are grateful to the staff of Argentario Divers (Porto Ercole, GR, Tuscany) for their assistance to deploy and recover the time-lapse device, and for the overall contribution to the acquisition of data. This work is included in the PRIN project "Corals and other benthic species LIfe HIstories – CHILI", financed by European Union – NextGenerationEU, Mission 4, Component CUP I53D23003300006, ref. code 2022NRM7NX_001.

Data Availability: The raw dataset generated from the images analysis is uploaded as supplementary material. **Conflict of interest:** The authors declare no conflicts of interest. **Fundings:** The present work was funded by the PRIN project "Corals and other benthic species LIfe HIstories – CHILI", financed by European Union – Next Generation EU, Mission 4, Component CUP I53D23003300006, ref. code 2022NRM7NX_001 and the National Biodiversity Future Center (Palermo, Italy). The work was funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP I33C22001300007, Project title "National Biodiversity Future Center - NBFC". **Ethical Approval:** The methodology used for data collection was observational and non-invasive. No organisms were disturbed, sampled or removed from their habitat during this study. **Author contribution:** Carlo Cerrano and Trevor J. Willis conceptualized the survey and designed the data collection. The underwater activities were performed by Martina Coppari, Teo Marrocco, Torcuato Pulido Mantas, Camilla Roveta and Chiara Gregorin. Image processing and data analysis were performed by Chiara Gregorin. The first draft of the manuscript was written by Chiara Gregorin and all authors reviewed, commented and edited the manuscript to the current form. Funds were provided by Carlo Cerrano and Trevor J. Willis.

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Supplementary Data

The following supplementary information is available online for the article:

Video V1_Patrolling: The female individual of the species Apogon imberbis patrolling the substrate at the base of the Paramuricea clavata colony. This activity was observed the night after the mating event, and lasted for approximately 6-7 hours, within the timeslots Early Night (20:00 – 23:59), Late Night (00:00 to 3:59) and, partially, Dawn (4:00 – 7:59) of day 4-5.

Video V2_Courtship: The pair formed by male and female Apogon imberbis performing courtship. Two activity patterns are distinct, the tail-to-tail crossing and the parallel circling. The courtship occurred during daytime of day 4 for approximately 10 hours, covering timeslots from Dawn (04:00 – 07:59) to Evening (16:00 to 19:59).

Fig. S1: a) scheme of the analysis of images. Ind. = individual; b) among the photos portraying A. imberbis (n= ***698),*** percentage and approximative time of observation of one or two individuals, male/female/unknown or pair/other, respectively.

Fig. S2: Activity budget of Apogon imberbis taken in totality (female, male, unknown) in the days of recoding, highlighting differences of their activity pattern between timeslots. LN = late night; Da = dawn; Mo = morning; Af = afternoon; Ev = evening; EN = early night.

Photographic references to the activity patterns of *Apogon imberbis* (Figures E1 – E5)

Photos may do not represent the real dimension of the frame, because they have been enlarged for better appreciation of the fish behavior.

Fig. E1 a – d: Sheltering behavior of single Apogon imberbis individual, male (a and b) and female (c and d). A. imberbis uses the branches of the gorgonian Paramuricea clavata for hiding, making it the territory to protect from intruders and the courtship arena.

Fig. E2 a, b: Wandering behavior performed by A. imberbis female, swimming around the colony with no clear direction, often out of focus because moving.

Fig. E3 a – d: Patrolling behavior, the sight of the fish is oriented towards the site where previous interactions with the substrate occurred. The fish moves in circles and keeps the eyes on the spot.

Fig. E4 a, b: Bottom interactions of single individual, whose gender is unknown. The white arrow indicates the fish, which is in a vertical position with head down and tail up.

Fig. E5: Parallel circling during courtship. The fish change orientation by keeping a parallel position to each other, while turning in circles. The female is on the left of the photo, keeping the outer side of the circle.

Fig. E6: Crossing of caudal fins during courtship to form a "X" shape in a sort of "tail-to-tail" position. The female is on the left of the photos.

Fig. E7: Bottom interactions by the two partners during courtship. White arrows indicate the two fish.