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First direct evidence of reproductive behaviour of the white grouper, *Epinephelus aeneus* (Geoffroy Saint-Hilaire, 1817)

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Abstract

This study describes the first ever reported direct observation of a pair spawning event and the reproductive liveries of the white grouper, *Epinephelus aeneus*. Spawning took place on a rocky bank located in the Marine Protected Area (MPA) of Tavolara-Punta Coda Cavallo (NE Sardinia, Italy), in the Western Mediterranean Sea. In the evening of August 4th, 2018, the spawning of two large-sized individuals (~90 cm total length), displaying distinct colour patterns, was observed using SCUBA diving. This direct observation is the first record of *E. aeneus* male reproductive livery, characterized by a darker coloration on the head, the dorsal part of the body and the caudal fin. In addition, information on the environmental conditions in which reproduction occurred was collected. At the study site, relatively high seawater temperatures were recorded at the time of the *E. aeneus* spawning (24 °C at 24.7 m), as well as over the 2018 summer months (July-September), even in deep waters (>35 m), compared to previous summers. The spawning event occurred in a coralligenous-dominated seascape where fishing is prohibited, while diving activities are allowed. The site hosts abundant populations of ecologically and commercially valuable fish species (e.g., groupers, sparid fishes), with significant proportions of large-sized individuals (i.e. reproducers). Further studies are needed to advance our knowledge of the white grouper, with a particular emphasis on reproduction and the importance of implementing effective protection measures. Prioritizing management actions at key reproductive sites, such as rocky banks, is essential for ensuring the protection and/or recovery of over-exploited species.

Keywords: Spawning; reproductive colour pattern; rocky banks; Tavolara-Punta Coda Cavallo MPA; Mediterranean Sea; Marine Protected Areas.

Introduction

The white grouper, *Epinephelus aeneus* (Geoffroy Saint-Hilaire, 1817), is reported to frequently occur throughout the southern Mediterranean Sea as well as along the Atlantic coasts of Portugal, Spain, and Africa, down to southern Angola (Heemstra & Randall, 1993; Pollard *et al.*, 2018). This species was considered to be present but rare in the central Mediterranean (from Spain to central Italy) and absent in the North-Western Mediterranean Sea, as well as in the Adriatic Sea (Tortonese, 1975; Glamuzina & Skaramuca, 1999; Pollard *et al.*, 2018). Since the late 1990s, few specimens of this species have been recorded for the first time in the Adriatic Sea (Glamuzina *et al.*, 2000; Dulčić *et al.*, 2006) and, more recently, in the North-Western Mediterranean Sea (Riutort, 2012; Pollard *et al.*, 2018). The ongoing northward expansion of geographical distribution of the white grouper is reported to be related with the current sea warming (Glamuzina *et al.*, 2000; Riutort, 2012).

Epinephelus aeneus has been heavily exploited throughout the southern and eastern Mediterranean Sea and especially along the Atlantic African coast, where it is considered as over-exploited (Cury & Roy, 1988; Gascuel & Ménard, 1997; Thiao *et al.*, 2012). Current conservation measures for the species are inadequate, and those in force (minimum catch sizes, fishing-gear restrictions, limited number of fishing permits, fishing seasonal closures) are considered ineffective due to a lack, in most countries, of enforcement (Thiao *et al.*, 2012; Pollard *et*

al., 2018). Consequently, the white grouper is listed as 'Near Threatened' on the IUCN Red List (Pollard *et al.*, 2018).

The white grouper is a large, long-lived predatory fish exceeding 120 cm in maximum total length (TL) and living approximately 17 years (Bouain & Siau, 1983; Pollard *et al.*, 2018). Adults of *E. aeneus* are generally found on rocky or mud-sand bottoms in the 20-200 m depth range, while juveniles can be found in estuarine habitats (Heemstra & Randall, 1993). Like most other groupers (Sadovy De Mitcheson & Liu, 2008; Erisman *et al.*, 2009) this species is a protogynous hermaphrodite (female to male sex change) (Bruslé & Bruslé, 1976a). Individuals mature first as females at 50-60 cm TL and 5-7 years, changing sex at 80-110 cm TL and 10-13 years (Bruslé & Bruslé, 1976b; Bouain & Siau, 1983; Bruslé, 1985).

In the Mediterranean Sea, reproduction is reported to occur in the summer season in June-August, according to gonadosomatic index (GSI), gonadal histology and oocyte diameter analyses (Bruslé & Bruslé, 1976a, 1976b; Bouain & Siau, 1983; Gökçe *et al.*, 2003). Divers and fishers reported the occurrence of a *E. aeneus* spawning aggregation off Senegal in April 2008, based on indirect indicators (grouper massive migration, aggregating behaviour, and increased catchability) (Luckhurst, 2009). However, to date, no direct record of actual white grouper reproduction has ever been reported. This paper describes the first ever documented spawning event of the species.

Materials and Methods

The observation was made in the Marine Protected Area of Tavolara - Punta Coda Cavallo (TPCC MPA), located in the north-east of Sardinia, Western Mediterranean Sea, Italy. The MPA was established in 1997 but became effectively managed around 2003-04. In accordance with Italian law, TPCC MPA includes three types of zones with different levels of protection. The 'A zones', corresponding to no-take and no-access zones, are surrounded by the 'B and C zones', corresponding to buffer zones where human uses are variously restricted: in B zones, only licensed local artisanal fishing and diving are allowed; in C zones, recreational fishing is also allowed. Spearfishing is prohibited throughout the whole MPA (Fig. 1).

Within the framework of a larger study aimed at investigating the reproductive behaviour of the dusky grouper, *Epinephelus marginatus,* a site located within the B zone of TPCC MPA was regularly monitored between June and September 2018, using SCUBA diving (28, 39 and 40 dives were conducted after sunrise, during daytime, and before sunset, respectively). During all dives, depth and seawater temperature data were retrieved from the dive computer. The monitored site consists of two coralligenous rocky banks (i.e., undersea mountain-like structures) off the eastern side of Tavolara island. These two rocky banks, named 'Papa 1' (40.91533° N; 9.74696° E) and 'Papa 2' (40.91558° N; 9.74775° E), consist of two groups of pinnacles rising from a coarse sandy bottom from approximately 45 m up to 15 m (Papa 1) and 24 m (Papa 2). These limestone pinnacles, rich in boulders and crevices, are covered by coralligenous formations, characterized by the widespread presence of sea fans (*Paramuricea clavata* and *Eunicella* spp.). These rocky banks also host the typical Mediterranean fish fauna associated with rocky reefs. In particular, large-sized individuals of commercially valuable fish species can be found in significant numbers, including dusky grouper (*E. margina-*

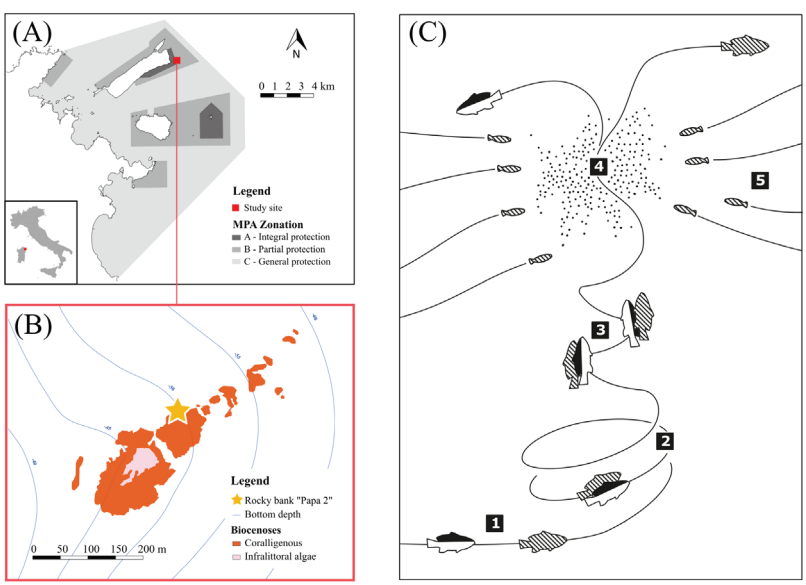

Fig. 1: (A) Tavolara – Punta Coda Cavallo MPA zonation; (B) Biocenotic map of 'Papa' rocky banks; (C) Illustration of the pair spawning behaviour of the white grouper, *Epinephelus aeneus*: 1) the bicolor male follows the female; 2) the individuals, side by side, perform horizontal circles; 3) ascent by the individuals, whose movements trace the vertical side of an imaginary cylinder; 4) release of sperm and eggs, and faster return of the male compared to the female to the mid-water; 5) fishes (*Boops boops*) prey on the eggs. The marine habitat mapping was produced in 2012 by Andromède Oceanologie, within the frame of the PIM (Petites Iles de Méditerranée) project. Graphics edited by L. Drago.

tus), white seabream (*Diplodus sargus*), common dentex (*Dentex dentex*), and brown meagre (*Sciaena umbra*) (Di Franco *et al.*, 2009; Sahyoun *et al.*, 2013). Solitary individuals or schools of pelagic fish can also be occasionally observed, including Atlantic bonito (*Sarda sarda*) and greater amberjack (*Seriola dumerili*). Around these rocky banks, diving activities are allowed, whereas fishing is prohibited within a radius of 100 m. The surveys were conducted within the 15–35 m depth range. During the whole summer, at least three adults of *E. aeneus* 85-90 cm long (TL) were observed at the rocky banks. Between July and early September, water temperatures were recorded with a ±0.5 °C resolution every hour by two sensors (iButton® devices, type DS1922L, Maxim Integrated Products, Inc), deployed at Papa 1 rocky bank at 20.5 m (July 12 – September 8) and 36/39.2 m of depth (August 1 – September 8).

Results

On August 4th 2018, during an underwater survey, the spawning of a couple of white grouper of similar size (around 90 cm TL) was observed at Papa 2 rocky bank. The spawning event was documented at 7:02 p.m., an hour and a half before sunset (day length: ~14 hours). While divers were hovering at 24.7 m depth, they observed the spawning taking place at an estimated depth of 20 m, with a sea bottom depth of 45 m. Digital photos and videos were recorded only immediately after the spawning event. The two mating individuals were characterized by two distinct liveries: the typical barred and the bicolor patterns (Fig. 2).

In the bicolor pattern, the upper head, the upper edge as well as the anterior half of the caudal fin, and the upper anterior border of the soft dorsal fin, were distinctly dark, while the belly, lower sides, lips, and the remaining parts of all fins were white. The typical color and the bicolor patterns can be presumably attributed to a female and a male, respectively, since the latter exhibited courtship behaviour towards the former. The courtship, which occurred both before and after the spawning event and lasted at least 1-2 minutes, consisted of the bicolor fish swimming closely behind the female. The spawning event involved a horizontal circular swim by the couple, followed by the ascent of the individuals side by side into the water column, culminating in the release of sperm and eggs, which small-sized silvery fish, probably *Boops boops*, preyed upon. After spawning, the male rapidly returned towards the rocky bank pinnacle in the mid-water (Fig. 1), while the female stayed almost still at 21.8 m depth for several minutes just above the rocky bank, before being followed by the male without any further ascent (supplementary video 1). The seawater temperature recorded by the dive computer at the observation depth (24.7 m) was 24 °C and there was a third quarter moon. At the thermocline depth (26 m), the temperature was 20 °C.

A couple of white groupers, characterized by the two distinct colour patterns and not displaying any reproductive-related behaviour, was also observed on two other occasions at Papa 2 rocky bank: on July 28th in the evening (7:12 p.m.; supplementary video 2), and on August 5th in the morning (around 7:30 a.m.). We cannot be sure whether or not these groupers were the same individuals observed reproducing on the evening of August 4th.

With respect to the hourly water temperatures recorded at Papa 1 over summer, temperatures ranged from a minimum of 18 °C to a maximum of 27.5 °C in shallower waters (20.5 m), and from 16.1 °C to 27.1 °C in deeper waters (36/39.2 m) (Supplementary Fig. S1 and S2). Considering only the period in which the spawning event and the male reproductive livery were observed, hourly temperatures ranged from a minimum of 22.5 °C to a maximum of 26.5 °C at 20.5 m, and from 16.6 °C to 18.1 °C at 39.2 m (Supplementary Fig. S1 and S2).

Discussion

This study documents for the first time the direct observation of a reproductive event of the white grouper. Moreover, it corroborates the previously reported reproductive season of the white grouper in the Mediterranean Sea, based on gonadal histology and GSI data (Bruslé & Bruslé, 1976a, 1976b; Bouain & Siau, 1983; Gökçe *et al.*, 2003). Witnessing the spawning event provides evidence

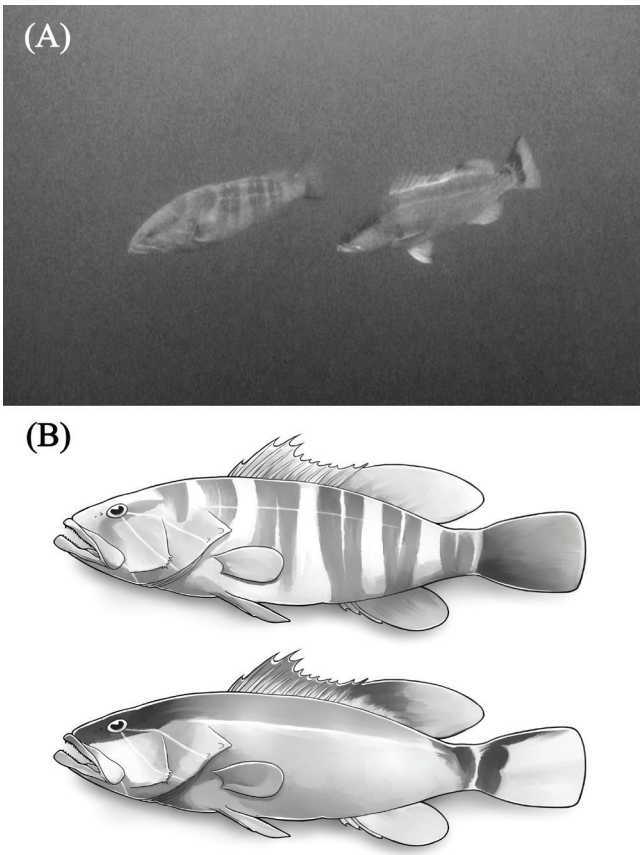

Fig. 2: (A) Frame extracted from the video taken after the spawning event was observed; (B) Colour patterns displayed by the apparent female (top) and male (bottom). Grouper silhouette redrafted from Bruslé, 1985. Graphics edited by L. Drago.

that the bicolor pattern, which has never been reported before, is a livery associated with spawning, and as such, it can be considered as an indirect indicator of reproductive activity. Therefore, the other two observations of bicolour specimens reported in this study suggest that more than one reproductive event may have occurred over a short time interval (within 10 days).

For many grouper species, spawning is clearly related to abiotic factors such as seawater temperature, day length and lunar phase (Sadovy de Mitcheson & Colin, 2012; Starr *et al.*, 2018). No robust literature data are available for establishing whether this is also the case for the white grouper. Luckhurst (2009) reported that white groupers were observed participating in a putative spawning aggregation off Senegal, based on indirect evidence (massive migration to the site, extraordinary density and easy catches, and almost impassive behaviour towards divers). This aggregation occurred several days after the full moon and fish were most numerous close to the bottom (28 m, 16 °C), below the thermocline. It is known that the species performs seasonal vertical migrations, possibly in relation to the thermocline depth (Domain, 1980; Bruslé, 1985). Specifically, it has been hypothesized that *E. aeneus* moves to deep waters to spawn in the eastern Mediterranean Sea and along the western African coasts, suggesting a relationship between low temperature values and *E. aeneus* spawning (Bruslé, 1985). The optimal thermal range reported for this species is 15-24 °C, with a preference for temperatures below 20 °C (Bruslé, 1985). Concerning our study, the summer of 2018 was particularly warm, with unprecedently high seawater temperatures, even in deep water, at both Papa 1 and 2 (Supplementary Fig. S1 and S2). Our observation does not offer an adequate basis, however, for drawing any definitive conclusion regarding the importance of temperature in determining white grouper spawning. Despite this, previous sampling data as well as reports from the local community of divers emphasize the exceptional nature of having observed not only the spawning event, but also more than one individual of *E. aeneus* over summer, especially considering that fish were present at relatively shallow depths (15-25 m). Further studies would help to better characterize white grouper reproductive behaviour and to determine what are the main environmental conditions affecting its spawning. Further studies would also help support the design and implementation of effective management measures, such as fishing restrictions or closures, at reproductive sites. The recent assessment of the conservation status of the white grouper reported that the management measures currently in place in the southern and eastern Mediterranean Sea (Pollard *et al.*, 2018), as well as off the African coast (Thiao *et al.*, 2012), are ineffective in mitigating the decline of the species (Pollard *et al.*, 2018). The observation described in the present study was made at a very peculiar site located in the partially protected zone of TPCC MPA, open to diving activities but closed to fishing. Our finding does not allow any conclusions to be drawn regarding whether or not this species is effectively recruiting in the area. It is worth noting, however, that the spawning events occurred just at a no-take rocky bank, which could suggest that spawning of this large predatory fish may be favoured by a combination of habitat suitability and protection measures (Sahyoun *et al.*, 2013; La Mesa *et al.*, 2006;1834 Erisman *et al.*, 2017).

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