# First records of dusky grouper *Epinephelus marginatus* settlement-stage larvae in the Ligurian Sea.

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#### **ABSTRACT**:

The presence of dusky grouper *Epinephelus marginatus* settlement-stage larvae in the Ligurian Sea is reported. *Epinephelus marginatus* populations are slowly recovering along the french Mediterranean coast since the establishment of a moratorium on spearfishing in 1993 but still remain spatially restricted to marine protected areas. The occurrence of *Epinephelus marginatus* larvae in a region where effective settlement had not been detected for decades is a new observation supporting the species' recovery.

**KEY WORDS**: *Epinephelus marginatus*; Epinephelidae; settlement-stage larvae; Ligurian Sea; NW Mediterranean Sea.

# Introduction

The dusky grouper *Epinephelus marginatus* (Lowe 1834) is distributed from Argentina to the Black Sea, although most abundant along the Brazilian coast and throughout the Mediterranean Sea (Heemstra and Randall, 1993). The species inhabits rocky bottoms almost exclusively, from shallow waters down to 200 m depth (Begossi and Silvano, 2008), with higher concentrations above 50 m depth when unfished (Harmelin and Harmelin-Vivien, 1999). Like other serranids, the dusky grouper is a protogynous hermaphrodite species, with individuals maturing at five years old into females and sex reversal around 9-17 years old for individuals of 80-90 cm in total length (TL) (Andrade et al., 2003; Harmelin and Harmelin-Vivien, 1999;

Marino, 2001; Reñones et al., 2010). Reproduction occurs during summer, in spawning aggregations (Reñones et al., 2010). The species is highly territorial, and shows high site fidelity with limited horizontal movements (usually < 1 km) (Pastor *et al.*, 2009); dispersal of adults is therefore assumed to be negligible and may occur mostly during the larval and juvenile phases (Chauvet and Francour, 1990).

In the Mediterranean Sea, groupers are among the most emblematic top-predator species (Prato et al., 2013). Their large size and fighting abilities made them of particular interest for sport-fishing (Coll et al., 2004) but, because of their slow growth and maturing, grouper populations are particularly sensible to overfishing (Coll et al., 2004). At few locations in the northwestern Mediterranean Sea, the species has even been completely depleted (Coll et al., 1999). The establishment of marine protected areas (MPAs) in the region has proved to be efficient to protect grouper populations, where they display significantly higher abundances and larger individuals (Guidetti et al., 2008; Hereu et al., 2006; Koeck et al., 2014; Pastor et al., 2009). In some countries, including France, the establishment in 1993 of a moratorium on spearfishing of groupers allowed their assemblages to increase both within and outside MPAs (Ganteaume and Francour, 2007); the current moratorium ends in 2023 (http://www.gemlemerou.org).

While recent studies using acoustic telemetry have been able to detect reproductive behaviors in grouper populations (Koeck et al., 2014; Pastor et al., 2009), actual observations remain scare. Before the 1990s, no successful recruitment occurred in the NW Mediterranean (Chauvet and Francour, 1990). It was assumed that new, small (< 40 cm TL) individuals reached the French Mediterranean coast through short-distance migrations during their juvenile phase only, originating from two southern populations (the Moroccan population for the western portion of the French coast and the Tunisian population for the eastern side; Chauvet and Francour 1990; Bodilis *et al.* 2003a). Since 1996, the locations of few breeding sites have been confirmed in the NW Mediterranean, in the north of Spain and in Corsica, while others were suspected in the MPAs of Cerbère-Banyuls and Port-Cros (Bodilis et al., 2003a; Hereu et al., 2006; Marinaro et al., 2005). Nevertheless, no settlement-stage larva of the species had been collected in the Ligurian Sea yet.

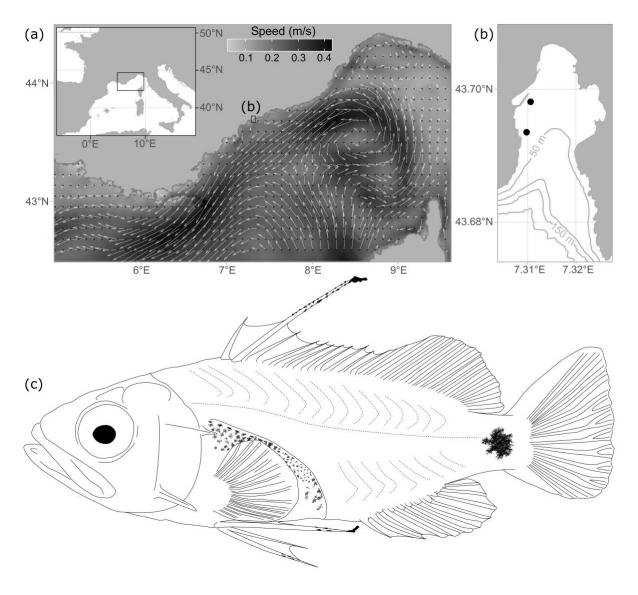
# **Records and methods**

This short note records the catches of two specimens of *E. marginatus* settlement-stage larvae in the Ligurian Sea, measuring 23 and 25 mm TL. The two individuals were collected in the Bay of Villefranche-sur-Mer (43.69°N, 7.31°E; Fig. 1a,b), on August 19<sup>th</sup> and 22<sup>nd</sup> 2014, respectively six and three days before the new moon.

The Bay of Villefranche-sur-Mer is open to oceanic waters (bottom depth drops to > 300 m at the mouth of the bay), and comprises rocky substrata on its sides and *Posidonia oceanica* meadows in the inner (northern) part. Fish larvae were collected with CARE light traps (Lecaillon, 2004). They are composed of a buoyant block with a 55 W LED light and a 2 m conical net made of 2 mm PVC mesh with a funnel in the middle. Settling larvae of many coastal fish species are attracted to light and descend in the net in search of a settlement substrate. Moorings were placed at two sites separated by over 450 m, above *P. oceanica* beds, with bottom depth between 10 m and 20 m (Fig. 1b). Light traps were set one hour before sunset (7:00 PM) and retrieved one hour after sunrise (8:00 AM). After collection, all fish larvae were sorted visually in 30 L buckets. After being identified as Serranidae, and potentially groupers, the two larvae were placed together in a dedicated aquarium in a temperature-controlled room

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at 20°C (slightly lower than seawater temperature measured *in situ*). Larvae were fed with wild-caught zooplankton (mostly decapod larvae) from plankton net tows. Every effort was made to minimise stress to the specimens: larvae were handled with water containers (never hand-nets) and protected from intense light and noise sources. Given their protected status, the two individuals were released at sea at their capture location after formal identification based on their morphology, pigmentation and size and shape of the dorsal spine I (i.e. about three times longer than the others and with a terminal spinelet; Cunha *et al.* 2013), as illustrated in Fig. 1c for further identification purposes of settlement-stage larvae of dusky grouper.



**Figure 1: (a)** Regional map depicting the average current in summer 2014, with flow vectors overlaid on top a linearly interpolated map of current intensity; it highlights the typical strong westward jet along the coast (source: MARS3DMed, PREVIMER). (b) Collection sites of fish larvae (dots) in the Bay of Villefranche-sur-Mer, France. (c) Morphology of wild-caught *Epinephelus marginatus* larva at settlement stage (TL=25 mm). The whole body is pigmented but only major pigments on the dorsal side of the gut, the main spines and on the caudal peduncle are represented here. The larva is characterized by a very long and pigmented dorsal spine II and pelvic spines, a large orange dot on the caudal peduncle and a strong pre-opercular spine. (Drawn from pictures of Faillettaz R. and Crec'hriou R.).

# Discussion

In the Mediterranean Sea, high abundances of top-predators, groupers in particular, are almost only found within marine reserves (Hackradt *et al.*, 2014). The reproduction site from which these larvae originated was therefore likely located in one such MPA. The two closest MPA from the Bay of Villefranche-sur-Mer are the MPA of Cap Roux (50 km westward, 445 ha, France) and the small MPA of Larvotto (20 km eastward, 14 ha, Monaco). Few scientific data are available for these MPA, but in the Larvotto MPA, the abundance of *E. marginatus* is comparable to that observed in other French Mediterranean MPAs, relatively to its size (http://www.gemlemerou.org). Large dominant males display typical mating behaviour (sensu Zabala *et al.*, 1997a) in summer since several years ago (Francour P., ECOMERS, University of Nice, pers. obs.). The dominant offshore current is westward, but with occasional and small scale reversals in the eastward direction along the coast (Andersen and Prieur 2000; Fig. 1), so larvae could therefore originate from any of these two MPA.

Little is known regarding the dispersal of *E. marginatus* larvae, although their pelagic larval duration is around 25 days (Macpherson and Raventos, 2006). Nevertheless, the larval supply of settlement-stage larvae has been monitored in the Bay of Villefranche-sur-Mer for five years on a weekly basis, since October 2012 (Faillettaz, 2015), and the fact that *E. marginatus* larvae were collected on a single year on two separate occasions, dozens of kilometres away from the closest MPA, suggests that 2014 may have been a particularly successful year for grouper reproduction.

In the NW Mediterranean Sea, most *E. marginatus* population replenishment was assumed to occur from short distance migrations of juveniles until the 2000s due to the lack of effective reproduction within local populations (Chauvet and Francour 1990; Bodilis *et al.* 2003a). New potential breeding sites have emerged in this region since then (Bodilis et al., 2003b; Hereu et al., 2006), and the present observations suggest that they may have started to replenish the local populations. These observations are in line with the general positive trend in the rebuilding of adult grouper populations observed in the NW Mediterranean (García-Rubies et al., 2013; Hackradt et al., 2014; Pastor et al., 2009), which may continue to expand beyond the limits of marines reserves following the enforcement of protection measures since the 1990s (Ganteaume and Francour, 2007).

## **Ethical declaration**

Samples were collected under permit n°36, delivered by the Direction interrégionale de la Mer Méditerranée to the Laboratoire d'Océanographie de Villefranche-sur-Mer. Ethical permission was not required for this study as purely observational work was carried out.

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