Extremely rapid withdrawal behaviour of the sea pen *Protoptilum* cf. *carpenteri* in the deep Mediterranean

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Abstract

Sea pens (Octocorallia, Pennatulacea) are a specialized and morphologically distinct group of octocorals. The majority of them have adapted to survive on soft sediments with the help of an anchoring muscular peduncle. The whip-like sea pen *Protoptilum carpenteri* is considered a deep-sea North Atlantic species, which recently has been documented also in the Mediterranean Sea, where its actual distribution and abundance are still unknown. Even less is known about its ecology and behaviour, its reactions after disturbance, and its possible escape strategies. Several species of pennatulaceans can withdraw partially or completely into the sediment, following an apparent rhythmic but unsynchronized procedure that is usually preceded by the closure of the autozooids and the expulsion of the water contained within the colony. The present study reports and discusses for the first time the extremely fast withdrawal behaviour of *P.* cf. *carpenteri* after *in-situ* disturbance.

Key words: Burrowing behaviour, Mediterranean Sea, soft bottoms, ROV, mega-epibenthic communities.

Introduction

Sessile marine organisms respond to environmental disturbance and predation by means of different adaptive strategies (Williams 2011). Sea pens (Octocorallia, Pennatulacea) are colonial cnidarians

and the majority of them are adapted to inhabit muddy and sandy bottoms of the continental shelf and slope. *Protoptilum carpenteri* Kölliker, 1872 is a deep-sea species that can live in a wide bathymetric range, between 400 and 4270 m depth, with a predominant North Atlantic distribution (Gilkinson and Edinger 2009; Mastrototaro et al. 2015). However, since its description at the end of the 18th century, only five sightings in deep-sea Mediterranean environments have been documented, which were between 400 and 800 m depth (Mastrototaro et al. 2015; Prampolini et al., 2020).

Although in the last decades a substantial increase in the exploration of the Mediterranean deep-sea environment has occurred, soft sediment environments have remained widely disregarded here (Grinyó et al. 2020). Consequently, there is a wide knowledge gap on the ecology and biogeography of deep-sea Mediterranean megabenthic species living in soft substrates. This is especially true for species showing a burrowing or withdrawing behaviour (Durden et al. 2015; Chimienti et al. 2018). Most sea pen species have the capacity to withdraw into the sediment following an apparent rhythmic but unsynchronized procedure (Langton et al. 1990). By means of few contractions of the peduncle, sea pens can expel part of the water contained within the colony withdrawing themselves partially or completely into the sediment. Although it has been suggested that food availability and light may influence pennatulacean withdrawal behaviour, most existing information based on laboratory studies is ambiguous and withdrawal triggering factors are not well understood (Buisson, 1964; Magnus, 1966). Hoare and Wilson (1977) described a possible tidal-based rhythm for *Virgularia mirabilis* while Wilson (1975), using time-lapse photography, found a 22- to 27-h rhythm for this species that was independent of lighting and tidal regime. For *Veretillum cynomorium*, Buisson (1964) documented that light was controlling the rhythm of extension and withdrawal. Magnus (1966) found a strong diurnal activity in *Scytaliopsis djiboutiensis*, which extended only at night. On the other hand, *Ptilosarcus gurneyi* expands only for feeding (Birkeland 1974) and *Echinoptilum* sp. can avoid dangerous situations such as predation via migration while being retracted inside the sediment (Kushida et al. 2020).

Observations of withdrawal behaviour *in situ* are still quite scarce, particularly in deep-sea environments (Kushida et al. 2020). The only in situ observations were reported by Birkeland (1974) for *P. gurneyi*, by Langton et al. (1990) for *Pennatula aculeata*, by Ambroso et al. (2013) for *Virgularia mirabilis*, and by Chimienti et al. (2018) for *Pennatula rubra*.

Pennatulacean withdrawal behaviour occurs at different velocities. For *P. rubra*, colony withdrawal is preceded by polyp retraction and is proved to be a slow process requiring more than 3 min to be completed (Chimienti et al. 2018). On the other hand, *V. mirabilis* has been reported to withdraw itself into the seabed in a few seconds when disturbed (Ambroso et al. 2013). The present

study reports for the first time the fastest withdrawal of *P.* cf. *carpenteri*, representing a behaviour never reported before for a sea pen.

Material and methods

Video transects were made during the research cruise ABRIC 1, from 13 to 29 February 2020, on board the *R/V Sarmiento de Gamboa*, using the ROV (Remotely Operated Vehicle) *Liropus 2000*. The main goal of this cruise was to explore and characterize deep-sea benthic habitats between 600 and 1200 m depth in the Blanes submarine canyon. Study sites were chosen based on the seafloor morphology, targeting canyon wall areas that could host habitat-forming anthozoans, such as framework building scleractinians, gorgonians and antipatharians as well soft bottom areas hosting pennatulaceans and soft corals. The ROV was equipped with a low-definition video camera for navigation and a high-definition video camera for the detailed observation during the video transects. The ROV also hosted a depth sensor, a compass, a grabber arm and two laser beams providing a 10-cm scale as reference for dimensional measurements.

Results and discussion

The withdrawal behaviour of *P.* cf. *carpenteri* was observed just one time for a single colony at 617 m depth in the east flank of Blanes Canyon (41°30'16.98"N, 2°57'2.71"W; Fig. 1), showing that this species retracts extremely fast and with its polyps fully expanded (Fig. 2; Online Resource 1). Mobility was also observed in free-living mushroom corals allowing them to escape from interactions with other organisms (Chadwick 1988; Hoeksema and de Voogd, 2012) and in solitary zoantharians by means of horizontal locomotion on sandy substratum via peristalsis (Nagabhushanam and Jothinayagam 1982). Mushroom corals can also turn themselves over and using peristalsis to shed sediment from their bodies (Bongaerts et al. 2012; Hoeksema and Bongaerts 2016). Withdrawal behaviour, peristalsis and active movements may be a somewhat common feature for anthozoans in sandy bottom environments but the reasons why some species burrows and moves are still unresolved (Kushida et al. 2020). The withdrawal behaviour of *P.* cf. *carpenteri* was observed after a disturbance caused approaching the arm of the ROV in an attempt to sample it and it was calculated by counting the number of frames per second (30 of 50 frames corresponding to 0.6 sec) it took the animal to hide (Online Resource 1).

The observed morphology of our specimen agrees well with the expanded colonies of the genus *Protoptilum* photographed and videotaped in the Mediterranean Sea, although a darker colouration is observed in the pharynx of autozooids. *Protoptilum carpenteri* is the only species of

this genus reported so far for the Mediterranean Sea (Mastrotorato et al. 2015; Altuna and Poliseno 2019). Unfortunately, the observed specimen was not available for examination.

The species delimitation in this genus is still an open question since part of the variability of characters currently in use can be found in a single colony (López-González. com. pers.). Molecular information for this genus is scarce, but suggests that species diversity is higher and that the wide geographic distributions are more restricted than those assumed today (López-González, com. pers.). Taking all this into account, and awaiting a revision of the genus, including Mediterranean forms, we prefer to maintain the species identification based on the present observations as *Protoptilum* cf. *carpenteri*.

Habitat-forming species have been recognized as important components of deep-sea ecosystems, whilst being very vulnerable to anthropogenic impacts (Auster et al. 2011). Sea pen aggregations can form important soft-bottom communities providing a three-dimensional complexity from which several associated species can benefit (Chimienti et al. 2018). They are relatively understudied and basic information on their ecology is largely lacking. Thus, there is a need to improve the data collection of sea pen species, in particular for offshore and deep areas, in order to improve the identification of Mediterranean VMEs.

The present study confirms that *P.* cf. *carpenteri* can completely withdraw into sediments within 0.6 sec. This process is similar to other species, such as *V. mirabilis*, which is able to withdraw in a few seconds (between 15 and 40 sec) (Ambroso et al. 2013). On the contrary, *P.rubra* showed a slow process requiring between 3 and 5 min for the complete withdrawal (Chimienti et al. 2018). The ability of some sea pen species (e.g. *Pennatula rubra, Pennatula aculeata, Virgularia mirabilis, Echinoptilum* sp. and *Protoptilum carpenteri*) to withdraw into the sediment should make them less sensitive to physical disturbance, such as the one caused by bottom trawling activities (Langton et al. 1990; Kenchington et al. 2011; Ambroso et al. 2013; Chimienti et al. 2018; Kushida et al. 2020).

Dominant species found in the Mediterranean bathyal mud are the sea pen *Funiculina quadrangularis* or the gorgonian *Isidella elongata* which are often found together with crustacean species, such as *Aristeus antennatus, Aristeomorpha foliacea, Parapenaeus longirostris* and *Nephrops norvegicus* (Pérès and Picard 1964). Both species *I. elongata* and *F. quadrangularis* have almost completely disappeared from the trawlable bottoms of the most Mediterranean areas (D'Onghia et al. 2011; Sardà et al. 2004). *F. quadrangularis* is one of the sea pen species that are unable to withdraw into the sediment (Mac Donald et al.1996). This shows that *F. quadrangularis* may act as an indicator of the state of health of deep-sea mud habitats and that it can be considered a Vulnerable Marine Ecosystem (VME) indicator species (Rogers and Gianni 2010). This is still

unknown for sea pens such as *P. carpenteri* due to the lack of knowledge about its distribution and abundance in the Mediterranean Sea. The low number of *P. carpenteri* colonies found until now in the Mediterranean Sea could be related to the highly patchy distribution of the species, which usually characterizes sea pens and other sessile organisms in deep-sea habitats (Carpine and Grasshoff 1975; Marshall 1988; Morris 2011).

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Author Contribution Statement: SA and JG conceived and designed research. SA wrote the manuscript. All authors read and approved the manuscript.

References

156 Altuna A, Poliseno A (2019) Taxonomy, genetics and biodiversity of Mediterranean deep-sea
157 corals and cold-water corals. In: Orejas C, Jiménez C (eds.) Mediterranean Cold-Water Corals: Past,
158 Present and Future. Springer, Cham, Switzerland, pp. 121–156.

159 Ambroso S, Dominguez-Carrió C, Grinyó J, López-González PJ, Gili J-M, Purroy A, Requena S,
160 Madurell T (2013) In-situ observations on withdrawal behaviour of the sea pen *Virgularia mirabilis*.
161 Mar Biodivers 43:257–258.

162 Auster PJ, Gjerde K, Heupel E, Watling L, Grehan A, Rogers AD (2011) Definition and detection
163 of vulnerable marine ecosystems on the high seas: problems with the "move-on" rule. ICES J Mar
164 Sci 68:254-264.

165 Birkeland C (1974) Interactions between a sea pen and seven of its predators. Ecol Monogr 44:211–
166 232.

167 Bongaerts P, Hoeksema BW, Hay KB, Hoegh-Guldberg O (2012) Mushroom corals overcome live
168 burial through pulsed inflation. Coral Reefs 31:399.

169 Buisson B (1964) Données physiologiques sur l'intégration et la polarité dans les colonies de
170 *Veretillum cynomorium* Pall. (Cnidaria, Pennatulacea). Compt Rend Hebd Séanc Acad Sci Paris
171 259:3361–3362.

172 Carpine C, Grasshoff M (1975) Les gorgonaires de la Méditerranée. Bull Inst Océanogr Monaco
173 71:1–140.

174 Chadwick NE (1988) Competition and locomotion in a free-living fungiid coral. J Exp Mar Biol
175 Ecol 123:189–200

176 Chimienti G, Angeletti L, Mastrototaro F (2018) Withdrawal behaviour of the red sea pen
177 *Pennatula rubra* (Cnidaria: Pennatulacea). Eur Zool J 85:64–70.

178 D'Onghia G, Indennidate A, Giove A, Savini A, Capezzuto F, Sion L, Vertino A, Maiorano P (2011)
179 Distribution and behavior of deep-sea benthopelagic fauna observed using towed cameras in the
180 Santa Maria di Leuca cold-water coral province. Mar Ecol Prog Ser 443:95–110.

181 Durden JM, Bett BJ, Jones DOB, Huvenne VAI, Ruhl HA (2015) Abyssal hills-hidden source of
182 increased habitat heterogeneity, benthic megafaunal biomass and diversity in the deep sea. Prog
183 Oceanogr 137:209–218.

Gilkinson K, Edinger EN (2009) The ecology of deep-sea corals of Newfoundland and Labrador waters: Biogeography, life history, biogeochemistry and relation to fishes. Canadian Technical Report of Fisheries and Aquatic Sciences 2830, VI. 136 pp.

Grinyó J, Garriga A, Soler-Membrives A, Santín A, Ambroso S, López-González PJ, Díaz D (2020) Soft corals assemblages in deep environments of the Menorca Channel (Western Mediterranean Sea). Prog Oceanogr 188:102435.

Hoare R, Wilson EH (1977) Observations on the ecology of the pennatulid *Virgularia mirablis* in Holyhead Harbor. In: Keegan, B. F., Ceidligh, P. O., Boaden, P. J. S. (eds.) Biology of benthic organisms. Pergamon Press, Oxford, p. 329–337.

Hoeksema BW, Bongaerts P (2016) Mobility and self-righting by a free-living mushroom coral through pulsed inflation. Mar Biodivers 46: 521–524

Hoeksema BW, de Voogd NJ (2012) On the run: free-living mushroom corals avoiding interaction with sponges. Coral Reefs 31:455–459

Kenchington E, Murillo FJ, Cogswell A, Lirette C (2011) Development of Encounter Protocols and Assessment of Significant Adverse Impact by Bottom Trawling for Sponge Grounds and Sea Pen Fields in the NAFO Regulatory Area. Dartmouth, NS: Northwest Atlantic Fisheries Organization.

Kükenthal WG. 1915. Pennatularia. Das Tierreich 43:i–xv. Berlin, R. Friedländer und Sohn. 132 pp.

Kushida Y, Higashiji T, Reimer JD (2020) First observation of mole-like burrowing behavior observed in a sea pen. Mar Biodivers 50(3):29.

Langton RW, Langton EW, Theroux RB, Uzmann JR (1990) Distribution, behavior and abundance of sea pens, *Pennatula aculeata*, in the Gulf of Maine. Mar Biol 107:463–469.

Mac Donald, DS, Little M, Eno NC, Hiscock K (1996) Disturbance of benthic species by fishing activities: a sensitivity index. Aquat Conserv Mar Freshw Ecosyst 6:257–268.

Magnus DBE (1966) Zur Ökologie einer nachtaktiven Flachwasser-Seefeder (Octocorallia, Pennatularia) im Roten Meer. Veröff Inst Meeresforsch Bremerhaven10(2):369–379.

Marshall AM, Fowler GH (1888) Report on the Pennatulida dredged by H. M. S. Porcupine. Trans R Soc Edinb 33:453–464.Mastrototaro F, Chimienti G, Capezzuto F, Carlucci R, Williams G (2015)

First record of *Protoptilum carpenteri* (Cnidaria: Octocorallia: Pennatulacea) in the Mediterranean Sea. Ital J Zool 82:61–68.

Morris KJ (2011) North Atlantic octocorals: Distribution, Ecology and Phylogenetics. PhD thesis. Faculty of Natural and Environmental Science School of Ocean and Earth Sciences. University of Southampton. 281 pp.

Nagabhushanam AK, Jothinayagam JT (1982) Observations on the zoanthid, *Sphenopus marsupialis* (Gmelin) (Anthozoa: Zoanthidea: Sphenopidae), along the southeast coast of India, with notes on its behaviour in aquaria. Rec Zool Surv India 80:17–26

Pérès JM, Picard J (1964) Nouveau manuel de bionomie benthique de la Méditerranée. Recueil des Travaux de la Station Marine d'Endoume 31:1–137.

Prampolini M, Angeletti L, Grande V, Taviani M, Foglini F (2020) Tricase Submarine Canyon: cold-water coral habitats in the southwesternmost Adriatic Sea (Mediterranean Sea). In: Harris P, Baker P (eds) Seafloor Geomorphology as Benthic Habitat. Elsevier, Amsterdam, pp. 793–810.

Rogers A, Gianni M (2010) The implementation of UNGA Resolutions 61/105 and 64/72 in the management of deep-sea fisheries on the high seas. International Programme of the State of the Ocean, London.

Sardà F, Calafat A, Flexas MM, Tselepides A, Canals M, Espino M, Tursi A (2004) An introduction to Mediterranean deep-sea biology. Sci Mar 68:7–38.

Williams GC (2011) The global diversity of sea pens (Cnidaria: Octocorallia: Pennatulacea). PLoS ONE 6(7):e22747.

Wilson EW (1975) Some aspects of the biology of *Virgularia mirabilis* O. F. Mfiller (Octocorallia, Pennatulaceae). M.Sc. thesis, University of Wales, Bangor, Wales, U.K.

Figure captions

Fig. 1 Map showing the location where the withdrawing behaviour of *P.* cf. *carpenteri* was observed (Eastern Blanes canyon, north-western Mediterranean Sea). Projected view (UTM Zone 31N (WGS84)) with geographic (WGS84) coordinates indicated for reference.

Fig. 2a *Protoptilum* cf. *carpenteri* on the east flank of Blanes Canyon (41°30'16.98"N, 2°57'2.71"W). **b** Close-up showing expanded polyps.

Online Resource 1 Video footage of the withdrawal behaviour of the sea pen *Protoptilum* cf. *carpenteri.*