Coral Reefs (2019) 38:1–14
https://doi.org/10.1007/s00338-018-01764-7

REPORT

Fragmentation, re-attachment ability and growth rate of the Mediterranean black coral *Antipathella subpinnata*

M. Coppari[1] · F. Mestice[1] · F. Betti[1] · G. Bavestrello[1] · L. Castellano[2] · M. Bo[1]

Received: 16 July 2018 / Accepted: 24 December 2018 / Published online: 2 January 2019

Abstract Cnidarians are known for their simple body plan and their complex life cycles, involving high regenerative and asexual-reproduction potential. In particular, several asexual reproductive strategies are known for anthozoans, including fragmentation, carried out by tentacles, by groups of polyps or by portions of colonies. Here, we report the first observation of an extensive event of fragmentation in the Mediterranean black coral species *Antipathella subpinnata* (Antipatharia: Myriopathidae) in rearing conditions. Once detached, fragments lose their polarity and new anchorages are rapidly created with polyps and cnidocysts participating in the adhesion phases. Multiple attachments are frequently observed, with new skeletal plates produced through the expansion of spines. Dendritic spines gradually arise on these new plates. Fragments start to generate numerous new branchlets orientating upward and with a fixed arrangement. In 7 months of monitoring, fragments revealed fast growth rates, up to 1.85 and 1.58 cm month^{-1}, for the whole fragments and new branchlets, respectively. Attachment of black coral fragments has never been recorded in the field; nevertheless, frequent adhesions observed in aquaria suggest that fragmentation could be a successful reproductive strategy in these anthozoans.

Topic Editor Dr. Anastazia Banaszak

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00338-018-01764-7) contains supplementary material, which is available to authorized users.

✉ M. Coppari
coppari.martina85@gmail.com;
martina.coppari@edu.unige.it

[1] Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Università degli Studi di Genova, Corso Europa, 26, 16132 Genoa, Italy

[2] Acquario di Genova, Costa Edutainment spa, Area Porto Antico, Ponte Spinola, 16128 Genoa, Italy

Introduction

Phylum Cnidaria shows an incredibly high variability of reproductive strategies (Fautin 2002); both sexual and asexual reproductions are widespread, frequently alternating in the life cycle of several species (Schiariti et al. 2008). Sexual reproduction normally occurs in stable environmental conditions, which ensure completion of gametogenesis and reproductive cycles (Foster et al. 2007) and maximize the survival of genetically diverse individuals or colonies (Miller and Ayre 2004). Contrarily, asexual reproduction is considered to be an adaptation to both unstable and unfavorable local conditions (Foster et al. 2007) allowing the temporal persistence of a species in the environment or its fast colonization after a disturbance event.

Asexual reproduction is common in anthozoans, occurring with a wide variety of strategies (Fautin 2002). Within hexacorals, asexual reproduction is particularly well represented in sea anemones, in which transverse and longitudinal fissions are commonly observed together with pedal laceration and tentacles autotomy (Bocharova 2016). These typologies of asexual reproduction have been related to environmental stress conditions (temperature and light fluctuations, desiccation, substrate instability) (Ivanova-Kazas 1977; Geller et al. 2005), or approaching neighboring individuals (Purcell 1977; Bickell-Page and Mackie 1991). In some species [e.g., *Acropora longicyathus* (Milne Edwards, 1860)], asexual reproduction takes place all year

round without any relation with external stressors (Wallace 1985). Hexacorals might also reproduce through polyp bailout, as observed for antipatharians (Miller and Grange 1997; Bo 2008; Gonçalves Ferreira 2016) in which the tentacles under stressful conditions separate from the colony forming highly mobile asexual propagules.

Both hexacorals and octocorals are also known to commonly reproduce by fragmentation (Gilmore and Hall 1976; Walker and Bull 1983; Coffroth and Lasker 1998; Acosta et al. 2001), which is the separation from the coral colony of a living portion that, after re-attachment, produces a new colony. In the gorgonian *Junceella fragilis* (Ridley, 1884), fragmentation starts with polyps' resorption in a small area (0.5–1 cm wide) of the subterminal region of the colony, followed by a terminal phase in which the daughter colony, connected only by the hair-like axial core to the mother, detaches (Walker and Bull 1983). Newly generated fragments (about 9 cm long) fall close to the mother colonies and actively lift themselves to an upright position during holdfast formation. The unbranched whip shape of this species avoids competition between mother and daughter colonies that grow close to each other forming dense meadows (Walker and Bull 1983). In some gorgonians of the genus *Plexaura*, fragmentation is considered extremely common and, in some cases, 94% of the populations develop in this way (Lasker 1984, 1990).

The occurrence of fragmentation has been hypothesized also for black corals (reviewed in Wagner et al. 2012) based on the evidence that the terminal branches in various Hawaiian ramified antipatharians and the apical part of Indonesian whip *Stichopathes* and *Cirrhipathes* colonies are commonly subjected to current-induced breakages and subsequent regeneration (Grigg 1964; Bo et al. 2009). Moreover, Montgomery (2002) has observed loose natural fragments around the parent colonies, and the study of the genetic population structure of *Antipathella fiordensis* (Grange, 1990), in New Zealand, has found that genetic clones are common in this species and occur over very long geographic distances (over 60 km), suggesting a role of asexual reproduction in antipatharians (Miller and Grange 1997; Miller 1998). Different transplanting experiments have also been performed with black corals suggesting, overall, relatively high survival rates of fragments (González et al. 1997; Montgomery 2002; Bo et al. 2009).

Up to now, fragments attachment has not been observed for any antipatharian species. This study documents for the first time the occurrence of fragment attachment in adult colonies of the Mediterranean black coral species *Antipathella subpinnata* (Ellis and Solander, 1786) in reared conditions. The re-settlement of fragments and the formation of new anchorages on various substrates are highlighted with particular regard to the polypar and skeletal rearrangements. The growth rates of newly settled fragments and the growth patterns of new branchlets are also described.

Materials and methods

Description of the target species

Antipathella subpinnata is an Atlantic-Mediterranean species living on hard substrates from 51 to 600 m, but mostly found between 65 and 200 m depth (Fig. 1a) (Bo et al. 2008; Gaino and Scoccia 2010). It is a common component of the lower fringe of the circalittoral twilight environment (Bo et al. 2008) where it can reach high densities, creating forest-like aggregations (Bo et al. 2008; de Matos et al. 2014). *A. subpinnata* is an arborescent species reaching more than 1 m in height and is characterized by long, numerous and flexible ramifications. The major branches converge basally in a single stem, while ramifications hold branchlets of different length, irregularly arranged in 1–4 rows and oriented upward with a 30°–45° angle, reaching a density of 2–3 branchlets cm^{-1} (Bo et al. 2018). Distal branchlets show an alternate biserial arrangement, whereas the highest-level ramifications can be monoserially arranged (on average 0.20 mm apart, author's pers. comm.). As for other myriopathids, the skeleton is characterized by the presence of dendritic spines at the stem's base and anchorage (ranging between 100 and 500 µm high, authors' pers. comm.), of needle-like cylindrical spines on the stem (ranging between 200 and 300 µm high) and of simple, triangular spines on the branchlets (ranging between 100 and 160 µm high). Polyps are white, monoserial and as numerous as 8–10 cm^{-1} in adult colonies (Bo et al. 2018).

Sampling and rearing conditions

The study considers five adult colonies of *A. subpinnata*. One colony sampled on December 2016 in Portofino (44°17.63′N; 9°13.27′E, East Ligurian Sea) at 67 m depth by technical divers. The other four colonies sampled on the 8th of August 2017 in Bordighera (43°46.11′N; 7°40.82′E, West Ligurian Sea) at 63 m depth by technical divers. These latter specimens (varying in size between 50 and 70 cm) are fertile females, while the 50-cm-tall colony from Portofino is unfertile during all the study period. Colonies are kept in cooler tanks filled with seawater collected at 60 m depth with the use of a 10-L Niskin bottle operated manually. Samples are then transported to the Genoa Aquarium (Acquario di Genova) facilities where they are slowly acclimatized to 16 °C and then moved into a 1860-L tank of a 6 fiberglass tanks system, called Holding A (total volume of 14 m^3). The Holding A Life

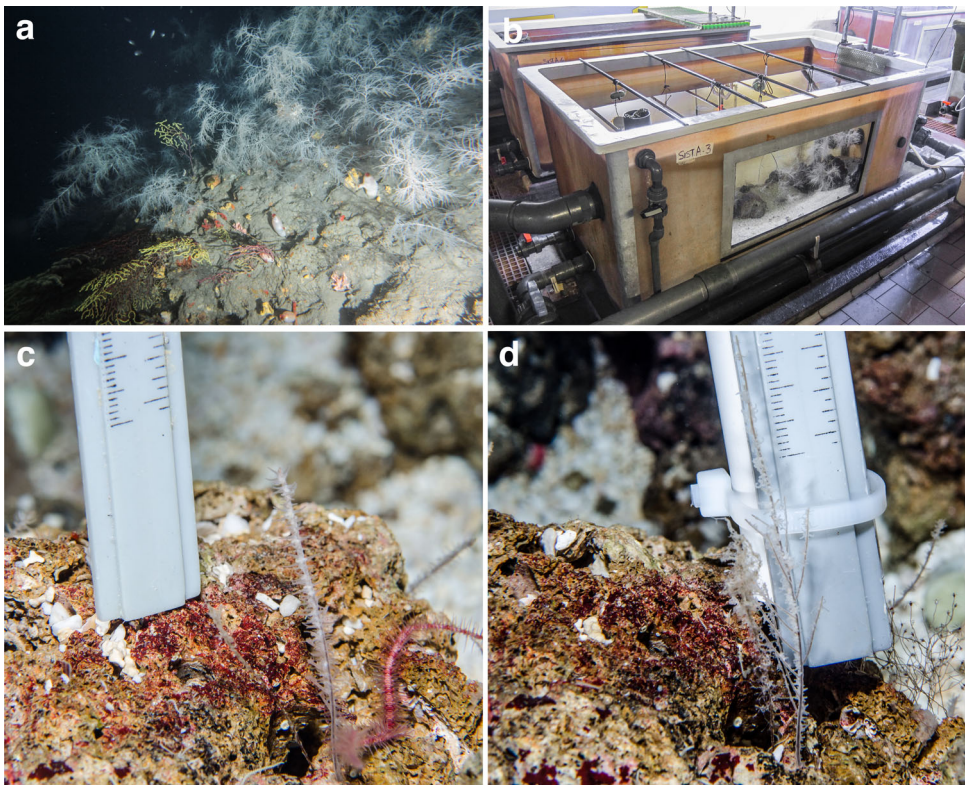

Fig. 1 A Forest of *Antipathella subpinnata* at one of the sampling sites, Bordighera, 63 m depth (**a**). Acquario di Genova facilities: the 1860-L tank where the colonies were reared. Colonies are visible inside the tank, hanged upside-down (**b**). Detail of one of the fragment with the caliper on its side after 2 months, M2 (**c**) and after 4 months, M4 (**d**)

Support System is composed by: 2 pumps, sand filter, UV sterilization, biological filtration and heat exchanger. The colonies are hung upside-down from the basal stem (Fig. 1b) and are initially kept for 4 weeks at 16 °C, and then the temperature is slowly lowered to 14.5 °C, the average ambient temperature at the collection depth. A submersible pump produces current inside the tank (Tunze-Turbelle® stream 6125). Substrate is composed mainly by small coralligenous rocks (*Lithothamnion* sp.) and small carbonatic grains. The tank is covered by a fence to reduce light intensity, and the photoperiod is 12 h day and 12 h night. Feeding occurs three times a day with a juice of minced mussel and a mixture of the microalgae *Tetraselmis* sp., *Artemia* nauplia and dry *Spirulina* sp. algae, in the morning, midday and afternoon, respectively. During feeding periods, the tank is isolated from the system for half an hour in order to maintain a high food concentration and optimize the possibility of polyps' feeding while avoiding changes in the water parameters.

A small glass container (5 L) is used in parallel to the main tank to observe the attachment phase of one fragment to the glass wall from a privileged backside point of view.

Long-term monitoring

The monitoring period lasts 7 months, from August 8, 2017 to March 19, 2018. Colonies are monitored every day to check for different biological events, including spawning, feeding and fragmentation.

Of all the fragments produced in the study period, 16 are tagged and monitored (Table 1). All the fragments are

Table 1 Height (cm) of the fragments at the beginning of the sampling period (initial measurement, M0, performed on the August 30, 2017)

Fragment code	Height (cm) M0
1	4.06
2	3.49
3*	3.44
4	2.02
5	1.88
6	8.62
7	6.73
8	1.64
9	1.24
10	4.28
11*	4.49
12	4.81
13	0.26
14	2.80
15	1.00
16	1.50

*Indicates the fragments collected for SEM analysis

naturally produced, except for fragment no. 6, which is mechanically induced. Fragments are photographed and measured every 15 days; measurements are then converted to a monthly growth rate (expressed as mean ± standard deviation (SD), cm month^{-1}). A Nikon D7000 equipped with 60-mm macrolens in an Isotta housing, with two Inon Z240 strobes is used to obtain high-resolution photographs. Fragments are photographed with a caliper on their side in order to measure their initial overall height, linear length and growth rate (Fig. 1c, d). Of the 16-tagged fragments, seven are unbranched; therefore, their growth rate is calculated as a variation in the linear length (Table 2). For the remaining 11 fragments, showing a branched habitus, the growth rate is based on the length variation of 2–6 pre-existing branchlets for each sample (for a total of 16 studied ramifications) (Table 2).

Pictures are also used to study the formation and development pattern of newly formed branchlets and to calculate their growth rate. In this latter case, as a standard procedure, five new branchlets per eight selected fragments are measured during the 7-month study for a total of 40 monitored ramifications (Table 3). The free software Fiji-ImageJ is used for this purpose (Schindelin et al. 2012).

Fragments nos. 3 and 11 are sampled, respectively, after 2 (M2) and 6 months (M6) from the beginning of the experiment (Table 3). Fragments are preserved in 95% ethanol and morphological analyses are carried out under stereomicroscopy. We monitor the morphological modifications of the polyps involved in the adhesion and the subsequent formation of new anchorages, growth of new branchlets and skeletal modification of the spines. Fragments are cleaned with gentle rinses in diluted sodium hypochlorite to remove coenenchyme. Naked skeletons are

Table 2 Growth rate (cm month^{-1}) of the fragments or pre-existing branchlets during the study period

Fragment code	Number of existing branchlets	M0 Initial size (cm)	M1 Δ (cm)	M2 Δ (cm)	M3 Δ (cm)	M4 Δ (cm)	M5 Δ (cm)	M6 Δ (cm)	M7 Δ (cm)	Av. ± SD (cm)
1	/	4.15	0.23	0.46	0.81	0.92	0.38	0.71	0.74	0.61 ± 0.25
2	i	3.58	1.07	0.85	0.66	1.85	0.90	0.94	0.11	0.91 ± 0.52
	ii	0.52	0.45	0.95	1.02	1.14	1.33	0.24	0.96	0.87 ± 0.39
3*	i	0.85	0.45	–	–	–	0.63	0.11	–	0.40 ± 0.26
	ii	1.47	0.71	–	–	1.20	0.90	0.61	0.22	0.73 ± 0.36
	iii	0.22	0.34	0.77	0.67	0.44	0.69	*		0.58 ± 0.18
4	/	2.10	0	0	0	0	0	0.10	0.16	0.04 ± 0.07
5	/	1.96	0.30	0.04	0.79	0.10	0.26	0.81	0.47	0.40 ± 0.31
6	/	8.72	0	0	0	0	0	0	0	No growth
7	i			**1.03**	0.15	0.10	0.13	0.26	0.04	0.14 ± 0.08
	ii			**5.8**	0.15	0.10	0.20	0.12	0.06	0.13 ± 0.05
	iii			**1.94**	0.30	0.29	0.03	–	–	0.21 ± 0.15
8	i	1.52	0.30	0.04	0.39	0.12	0.24	0.21	0.17	0.21 ± 0.12
	ii	1.58	0.56	–	–	–	0.26	0.20	0.05	0.27 ± 0.22
9	/	1.26	1.2	0.65	0.83	0.85	0.72	1.17	0.17	0.80 ± 0.35
10	/				**4.22**	0.20	–	0.92	0.12	0.41 ± 0.44
11	/	4.49	0.29	*						ND
12	i			**1.19**	0.37	1.06	0.88	1.15	1.65	1.02 ± 0.46
	ii			**1.30**	0.79	0.59	1.31	1.50	1.20	1.08 ± 0.38
	iii			**0.76**	0.71	0.77	0.70	1.45	1.57	1.04 ± 0.43
	iv			**1.12**	–	–	1.37	0.98	1.07	1.14 ± 0.21
	v			**1.27**	0.78	1.37	0.83	–	1.42	1.10 ± 0.34
	vi			**0.41**	0.35	0.72	0.73	0.79	1.31	0.78 ± 0.34

'/' Indicates that the fragment is unbranched; '–' indicates the lack of measurement. '*' indicates the fragments collected for SEM analysis. Monthly measurements are indicated with a code, from M0 to M7. Under M0 are reported the initial sizes (linear length) of either the unbranched fragments or the considered branchlets per fragments. Columns from M1 to M7 show the increment of growth in time (Δ). Av. ± SD indicates the average growth rate and the corresponding standard deviation. Bold characters indicate the initial size (linear length) of late-reported new fragments. ND indicates that the average value and SD could not be determined

Table 3 Growth rate (cm month^{-1}) of the newly formed branchlets during the study period

Fragment code	Number of new branchlets	M0	M1	M2 Initial size (cm)	M3 Δ (cm)	M4 Δ (cm)	M5 Δ (cm)	M6 Δ (cm)	M7 Δ (cm)	Av. ± SD (cm)
1	i	–	–	**0.09**	0.49	0.73	0.41	0.63	0.12	0.48 ± 0.23
	ii	–	–	**0.17**	0.49	0.70	0.33	0.43	0.16	0.42 ± 0.20
	iii	–	–	**0.20**	0.45	0.55	0.60	0.47	0.08	0.43 ± 0.21
	iv	–	–	**0.19**	0.50	0.39	0.55	0.40	0.10	0.39 ± 0.17
	v	–	–	**0.23**	0.76	0.36	0.77	0.27	0.21	0.47 ± 0.27
2	i	–	–	**0.15**	0.63	1.11	0.72	0.79	0.08	0.66 ± 0.37
	ii	–	–	**0.13**	0.61	0.90	0.85	0.91	0.08	0.67 ± 0.35
	iii	–	–	**0.08**	0.40	0.48	0.72	0.98	0.15	0.55 ± 0.32
	iv	–	–	**0.27**	0.81	0.93	0.75	0.91	0.16	0.71 ± 0.32
	v	–	–	**0.15**	0.65	1.14	0.96	0.71	0.10	0.71 ± 0.39
3*	i	–	–	**0.05**	0.57	0.39	0.53	*		0.50 ± 0.09
	ii	–	–	–	**0.07**	0.31	0.59	*		0.45 ± 0.20
	iii	–	–	–	**0.23**	0.40	0.42	*		0.43 ± 0.01
	iv	–	–	–	**0.25**	0.42	0.46	*		0.44 ± 0.03
	v	–	–	–	**0.10**	0.30	0.23	*		0.27 ± 0.05
5	i	–	–	–	**0.16**	0.37	0.18	0.23	0.22	0.25 ± 0.08
	ii	–	–	–	**0.17**	0.65	0.13	0.12	0.12	0.26 ± 0.26
	iii	–	–	–	**0.24**	0.37	0.13	0.20	0.33	0.26 ± 0.11
	iv	–	–	–	**0.28**	0.31	0.28	0.33	0.25	0.29 ± 0.04
	v	–	–	–	**0.13**	0.31	0.27	0.31	0.39	0.32 ± 0.03
8	i	–	–	–	**0.19**	0.45	0.25	0.30	0.06	0.26 ± 0.16
	ii	–	–	–	**0.10**	0.24	0.14	0.18	0.11	0.17 ± 0.06
	iii	–	–	–	**0.14**	0.24	0.18	0.18	0.11	0.18 ± 0.05
	iv	–	–	–	**0.08**	0.23	0.16	0.18	0.07	0.16 ± 0.07
	v	–	–	–	**0.08**	0.16	0.25	0.20	0.06	0.17 ± 0.07
9	i	–	–	–	–	**0.19**	0.33	0.34	0.45	0.38 ± 0.07
	ii	–	–	–	–	**0.12**	0.26	0.28	0.18	0.24 ± 0.03
	iii	–	–	–	–	**0.09**	0.21	0.32	0.17	0.23 ± 0.08
	iv	–	–	–	–	**0.06**	0.43	0.28	0.40	0.37 ± 0.08
	v	–	–	–	–	**0.14**	0.30	0.28	0.17	0.25 ± 0.07
10	i	–	–	–	**0.12**	0.22	0.29	0.73	0.08	0.33 ± 0.28
	ii	–	–	–	–	**0.18**	0.34	0.31	0.16	0.27 ± 0.10
	iii	–	–	–	–	**0.19**	0.39	0.32	0.18	0.30 ± 0.10
	iv	–	–	–	–	**0.11**	0.35	0.26	0.17	0.26 ± 0.09
	v	–	–	–	–	**0.14**	0.21	0.53	0.13	0.29 ± 0.21
12	i	–	–	**0.16**	0.38	0.44	1.05	0.42	1.58	0.78 ± 0.53
	ii	–	–	**0.03**	0.55	0.54	1.01	0.40	0.52	0.60 ± 0.24
	iii	–	–	–	**0.21**	0.42	0.66	0.72	1.08	0.72 ± 0.27
	iv	–	–	–	**0.21**	0.46	0.73	0.88	1.06	0.78 ± 0.25
	v	–	–	–	**0.05**	0.55	0.53	0.51	0.69	0.57 ± 0.08

'–' Indicates the absence of new branchlets. '*' Indicates the fragments collected for SEM analysis. Monthly measurements are indicated with a code, from M0 to M7. Under M2 are reported the initial sizes (linear length) of the newly formed branchlets. Columns from M3 to M7 show the increment of growth in time (Δ). Av. ± SD indicates the average growth rate and the corresponding standard deviation. Bold characters indicate the initial size (linear length) of late-reported new branchlets

then positioned on stubs successively coated with gold–palladium in a Balzer Union evaporator and examined with a Philips EM 515 Scanning Electron Microscope (SEM). To study the intact coenenchyme of the settled portions, some portions of the fragments are washed with distilled water, dehydrated in a graded ethanol series and dried with

the Critical Point Dryer before proceeding with evaporator and SEM analysis.

Results

Fragmentation

At the beginning of the study, fragmentation is observed only from the four fertile female colonies of Bordighera. Fertility is evident also in the fragments due to the pinkish coloration of polyps, as already described for this species, as well as other black corals (Bo et al. 2008, 2015). Fragmentation starts few days after the arrival of the colonies, on the 13th of August; all detached pieces, ranging from 1 cm to up to 8.62 cm long (Table 1), fall and spontaneously re-attach to the substrate. After 1 month, many fragments are observed on the bottom of the tank and after 7 months monitoring, over 30 fragments are counted, only two coming from the unfertile colony.

The fragments re-attach on three different types of substrate: biogenic coralligenous rocks and small-sized carbonatic grains (most commonly) as well as glass (in one case). Half of the fragments are on coralligenous rocks and the other half on small carbonatic grains.

Detached fragments may settle in a vertical position, horizontally on the substrate and/or in an upside-down position (Fig. 2a–c).

Adhesion

Polyps play a key role in the first adhesion phase (Fig. 3a). The polyps in contact with the substratum widely flatten adhering with the mouth and embracing the substrate with the tentacles (Fig. 4a, b). This is especially evident in polyps placed at the apex of the branchlets. SEM footage shows the occurrence of a conspicuous nematocysts employment along the borders of the developing basal plate together with the release of a large quantity of flocculating unidentified material, tentatively identified as mucous (Fig. 4c–e). Among the identified nematocysts, we find basitrich isorhizas about 10 * 3 μm (length * width) (Fig. 4e). During this phase, the skeleton of the ramification starts to expand setting the base for a new basal plate. This phenomenon is clearly visible in the specimen attached to glass (Fig. 3).

Generally, fragments re-attach in one site; however, multiple new anchorages are observed for pieces landing horizontally on small cobbles, showing emerging tiny anchorages from all polyps touching the grain (Figs. 2a–c, 5a–c). Instead, when fragments attach to big coralligenous rocks (or glass), they create a single anchorage. The only exception is fragment no. 3, which is attached to the substrate with lateral branchlets by means of multiple anchorages (Fig. 2a).

On a smaller scale of observation, various modifications occur at the skeletal level as demonstrated by the SEM footage. In all the adhesion sites, the portion of ramification, whether apical (Fig. 5d, e) or central (Fig. 5f), completely loses its original shape during the fusion. In the case of adhesion along the central portion of a branchlet, it is possible to depict various phases: spines initially become rounded at their tip (Fig. 5g), produce new abundant skeletal material that flattened around the tip (Fig. 5h) and fuse with adjacent spines' expansions as well as substrate, creating the basal plate (Fig. 5i, j).

Skeletal modifications occur also at the anchorage level: initially, the basal plate is a very thin and transparent sheet of golden-brown skeleton (Fig. 3b). In the backside view, overlaying coenenchyme and polyps are visible through the translucent skeletal material together with mesenterial filaments and acontiae at the base of the gastric cavities

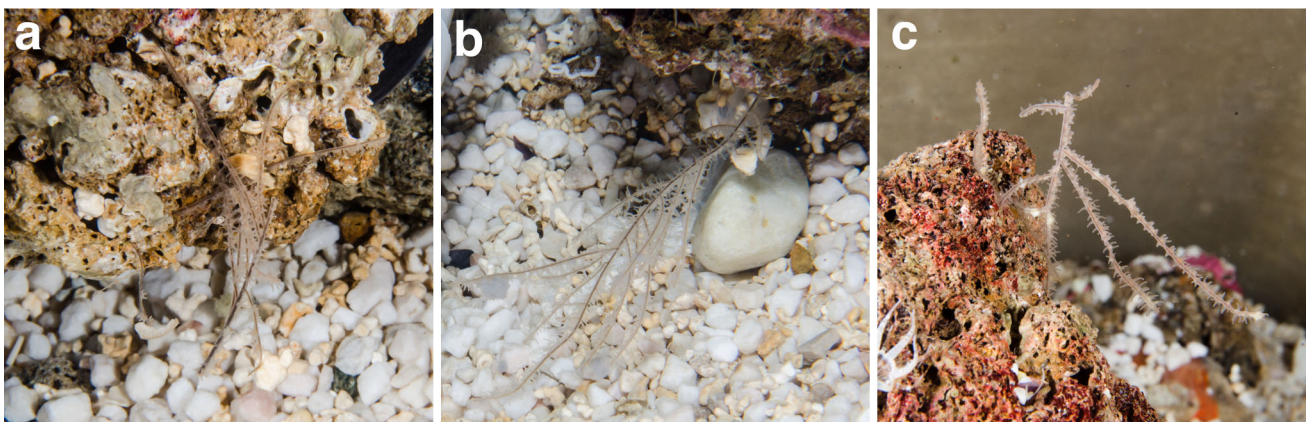

Fig. 2 Fragments settle and re-attach in different positions: vertical position with the attachment mediated by the lateral branchlets (**a**); horizontal position (**b**); upside-down position (**c**)

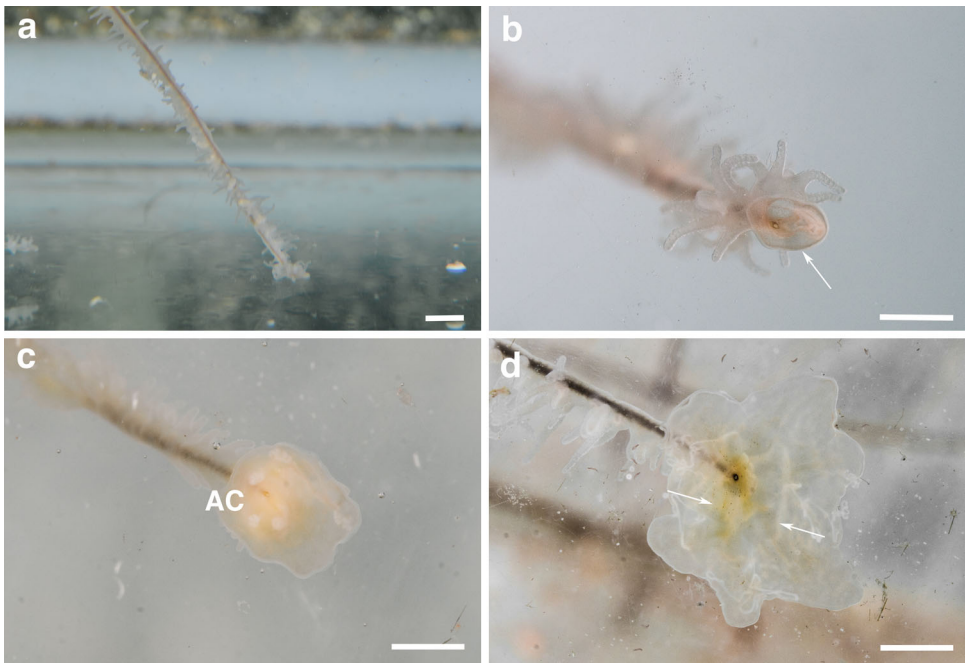

Fig. 3 Role of the apical polyp in the first phase of the adhesion to a new substrate: the polyp touches the wall of the tank and adheres to it (**a**); the polyp starts modifying its shape by flattening and creating the first basal plate (a thicker, golden border is visible as indicated by the white arrow) (**b**); the basal plate expands, but it is still thin enough to see right through it and small masses of acontiae (AC) are visible inside the gastric cavities (**c**); the new basal plate is well formed, irregularly shaped with thicker strips of skeleton (white arrows) emerging from the central area (**d**). Scale bars: **a** 1 mm; **b**–**d** 3 mm

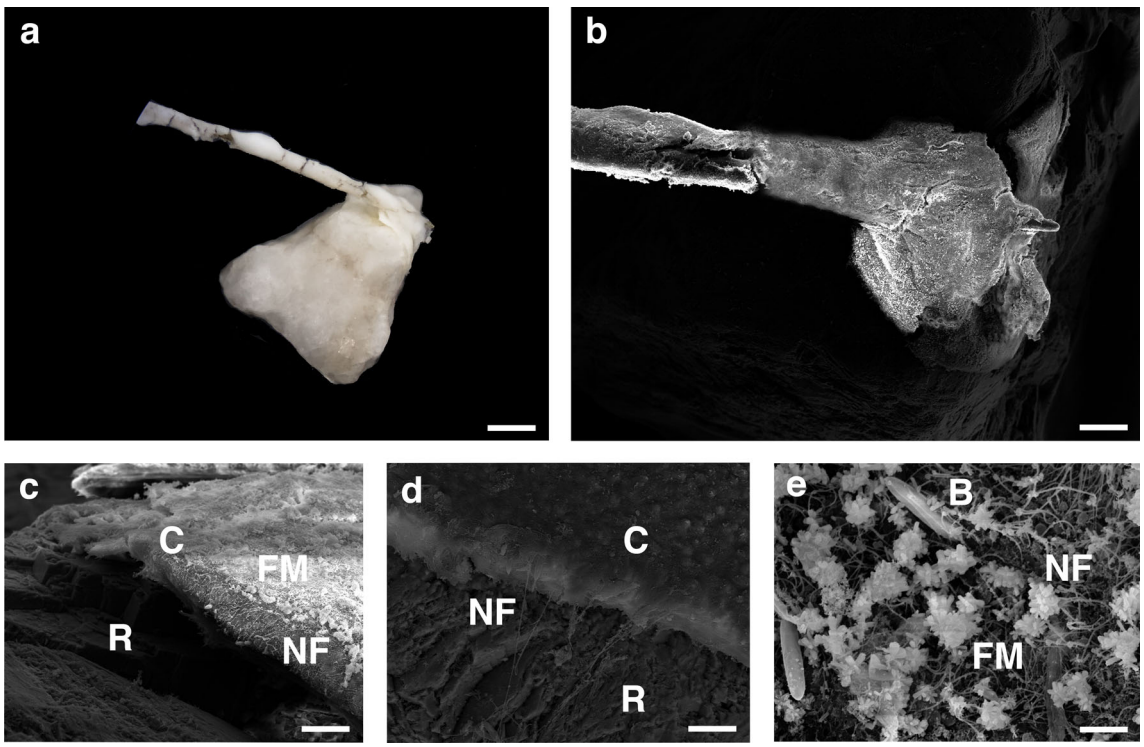

Fig. 4 Stereomicroscopes image showing an apical polyp attached to a small carbonatic grain (**a**); SEM images of the same polyp after treatment with Critical Point Dryer (**b**); coenenchyme (C) of the polyp covering the rock (R): a net of exploded nematocyst filaments (NF) and flocculating material (FM) are visible (**c**); close-up of the coenenchyme over the rock with exploded nematocyst filaments (**d**); close-up showing the nematocyst capsules (basitrich isorhizas) embedded in flocculating material and spiny filaments **e**. Scale bars: **a** 1 mm; **b** 100 μm; **c** 30 μm; **d** 20 μm; **e** 5 μm

(Fig. 3c). After 6 months, the basal plate becomes larger, irregularly shaped with strips of thicker skeleton emerging from the central area (Fig. 3d). The initial adhesion plate differentiates into thin, triangular, pointed spines, 18.37 ± 9.97 μm in height (mean ± SD), scattered on the surface (Fig. 5k). The basal plate progressively becomes

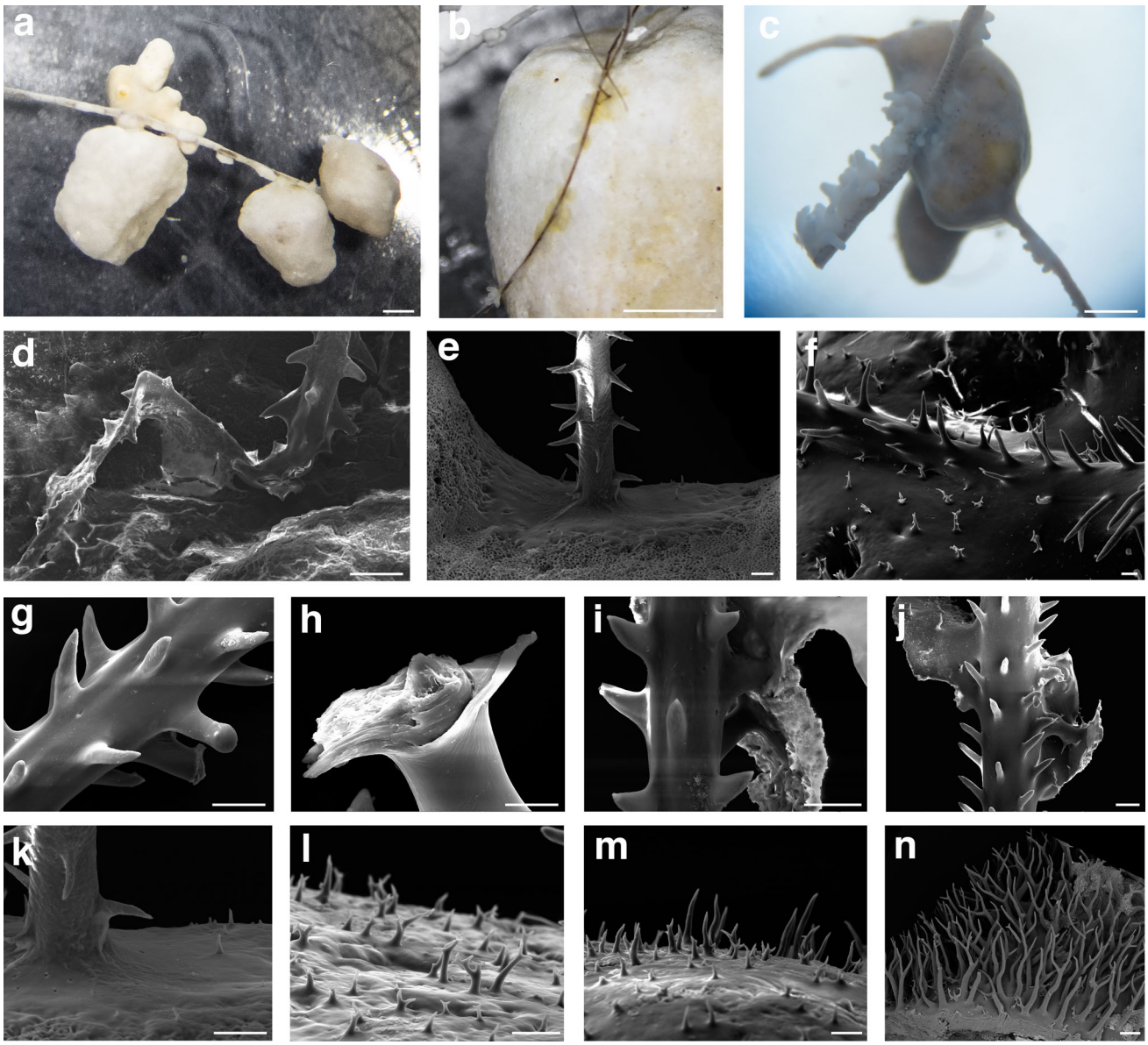

Fig. 5 Stereomicroscopes images showing multiple adhesions to small grains (**a**), with the polyps modifying their shape to adhere to the rock and create new, multiple basal plates (**b**), later stage of the adhesion with coenenchyme and spines completely covering the rock and new young stems arising from it (**c**). SEM images showing a multiple apical adhesion (**d**); early phase of an apical attachment with a thin layer of spineless skeleton covering the rock, the latter identified by a porous texture. Note downwards looking spines along the ramification indicating an upside-down settling (**e**); later stage of a central adhesion with the complete fusion of the fragment and the underlying rock; numerous branched spines are visible in this area already differentiated in an anchorage (**f**). Gradual modification of the spines during the adhesion: spines' tip turning rounded (**g**), then getting externally flat (**h**) and, lastly, completely fused with the substrate (**i–j**). Modification of the spines' shape through time over the new basal plate: few and small triangular spines are visible on a newly formed base (**k**), spines become dendritic and more numerous after 6 months (**l**), reaching larger sizes (**m**). Basal plate of an adult specimen of *A. subpinnata* sampled in Santa Eufemia (Calabria) at 100 m (**n**). Scale bars: **a–c** 2 mm; **d–m** 50 µm; **n** 100 µm

thicker embracing the totality of the rock or expanding on the glass surface (Figs. 3d, 5c). At this stage, the basal plate is densely characterized by dendritic spines, 84.54 ± 20.89 mm high (Fig. 5l, m). Polyps are not present or well developed on these larger basal plates.

Fragments landing in an upside-down position invert growth directionality: young stems emerge vertically from the basal plate (Fig. 5c), and upward spines emerge from the new anchorages (Fig. 5e).

Newly formed branchlets

SEM data indicate that each new branchlet (Fig. 6a) emerges as a progressive extension and expansion of one or

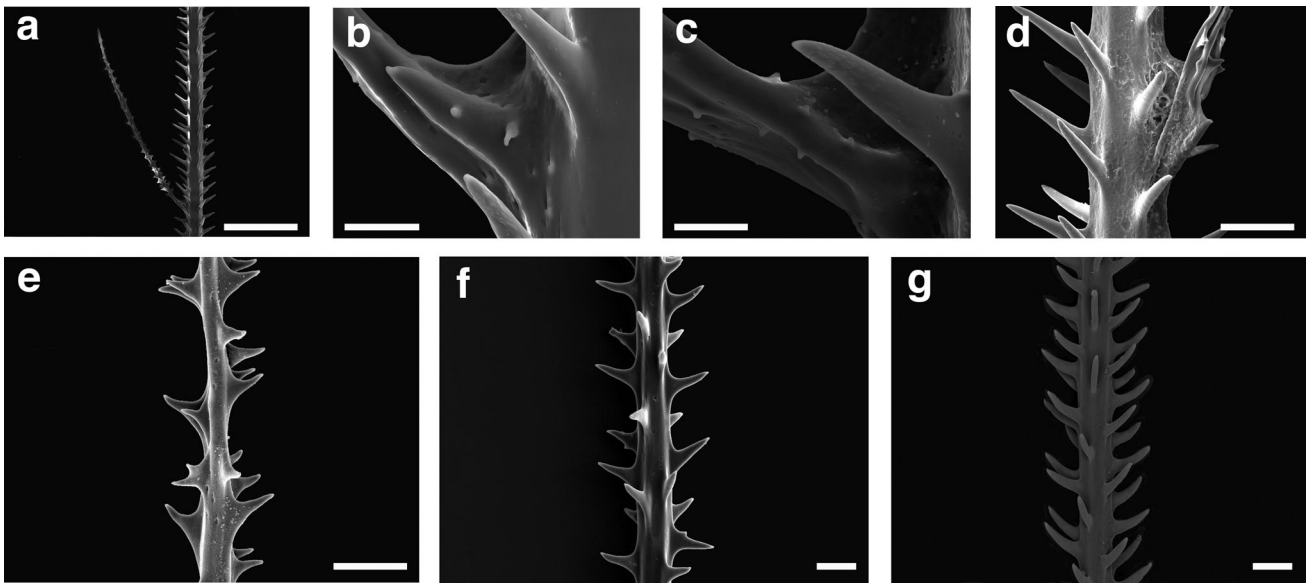

Fig. 6 Different phases of the formation of a new branchlet: overall view of a new branchlet emerged after 2 months (M2) (**a**); close-up of the basal portion of the new branchlet (**b–d**) in which the spine that originated the branchlet is still visible, with small spines emerging above it highlighting a complete reorganization of the original spine (**b**); late evolution of the basal portion of the new branchlet, with the involved basal spines completely embedded within the new branchlet (**c**, **d**) and more densely covered by numerous small spines (**c**). Different growth stages of the spines of a new branchlet: newly formed branchlet (M2) with small, simple and irregularly arranged triangular spines (**e**); 4-month branchlet (M6) with spines becoming slightly cylindrical and arranged in four longitudinal rows (**f**); branchlet of an adult colony of *A. subpinnata* sampled in Santa Eufemia (Calabria) at 100 m with cylindrical upward spines arranged in five regular rows (**g**). Scale bars: **a** 500 μm; **b** 50 μm; **c–g** 100 μm

more spines (Fig. 6b–d). Along the newly formed thin ramifications, spines are initially simple, triangular and irregularly distributed, but already after 3 months, they assume their final morphology, acute and cylindrical, and a regular arrangement in distinct longitudinal rows (Fig. 6e, f). New branchlets are observed for the first time after 2 months in three of the monitored re-settled fragments. After 4 months, they are observed in half of the tagged fragments (Table 3).

Emergence of new branchlets occurs at regular distance along the ramification bearing them, ranging from a minimum average of 0.20 ± 0.04 cm (no. 5) to a maximum average of 0.26 ± 0.06 cm (nos. 2 and 10) (Fig. 7a, Table ESM1); in some cases, the branchlets' arrangement pattern is monoserial, in other cases biserial alternate, regularly directed upward (Fig. 7).

Growth rates

The average growth rate of the fragments or their branchlets varies between 0.13 ± 0.05 and 1.14 ± 0.21 cm month^{-1} with one fragment that started growing only after 6 months after the detachment (no. 4). The minimum and maximum growth rates observed here are 0.03 and 1.85 cm month^{-1}, respectively (Table 2). The growth rate is constant and positive (Fig. 8) over the sampling period. The highest growth rates and overall well-being of the fragments are observed in the proximity of the pump in a well-exposed orientation presumably due to the moderate hydrodynamic flow. This is particularly true for fragments nos. 2 and 12.

With respect to the new branchlets, their average growth rate varies from 0.16 ± 0.07 to 0.78 ± 0.25 cm month^{-1} with the minimum and maximum observed growth of 0.06 and 1.58 cm month^{-1}, respectively (Table 3). A positive linear correlation ($y = 3.7308x + 0.713$, $R^2 = 0.91$) is found between growth rate and increase in polyps' number (2–7 polyps month^{-1}).

Discussion

In this study, we document, for the first time, fragmentation of the Mediterranean arborescent black coral *Antipathella subpinnata* in aquaria.

The potential existence of fragmentation is stated previously in black corals based on indirect evidences (Grigg 1964, Bo et al. 2009). In particular, in *Antipathes grandis* Verrill (1928), Grigg (1964) observes a softening and successive degeneration of a portion of the skeleton resulting in the breakage of the fragment. Prior to the detachment, a circle of mucus is observed around the point of detachment. In our study, we do not observed any sign suggesting forthcoming fragmentation. The three-

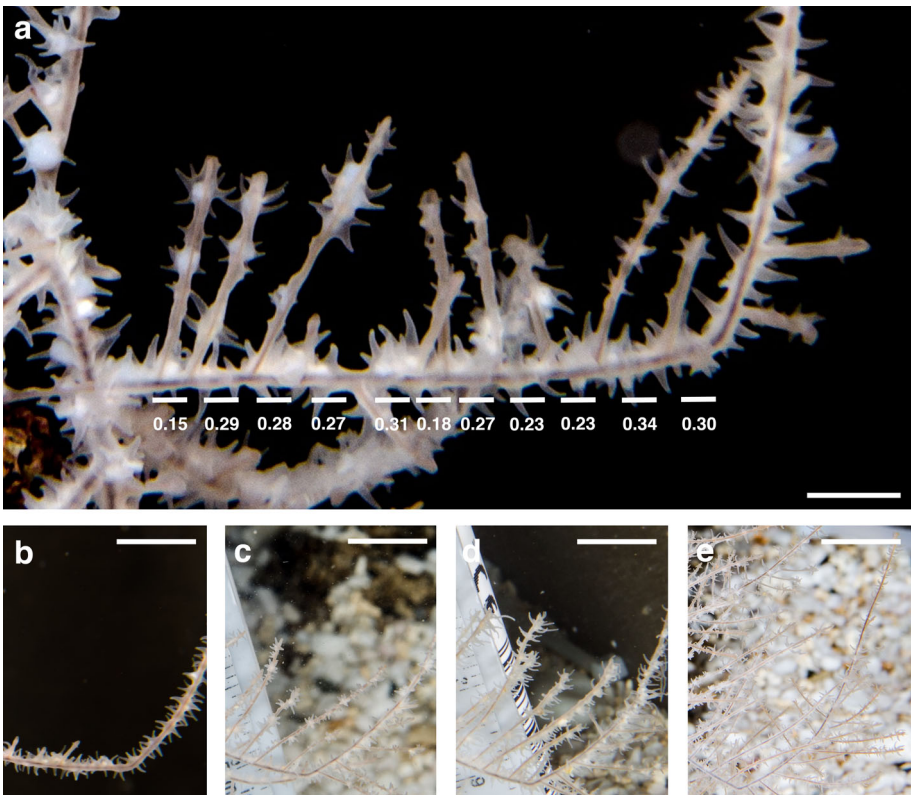

Fig. 7 Distance between new branchlets (expressed in cm) (**a**). Growth of the new branchlets through time (**b**–**e**): M2 (**b**); M4 (**c**); M5 (**d**); M7 (**e**). Scale bars: **a**–**e** 5 mm

dimensionality of the canopy of *A. subpinnata*, together with the reduced diameter of the ramifications involved (around 1.2 mm) and a putative fast recovery of the coenenchyme in the autotomy sites (Grigg 1964), could have complicated the possibility to clearly spot the detachment sites.

The difficulty in localizing rupture points in these arborescent colonies still keeps open further investigations regarding the biochemistry and enzymatic pathways regulating breakages in the skeleton, probably involving localized production of chitinases as observed in other cnidarian taxa (Mali et al. 2004).

This modality of asexual reproduction is difficult to be predicted, hence to be correlated with specific biological or environmental parameters. The factors triggering fragmentation still remain elusive and need focused experiments to be clearly understood; nevertheless, some generic considerations can be pointed out.

In natural conditions, monopodial, unbranched species such as black corals and gorgonians seem to undergo evident apical fragmentations in response to heavy currents (Bo et al. 2009) or as a control mechanism of the colony's growth (Walker and Bull 1983), occurring with a relatively regular periodicity as observed in other anthozoans such as *Acropora longicyathus* (Milne Edwards and Haime, 1860) (Wallace 1985). Russo and Vari (1997), while studying the precious red coral *Corallium rubrum* (Linnaeus, 1758) reared in a tank describe the first case of fragmentation for this species after two weeks. They observe the production of fragments after a strong increase of temperature and salinity in the tank, concluding that fragmentation could have been triggered by stress conditions associated with sampling and transport and, in a second moment, by the change of physical parameters in the tank. Other studies suggest that fragmentation in reared conditions may depend on the food availability or food typology (Hand and Uhlinger 1995; Orejas et al. 2008). Biological factors such as predation or diseases are considered important in triggering colonies' fragmentation in both hexacorals and octocorals (Acosta et al. 2001; Brazeau and Lasker 1992).

In our study, no evidence of biotic factors' implication can be reported in the fragmentation of *A. subpinnata*; instead, more evident interactions with the abiotic conditions can be highlighted.

Since *A. subpinnata* fragmentation occurs after a very short period of time (i.e., 5 days after sampling), one could argue that it is initially related to a thermal stress due to the difference in water temperature between the tank (16 °C) and the sampling site (14.5 °C). However, even after the temperature reduction the phenomenon is still continuously observed. Moreover, the unfertile colony collected in Portofino, previously kept at 16 °C for 5 months, does not show signs of detachment at first. The responses observed

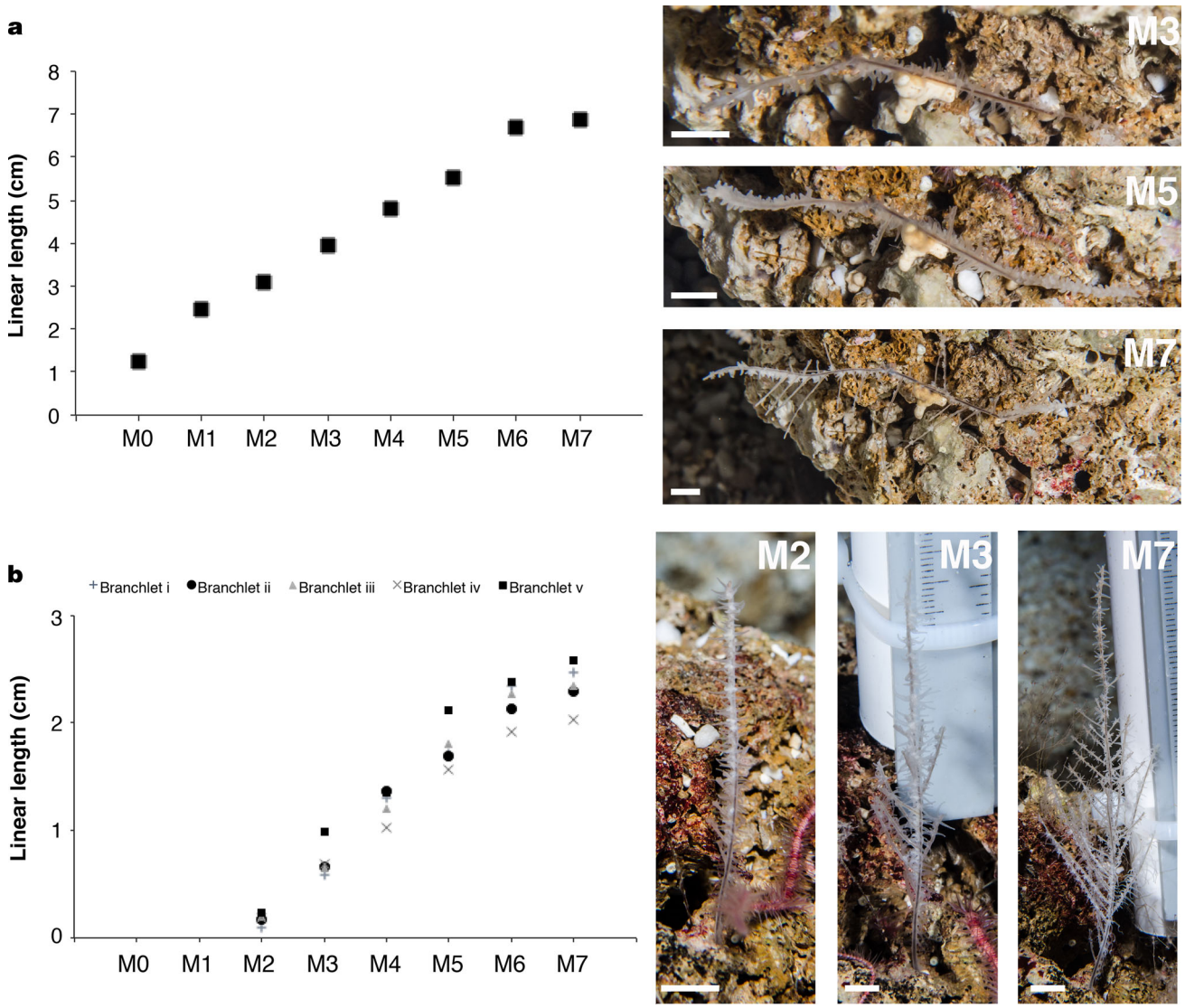

Fig. 8 Variation in growth (linear length increase) of one of the fragments (no. 9), with the relative picture at times M3, M5, M7 (**a**); growth rate of the new branchlets in one of the fragments (no. 1) with relative pictures at times M2, M3, M7 (**b**). Scale bars: 5 mm

in this experiment would lead to think that, in natural conditions, *A. subpinnata* does not fragment regularly.

Possibly, a synergic effect among transport/sampling stress, temperature variations, age and sex-dependent allocation of energy and responses to stress, as well as rearing conditions in general (e.g., food availability, hydrodynamic regime, upside-down position), can be hypothesized. However, no typical external signs of stress such as tissue loss, closed polyps, epibiosis or necrosis of the colonies are reported in the mother colonies.

Our observation shows that not only fragments survive, but they also re-attach on different substrates and continue to grow demonstrating a high morphological plasticity. Therefore, fragmentation might potentially be a viable asexual reproductive strategy for this taxon.

The fragments' capability to rapidly re-attach to the substrate, in fact, increases their survival rates and their ecological success. Fragments of *A. subpinnata* show a high versatility in terms of settlement preferences, being able to attach on different substrata, even on the glass wall of the tank. This adhesion on the glass wall was also observed in a gorgonian species, *Leptogorgia sarmentosa* (Esper, 1789) kept in the same Aquarium facilities (authors' pers. comm.). Different factors can impede re-settlement in black corals in natural conditions: fragments can be dislodged by water movement (Grigg 1965) making the re-colonization of surf zones or very steep walls impossible (Highsmith 1982). Contrarily, a stable environment (low surge and current) might ensure by itself a higher fragment success. *A. subpinnata* normally avoids vertical walls preferring slightly sloping rocks (Bo et al. 2008), which

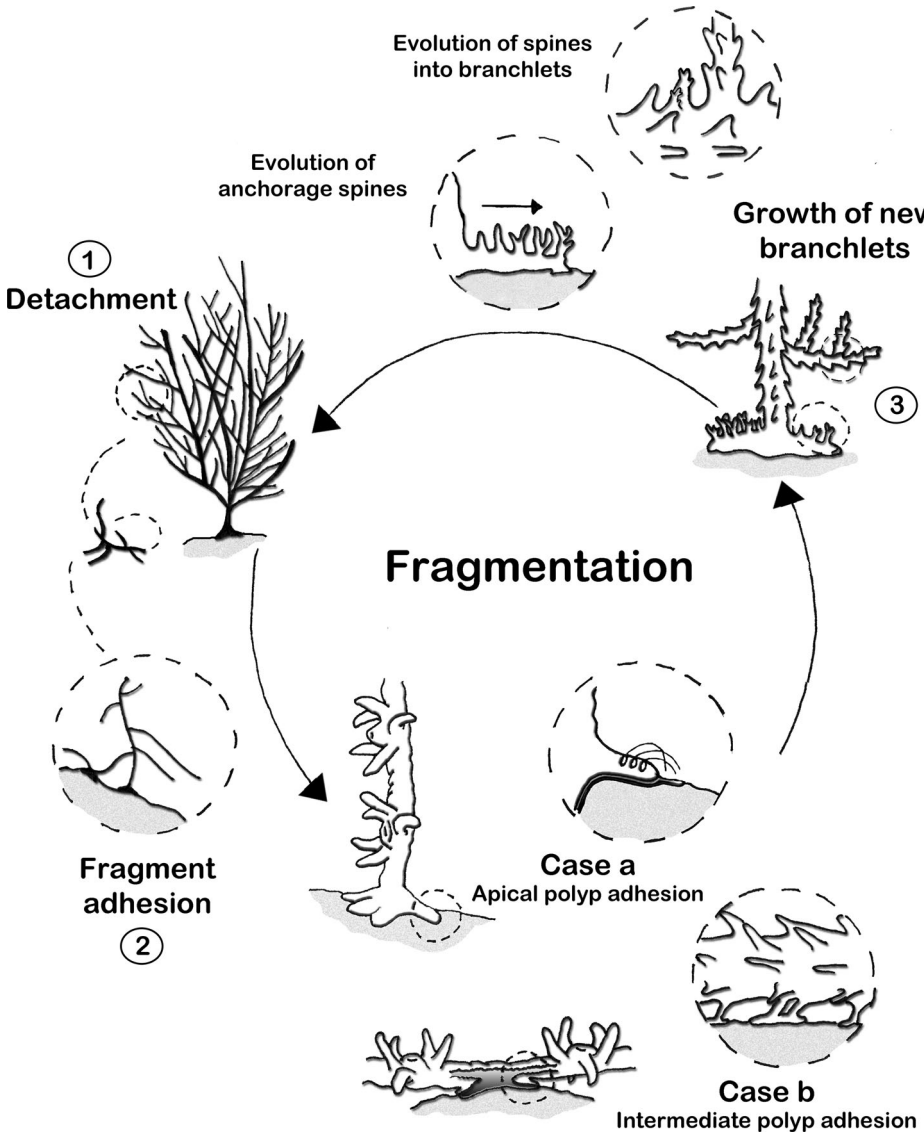

Fig. 9 Schematic drawing of the fragmentation steps

might facilitate the attachment of newly produced fragments. In controlled conditions, fragments might still be displaced by the current flux but remain very close to suitable substrata inside the tank, favoring a very rapid attachment and limiting the possibility of tissue abrasion and eventually death of the fragment. The possibility to create multiple attachments (especially when settling occurs on small cobbles) with different ramification types (not only basal) greatly enhances the success of this phase.

Orientation of re-settled fragments is random, depending on the landing conditions; fragments are found in vertical, horizontal and in upside-down positions, the latter probably favored by the holding of the colonies in the tank. A clear photophilic growth pattern is observed as upside-down fragments lose directionality and start growing upwards, toward the light source, as already suggested by other studies (Grigg 1965; Highsmith 1982; Bo et al. 2009).

Adhesion implies an important tissue rearrangement at both coenenchyme and skeleton level, probably involving fast cellular differentiation in the settling sites. Based on the collected evidence, it is possible to depict a generalized cycle of the fragmentation event (Fig. 9) including three fundamental steps: 1. Detachment of the fragment from the mother colony. 2. Initial adhesion mediated by the contact of an apical (case a) or intermediate polyp (case b) through the involvement of cnidocysts. Case a: polyp adheres to the substrate, flattening and radically changing its morphology and starting forming a new basal plate as an enlargement of the branchlet tip's spines. Case b: more polyps contribute to the adhesion; spines in the adhesion area apically enlarge and skeletal excess merges and fuses with the substrate creating an incipient basal plate, a thin layer enveloping the substrate and progressively thickening. In both cases, the original branch skeleton differentiates into a basal

anchorage, independently from its position or orientation. Scattered triangular spines increase in number and become dendritic. 3. Origin of a new colony thanks to the creation of new branchlets, emerging as a vertical extension and fusion of one or more spines at regular distance from each other.

Success of fragmentation as a reproductive strategy is supported by the high production rate, high survival rate (100% in this study) and high growth rate of the fragments in terms of both overall length and ramification pattern (3D complexity of the canopy). The maximum estimated annual growth rate observed in this study for a fragment of *A. subpinnata* is 22.2 cm yr^{-1}, which is not representative of the overall growth of the colony, supposedly much lower. Growth rates among branched black corals vary between 3 and 6.5 cm yr^{-1}: in *Antipathella aperta* (Totton, 1923) growth rate is 2.9 cm yr^{-1} (Grange 1985), whereas in *Antipathes griggi* (Opresko, 2009) and *A. grandis* it is 6.42 and 6.12 cm yr^{-1}, respectively (Grigg 1976); in *Plumapathes pennacea* (Pallas 1766) it is as high as 5.1 cm yr^{-1} (Oakley 1988), and in the cogeneric *Antipathella fiordensis* it is around 4.9 cm yr^{-1} (Grange and Singleton 1988). The growth rates of the fragments of *A. subpinnata* are more similar to those reported for unbranched, unpinnulated black corals focusing on the variation in linear length per year and reporting growth rates of up to various tens of cm in *Stichopathes* species (Goenaga 1977; Bo et al. 2009). Growth rates depend on age, morphology of the corallum and various environmental factors, including food availability that, in rearing conditions, can boost biomass accretion (Orejas et al. 2008). Possibly, fragmentation might accelerate growth rates increasing survival chances of fragments with respect to adult colonies, similarly to what happens with newly settled or juveniles.

A wide range of growth rates are measured among fragments, with specimens starting to grow immediately and others only late in the study period (Table 2), suggesting differences in the energy allocation among individuals (not necessarily clones) and also related with the different initial size of the fragments. Certain positions in the tank (e.g., near the pump) may have favored growth of certain fragments. A similar situation is observed for the formation of new branchlets (Table 3). Emergence of new ramifications is observed from both new basal plates and old branchlets. In this latter case evidence supports the origin of new branchlets by means of telescopic extension, as suggested in the past (Thomson 1905; Grigg 1964; Bo et al. 2009) as well as with the radial enlargement of the spines. Genetic determination of the ramification pattern is highlighted by the regular emergence of branchlets at constant distances of 0.2–0.4 mm, whether epigenetic factors (i.e., availability of space around a branchlet or minimum distance among neighboring polyps) might control this mechanism. Due to a lack of studies on the initial growth phases in black corals, our observations represent the first documentation of the development of the antipatharian corallum.

Mechanically induced breakage seems to negatively affect the growth of the fragment (as observed for fragment no. 6) and possibly its survivability. Indeed, at the end of our long-term monitoring the induced fragment does not appear healthy (e.g., thin tissue, closed polyps), a condition that might also be related to the position of this fragment, far from the current source.

Black corals share various biological and ecological traits with many fragmenting coral species, features that might contribute to increase the chances of fragmentation, including external fertilization, late sexual maturity, short reproductive periods, high longevity, populations with a reduced number of juveniles (Highsmith 1982). The occurrence and success of fragmentation in black corals population might explain their patchy distribution (Grigg 1964; Bo et al. 2014) and may potentially play an important ecological role in the survivorship of the populations in not optimal environmental conditions.

Acknowledgements Authors would like to thank the two reviewers who significantly improved this manuscript with their helpful suggestions, the Carabinieri Military Force (Centro Carabinieri Subacquei di Genova in particular Marshal Duilio Lenzini's team) and the diving center "Il Grande Blu" for their help during sampling procedures; Acquario di Genova Mediterranean Staff Department for the knowhow and support in black corals' husbandry and the Scanning Electron Microscopy Laboratory of the Department of Earth, Environment and Life Sciences, University of Genoa. This work was funded by BIOMOUNT project MIUR-SIR (RBSI14HC9O, Biodiversity patterns of the Tyrrhenian Seamounts).

Compliance with ethical standards

Conflict of interest The submitting author declares, on behalf of all the authors, no financial, personal or professional conflict of interests.

References

Acosta A, Sammarco PW, Duarte LF (2001) Asexual reproduction in a zoanthid by fragmentation: the role of exogenous factors. Bull Mar Sc 68:363–381

Bickell-Page LR, Mackie GO (1991) Tentacle autotomy in the hydromedusa *Aglantha digitale* (Cnidaria): an ultrastructural and neurophysiological analysis. Philos T Roy Soc B 331:155–170

Bo M (2008) Taxonomy and ecology of antipatharians. Ph.D. Dissertation in Marine Biology and Ecology, Università Politecnica delle Marche, Ancona, Italy, p 212

Bo M, Tazioli S, Spanò N, Bavestrello G (2008) *Antipathella subpinnata* (Antipatharia, Myriopathidae) in Italian seas. Ital J Zool 75:185–195

Bo M, Di Camillo CG, Addamo AM, Valisano L, Bavestrello G (2009) Growth strategies of whip black corals (Cnidaria:

Antipatharia) in the Bunaken Marine Park (Celebes, Indonesia). Mar Biodivers Rec 2:e54
Bo M, Canese S, Bavestrello G (2014) Discovering Mediterranean black coral forests: *Parantipathes larix* (Anthozoa: Hexacorallia) in the Tuscan Archipelago, Italy. It J Zool 81:112–125
Bo M, Bavestrello G, Angiolillo A, Calcagnile L, Canese S, Cannas R, Al C, D'Elia M, D'Oriano F, Follesa MC, Quarta G, Cau A (2015) Persistence of pristine deep-sea coral gardens in the Mediterranean Sea (SW Sardinia). PLoS one 10:e0119393
Bo M, Barucca M, Biscotti MA, Brugler MR, Canapa A, Canese S, Lo Iacono C, Bavestrello G (2018) Phylogenetic relationships of Mediterranean black corals (Cnidaria: Anthozoa: Hexacorallia) and implications for classification within the order Antipatharia. Invertebr Syst 32:1102–1110
Bocharova E (2016) Reproduction of sea anemones and other hexacorals. In: Goffredo S, Dubinsky Z (eds) The cnidaria, past, present and future. Springer, Cham, pp 239–248
Brazeau DA, Lasker HR (1992) Growth rates and growth strategy in a clonal marine invertebrate, the Caribbean octocoral *Briareum asbestinum*. Biol Bull 183:269–277
Coffroth MA, Lasker HR (1998) Population structure of a clonal gorgonian coral: the interplay between clonal reproduction and disturbance. Evolution 52:379–393
De Matos V, Gomes-Pereira JN, Tempera F, Ribeiro PA, Braga-Henriques A, Porteiro F (2014) First record of *Antipathella subpinnata* (Antozoa, Antipatharia) in the Azores (NE Atlantic), with description of the first monotypic garden of this species. Deep-sea Res Pt II 99:113–121
Fautin DJ (2002) Reproduction of cnidaria. Can J Zool 80:1735–1754
Foster NL, Baums IB, Mumby PJ (2007) Sexual and asexual reproduction in an ecosystem-engineer: the massive coral *Montastraea annularis*. J Anim Ecol 76:384–391
Gaino E, Scoccia F (2010) Gamete spawning in *Antipathella subpinnata* (Anthozoa, Antipatharia): a structural and ultrastructural investigation. Zoomorphology 129:213–219
Geller JB, Fitzgerald LJ, King CE (2005) Fission in sea anemones: integrative studies of life cycle evolution. Integr Comp Biol 45:615–622
Gilmore MD, Hall BR (1976) Life history, growth habits, and constructional roles of *Acropora cervicornis* in the patch reef environment. J Sedim Petrol 46:519–522
Goenaga C (1977) Two new species of *Stichopathes* (Zoantharia: Antipatharia) with observation on aspect of their biology. Thesis in Master Science, University of Puerto Rico, Puerto Rico, p 101
Gonçalves Ferreira J (2016) On the origin of bilaterality: insights from the study of black corals (Cnidaria: Antipatharia). Populations and evolution. Université Pierre et Marie Curie, Paris
González E, Guitart B, Garcia M (1997) Sown black coral in the Cuban southwest. In: Proceedings of the 5th congress on marine science, MarCuba'00, Havana, Cuba, p 73
Grange KR (1985) Distribution, standing crop, population structure and growth rates of black corals in the southern fiords of New Zealand. New Zeal J Mar Fresh 19:467–475
Grange KR, Singleton RJ (1988) Population structure of black corals, *Antipathes aperta*, in the southern fiords of New Zealand. New Zeal J Zool 15:481–489
Grigg RW (1964) A contribution to the biology and ecology of the black coral, *Antipathes grandis* in Hawaii. MS Thesis in Zoology, Hawaii, Honolulu, p 74
Grigg RW (1965) Ecological studies of black corals in Hawaii. Pac Sci 19:244–260
Grigg RW (1976) Fishery management of precious and stony corals in Hawaii. Sea Grant Technical, Report. UNIHI-SEAGRANT-TR-77-03, p 48
Hand C, Uhlinger KR (1995) Asexual reproduction by transverse fission and some anomalies in the sea anemone *Nematostella vectensis*. Invertebr Biol 114:9–18
Highsmith RC (1982) Reproduction by fragmentation in corals. Mar Ecol Prog Ser 7:207–226
Ivanova-Kazas OM (1977) Bespoloye razmnogeniye zhivotnykh (Asexual reproduction of animals). Izdatel'stvo LGU, Leningrad
Lasker HR (1984) Asexual reproduction, fragmentation and skeletal morphology of a plexaurid gorgonian. Mar Ecol Prog Ser 19:261–268
Lasker HR (1990) Clonal propagation and population dynamics of a gorgonian coral. Ecology 71:1578–1589
Mali B, Mohrlen F, Frohme M, Frank U (2004) A putative double role of a chitinase in a cnidarian: pattern formation and immunity. Devel Comp Immun 28:973–981
Miller K (1998) Short-distance dispersal of black coral larvae: inference from spatial analysis of colony genotypes. Mar Ecol Prog Ser 163:225–233
Miller K, Grange KR (1997) Population genetic studies of antipatharian black corals from Doubtful and Nancy Sounds, Fiordland, New Zealand. In: Proceedings of the 6th international conference on coelenterate biology, pp 353–363
Miller K, Ayre DJ (2004) The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral *Pocillopora damicornis*. Heredity 92:557–568
Montgomery AD (2002) The feasibility of transplanting black coral (Order Antipatharia). Hydrobiologia 471:157–164
Oakley SG (1988) Settlement and growth of *Antipathes pennacea* on a shipwreck. Coral Reef 7:77–79
Orejas C, Gori A, Gili JM (2008) Growth rate of live *Lophelia pertusa* and *Madrepora oculata* from the Mediterranean Sea maintained in aquaria. Coral Reefs 27:255
Purcell JE (1977) Aggressive function and induced development of catch tentacles in the sea anemone *Metridium senile* (Coelenterata, Actinaria). Biol Bull Mar Biol Lab Woods Hole 153:355–368
Russo GF, Vari L (1997) Life strategies of clone-forming anthozoans and the meaning of fragmentation. In: Cicogna F, Bavestrello G, Cattaneo-Vietti R (eds) Red Coral and other Mediterranean octocorals: biology and protection. Ministero per le politiche agricole, Roma
Schiariti A, Kawahara M, Uye S, Mianzan HW (2008) Life cycle of the jellyfish *Lychnohriza lucerne* (Sciphozoa: Rhizostomeae). Mar Biol 156:1–12
Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfed S, Schimd B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomankac P, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–682
Thomson JA (1905) Scotia collections: Scottish Antarctic expedition—report on the Antipatharians. Proc R Phys Soc Edinb 16:76–79
Wagner D, Luck DG, Toonen RJ (2012) The biology and ecology of black corals (Cnidaria: Anthozoa: Hexacorallia: Antipatharia). In: Advances in marine biology, vol 63, Academic Press, pp 67–132
Walker TA, Bull GD (1983) A newly discovered method of reproduction in gorgonian coral. Mar Ecol Prog Ser 12:137–143
Wallace CC (1985) Reproduction, recruitment and fragmentation in nine sympatric species of the coral genus *Acropora*. Mar Biol 88:217–233