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Turning casualty into opportunity: fragmenting dislodged colonies is effective for restoring reefs of a Mediterranean endemic coral

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ABSTRACT

Within the framework of ecosystem-based management, restoration appears as a sensible option to counteract the global decline of coral reefs. Several techniques involving sexual and asexual coral propagules have been used for the restoration of reefs. Culturing of fragments has proved fruitful since it takes advantage of the capability of corals to asexually reproduce, providing a number of novel colonies that can be replanted. This method however, when using fragments detached from a colony, might be stressful for the wild donor. *Astroides calycularis* is an endemic and endangered Mediterranean scleractinian coral forming massive colonies mostly at shallow depth. It is subject to anthropogenic impact, particularly from damage due to accidental contacts by SCUBA divers, and it is expected to suffer from sea storms of increasing power under the projected climate change scenarios. Corals of opportunity (i.e. dislodged colonies found alive on the seabed) may be a useful resource for the restoration of *A. calycularis* reefs, given that the fragment-based transplant technique is effective for this species as it is for other massive corals. A one-year transplant experiment was carried out along an exposed rocky shore in NW Sicily (Mediterranean Sea) to test the feasibility of using fragments of corals of opportunity for restoration purposes. The transplants revealed high survival rates and higher number of new polyps than in control colonies. The original size of transplanted fragments did not influence their capability to bud new polyps and was not related to their survival rate. The applied technique provides the opportunity to restore rocky reefs, even the very shallow ones, through direct transplant of coral fragments, thus making reef restoration a feasible option in ecosystem-based management plans for this species.

1. Introduction

Reefs built up by scleractinian corals are estimated to contribute with an astonishing 830,000 to the global species count being among the most diverse known habitats (Fisher et al., 2015). They also provide an array of goods (e.g., sea food, raw material for pharmaceutics, organisms for aquarium trade, construction material, etc.) and ecological services (e.g., shoreline protection, maintenance of biodiversity and habitats, burial of CO_2, support of recreation and tourism) that make coral reefs ranking first among world habitats in terms of economic value per unit area (Moberg and Folke, 1999; de Groot et al., 2012).

Human impacts like pollution, fishing, coastal development, and climate change, are responsible for degradation of coral reefs in tropical areas, impairment of their recovery ability and ultimately loss of the goods and services they provide (Moberg and Folke, 1999; Pandolfi et al., 2003). Evidence indicates that in some tropical regions, coral reefs under low threat levels are exceptions and predictions warn that, without a global plan of conservation and management, the majority of reefs will undergo severe threat during the next few decades. Halting such threats requires an integrated approach at the ecosystem level focused on the management of sea-related human activities, and stakeholders may play a paramount role in this process (Moberg and Folke, 1999; Burke et al., 2011; Chavanich et al., 2015). For instance, diving on coral reefs is a major tourist attraction in many tropical locations, often providing substantial incomes to local communities. In some cases, part of such incomes is reinvested to support conservation programs in positive cycles (Burke et al., 2011). However, unman-

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aged or poorly managed recreational diving may represent a severe threat to coral reefs (Rinkevich, 1995). It is apparent that accidental contacts by SCUBA divers and snorkelers (kicking, kneeling, trampling, etc.) cause physical stress to corals with a strong correlation between the number of divers and that of damaged colonies (see Hasler and Ott, 2008, and references therein). However, the risk of contacts with corals falls significantly when tourists undergo educational training held by diving centres, as they are among the main stakeholders involved in coral reef conservation (Camp and Fraser, 2012; Branchini et al., 2015).

Restoration (*sensu* Edwards and Gomez, 2007) or rehabilitation (*sensu* Edwards, 2010) interventions may be necessary when coral reef degradation reaches levels that are hardly reversible using traditional management and conservation plans. In this regard, techniques involving the use of sexual as well as asexual propagules have been tested (Chavanich et al., 2015). Sexual propagules give the advantage of providing a restored reef with a higher genetic diversity. In this case, reefs are restored or rehabilitated by transplanting gravid colonies, from which the planulae produced *in situ* can settle and develop into new colonies or by inducing settlement in laboratory conditions on a proper substratum (tile, plastic support, dead colony etc.) that is then positioned on the damaged reef (Rinkevich, 1995). As far as asexual propagules are concerned, the transplant of entire or fragmented colonies has been successfully used to create a self-sustaining reef in an area where the original reef has been damaged. Cultivating fragments harvested from wild corals is functional to several purposes other than restoration, including the production of colonies for aquarium trade or research (Shafir et al., 2001, 2006a), thus reducing the burden on wild populations. However, wild donors may suffer from the removal of large fragments (Henry and Hart, 2005; Shafir et al., 2006a). To overcome this issue, the use of small (<1 cm) colony fragments (i.e. coral nubbins) has proved an efficient coral gardening technique to produce novel colonies within nursery areas in the wild which are then transplanted in damaged reef areas (Rinkevich 1995; Shafir et al., 2001, 2006b). Successful gardening experiences have been realised in tropical areas with branching species (e.g. *Acropora* spp.), while massive corals have been used less frequently. Among the latter, *Dipsastraea favus* (Forskål 1775) appeared to be a good candidate, although its survival performance (50%) was lower than in branching species (Shafir and Rinkevich, 2008). Medium-sized fragments (3.5 × 3.5 cm) of the massive coral *Porites lutea* Milne Edwards and Haime 1851 had an even lower survival rate of 23.3% that dropped further to 2.2% for smaller fragments (2.5 × 2.5 cm) (Thongtham and Chansang, 2008).

Outside tropical regions and more generally beyond 30° latitude, temperate corals and other calcifying organisms form complex 3-dimensional habitats like shallow-water coralligenous and vermetid reefs and deep/cold-water reefs, which harbour biodiversity hotspots similar to those of tropical coral reefs (Roberts et al., 2006). These biogenic reefs suffer from local (e.g., fishing, coastal development, pollution from land-based sources) and global (e.g., warming, acidification) pressures that are likely to surpass their ability to adapt and survive (Hoegh-Guldberg et al., 2007; Fabricius et al., 2011). In the Mediterranean Sea, the endemic dendrophylliid coral *Astroides calycularis* (Pallas 1766) is considered a reef forming species (European Commission, 2013) and covers up to 90% of some rocky areas in shallow waters (Goffredo et al., 2011a). Even though sparse colonies have been observed in the Adriatic Sea (Kružić et al., 2002; Grubelic et al., 2004), *A. calycularis* mostly occurs in the south-western part of the basin from the Gibraltar Strait to Sicily and the south-western coast of the Italian peninsula (Terrón-Sigler et al., 2016a; Musco et al. in press), where it is abundant from the surface to 15 m depth, although it can be observed down to 50 m. *A. calycularis* is an azooxanthellate species growing in both low and high light conditions, in caves and on vertical walls preferentially

Fig. 1. Massive colonies of *Astroides calycularis* at 2 m depth along the Zingaro coast (NW Sicily, Mediterranean Sea). Top: close-up picture. Bottom: a reef with reference measure (distance between the two red laser spots = 20 cm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

exposed to intense hydrodynamics (Rossi, 1971; Cinelli et al., 1977; Zibrowius, 1995). The colonies have a massive-shaped morphology in shallow waters that are exposed to waves (Fig. 1), and a bush-shaped morphology at higher depths (Casado-Amezua et al., 2013). This species is gonochoric at colony level and brooder, and planulae are released from the tentacles from June to July (Goffredo et al., 2010; Casado-Amezua et al., 2013; Pellón and Badalamenti, 2016). The most frequent size class is generally from 3 to 4 mm as colonies tend to invest energy in increasing polyp size up to size at sexual maturity rather than in the growth of mature ones (Goffredo et al., 2010).

A. calycularis is considered a habitat former since its bioconstructions host a rich invertebrate fauna (Terrón-Sigler et al., 2014). It appears in the lists of strictly protected species of the Bern Convention (Annex II), endangered or threatened species of the Barcelona Convention (Annex II), and the CITES Convention. However, at present the species falls within the LC (Least Concern) category of the IUCN Italian Committee since it is spreading northwards in the Mediterranean Sea (Bianchi, 2007) and it does not appear to suffer from specific threats, although pollution and recreational diving may pose risks to its status (www.iucn.it). While studies aiming at a better understanding of the effects of pollutants on this species are needed, diving has already proved to cause serious damage to the colonies, especially in areas where its dense reefs attract SCUBA divers and snorkelers. In fact dislodged colonies on the seabed are

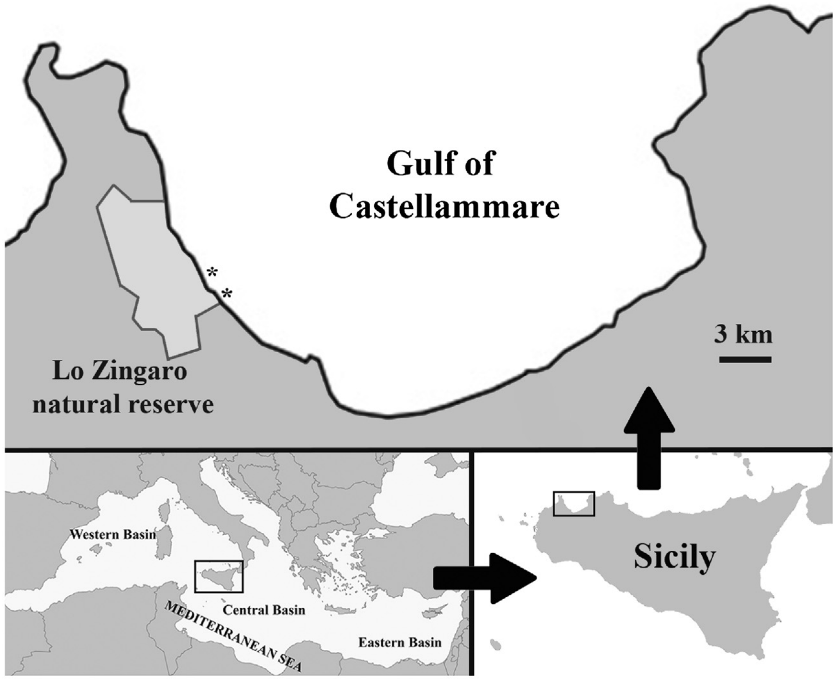

Fig. 2. Study area located along the rocky coast of the Lo Zingaro natural reserve, on the eastern side of the Gulf of Castellammare (NW Sicily, Mediterranean Sea: 38°09N 12°80E). ** indicate the study area boundaries.

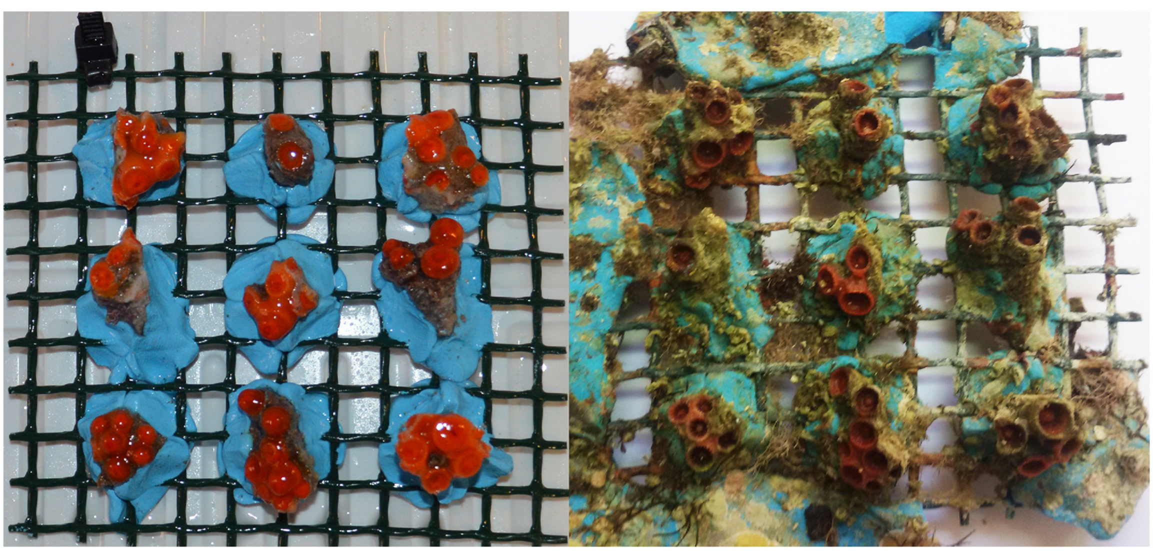

Fig. 3. Cluster of fragments of *Astroides calycularis* before transplant (left) and after collection 12 months later (right). Fragments on the right were frozen to allow an easier observation and count of polyps.

commonly observed and attributed to accidental contact by divers (Di Franco et al., 2009; Terrón-Sigler et al., 2016b). Moreover the increased frequency and intensity of extreme storms under global climate change scenarios (Slott et al., 2006) is likely to damage the colonies, especially those living in shallow waters. Since *A. calycularis* is not known to use fragmentation as a strategy of asexual reproduction, and the colonies laying on the seabed are not able to survive, dislodged colonies of this species can be considered as "corals of opportunity" *sensu* Monty et al. (2006) that might be used as donors of fragments for restoration purposes.

The aim of this study is to demonstrate that *A. calycularis* fragments obtained from corals of opportunity may be used as a feasible, rapid, potentially cost effective and environmentally friendly reef restoration technique at shallow depth, revealing that restoration/rehabilitation programs for this species may be feasible. Survival rate of *A. calycularis* fragments is expected to be comparable to that of other massive tropical corals used in analogous restoraton techniques. Furthermore, the prediction is that asexual reproduction is not altered, with transplanted fragments generating new polyps at a rate equal to that of wild (control) colonies. In particular, it is expected that the ratio between young polyps and adult polyps would be the same as in control colonies of the study area.

2. Material and methods

Dislodged *A. calycularis* colonies bearing apparently healthy polyps were collected along a 1500 m stretch of coast in September

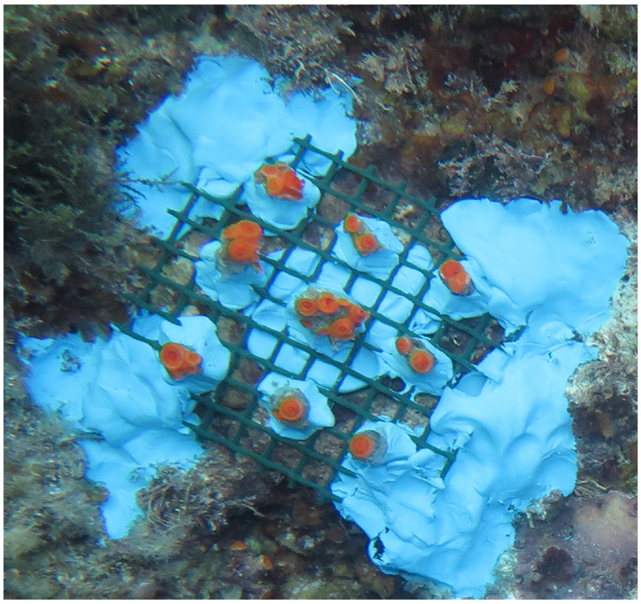

Fig. 4. Cluster of fragments of *Astroides calycularis* after transplant on a rocky wall at 2 m depth.

Fig. 5. Close-up view of three adult polyps and two newly budded polyps of *Astroides calycularis* in a transplanted fragment after 12 months. Fragments were frozen to allow easier observation and count of polyps.

2014 along the Zingaro rocky shore in the eastern side of the Gulf of Castellammare (NW Sicily, Mediterranean Sea: 38°09N 12°80E). In this area, which is exposed to NNE and may be subject to intense winter sea storms, *A. calycularis* is common from the surface to 30–40 m depth but is particularly abundant in shallow waters from 0 to 5 m depth where it forms dense patches (reefs) (Fig. 1). The area is a terrestrial natural reserve and among the most appreciated tourist destinations in Sicily (Fig. 2). Despite some restrictions applied to shipping and anchoring up to 300 m from the shoreline, these are limited to the few, small bays where bathing is practised.

The collected colonies (n = 12) were transferred to the facilities of the Istituto per l'Ambiente Marino Costiero (CNR-IAMC) at Castellammare del Golfo in aerated tanks filled with sea water collected at the sampling sites. Three colonies were randomly chosen for the transplant experiment and maintained in the aquaria, while the remaining nine colonies were used as control colonies. The latter were frozen for subsequent morphometric analyses, as freezing tends to relax the polyp tissues up to a point that corallites become evident allowing an easier and precise count and measurement of both adult and young polyps.

The size of the collected colonies (measured as the number of live polyps) varied from 30 to 70 (49.9 ± 12.3, mean ± SD). Among the colonies, 3 were haphazardly broken into pieces with a chisel in order to obtain fragments having various dimensions. Their size varied from 1 to 8 polyps (3.4 ± 1.9, mean ± SD). In order to create transplant clusters (TC), the 36 fragments obtained were mounted on four 10 × 10 cm quadrates of a plastic net (1 cm mesh side, 9 fragments per quadrate: Figs. 3 and 4) with a 2-component epoxy putty [pot life (20 °C): 1 h]. The 9 fragments in each TC were randomly chosen from the pool of fragments. Although *A. calycularis* is supposed to be resistant to air exposition being naturally found also in the intertidal zone, direct exposition to air was limited to a few minutes to avoid extra stress to the fragments. Each TC was tagged using cable ties and the position of each fragment was recorded (Fig. 3). Live polyps in each fragment were counted, then TCs were moved to an air-conditioned room (20 °C) and kept in aerated aquaria filled with sea water collected at the sampling sites until the epoxy putty appeared solid enough to allow TCs to be safely handled (ca. 4 h). The aquaria were previously sterilized with bleach and repeatedly rinsed with freshwater. After putty solidification the TCs were transferred back to the sea for transplant in a site with similar wave exposition and bottom slope as in the area of collection. Using a hammer, chisel and iron brush the extant algae-dominated assemblage was removed in order to obtain four 15 × 15 cm plots ca. 5 m apart for the fixation of TCs. An airlift sampler was used to remove the sediment from each plot allowing the TCs to be successfully fixed to the rocky substrate with epoxy putty (Fig. 4).

During September 2015, after ca. 12 months, all TCs were collected by carefully using a hammer and chisel and then frozen for subsequent morphometric analyses (Fig. 5).

Both the TCs and the 9 control colonies were analyzed using a calliper and a stereoscope to better count and measure young polyps. Polyp length i.e., the maximum axis of the oral disc – considered as good indicator in population dynamics studies on *A. calycularis* and other corals (Goffredo et al., 2011b and literature cited therein) – was measured. Following a conservative approach, a length of 3 (instead of 4) mm was selected as the threshold to distinguish young and adult polyps (Goffredo et al., 2011b).

Transplant success was measured as (1) the survival rate of fragments, (2) the survival rate of polyps, and (3) the ratio of young to adult polyps. The survival rate of fragments was calculated as the percentage of live fragments (i.e. fragments bearing at least one live polyp) in each TC after 12 months. The survival rate of polyps was calculated as the percentage of the originally transplanted polyps that survived in each TC after 12 months. The ratio of young to adult polyps was calculated for both the fragments and the control colonies.

Differences in the number of new polyps between experimental fragments 12 months after transplant and control colonies were tested through a Welch's *t* test on the ratio of young to adult polyps (Welch, 1938). This test was preferred over a classic *t* test (Gossett, 1908) due to concerns raised by the imbalance of the sample sizes and hence the possible inequality of their variances. The Welch's *t* test allows maintaining Type I error at nominal level while keeping Type II error unchanged. This is achieved by handling group variances independently, plus adjusting the degrees of freedom to the dataset used. The same test was applied to check *a posteriori* the potential effect of the size of the fragments on their capability to bud new polyps, i.e. the original number of polyps in reproductive frag-

Table 1
Astroides calycularis fragment/polyp survival and ratio of young over survived adult polyps after a 12 month transplant experiment. TC = transplant cluster; S% = percent survival; dead = apparently dead fragments; disl. = apparently dislodged fragments; OT = mean ± SD number of originally transplanted polyps; Y/A = mean ± SD ratio of young over survived adult polyps.

	FRAGMENTS			POLYPS		
	S%	dead	disl.	OT	S%	Y/A
TC1	77.8	1	1	2.56 ± 1.13	73.9	0.30 ± 0.37
TC2	100			5.00 ± 2.12	93.3	0.29 ± 0.34
TC3	66.7	1	2	2.89 ± 1.76	46.2	0.42 ± 0.66
TC4	88.9	1		3.33 ± 1.80	70.0	0.28 ± 0.38
total	83.3	3	3	3.44 ± 1.92	74.2	0.31 ± 0.41

Table 2
Output of the Welch's *t* tests on a) the ratio of young over adult polyps (Y/A) in survived fragments *versus* control colonies, and b) the *a-posteriori* comparison of the original number of polyps in reproductive fragments (R) *versus* the number of those in non-reproductive fragments (NR) of *Astroides calycularis*.

a)

	mean	SD	n
fragments	0.31	0.41	30
control colonies	0.01	0.01	9

Welch *t*'	d.f.	p
3.99	29.22	0.0004

b)

	mean	SD	n
R fragments	3.60	2.13	15
NR fragments	3.53	1.77	15

Welch *t*'	d.f.	p
0.09	27.07	0.9264

ments *versus* the number of those in non-reproductive fragments (Zimmerman, 2004; Ruxton 2006).

3. Results

Thirty out of 36 transplanted fragments of *A. calycularis* (=83.3%) survived 12 months after the transplant. At TC level, the survival rate ranged from 66.7% to 100%. Among what were considered non survived fragments, 3 had no live polyps (i.e. apparently dead fragments) and 3 were missing (i.e. apparently dislodged). The overall survival rate calculated ignoring the dislodged fragments was 91.7% (Table 1). Fragments that originally had 1 polyp had the lowest survival rate (60%, n = 5). The highest rate (88.9%, n = 9) was recorded in fragments with 2 polyps, followed by fragments with 4 (87.5%, n = 8) and 3 polyps (83.3%, n = 6).

The percentage of originally transplanted polyps that survived was 74.2% (82.9% excluding dislodged fragments from the count), ranging from 46.1% to 93.3% among TCs (Table 1). Considering the survived polyps, the 0.31 ± 0.41 (mean ± SD) ratio of young polyps over adults ones resulted significantly higher compared to control colonies (0.01 ± 0.01) (Table 1a). The same ratio ranged from 0.28 ± 0.36 (mean ± SD) to 0.42 ± 0.66 (mean ± SD) at TC level.

Among the survived fragments, 50% reproduced asexually i.e. produced at least one young polyp (Fig. 5). The original size of the reproductive fragments was not significantly different from the rest of the survived ones (Table 2b). There was a slight positive but not significant correlation between the original size of the reproductive fragments and number of newly budded polyps (Fig. 6: $R^2 = 0.06$, $p = 0.38$).

4. Discussion

The endemic Mediterranean scleractinian coral *A. calycularis* seems particularly well suited for environmental restoration programs. Preliminary studies carried out at 8 m depth along the Spanish coast for 9 and 12 months demonstrated that corals of opportunity of this species may be successfully transplanted (Terrón-Sigler et al., 2011; Terrón-Sigler, 2015). Here it is demonstrated that the restoration of *A. calycularis* reefs by fragments is feasible and environmentally friendly since the use of colonies of opportunity has no impact on wild populations. It should also be noted that restoration by asexual propagules seems the only feasible method for this species at present. The only known experiment with sexual propagules was made by Terrón-Sigler (2015) who used tiles placed in the proximity of gravid colonies as settling substrates for planulae to eventually transplant the new born colonies elsewhere, but the settlement did not occur. Hence, the present study suggests that the use of fragments from colonies of opportunity represents the most suitable method to obtain a large number of novel colonies to be transplanted.

The high survival rate detected in this study was somewhat surprising given that fragment-based restoration techniques proved to be more efficient with branching corals than with massive species. The massive coral *P. lutea* showed 23.3% and 2.2% survival for medium and small fragments respectively, while in *D. favus* nubbin survival was 50% (Shafir and Rinkevich, 2008; Thongtham and Chansang, 2008). Moreover, the fragment survival rate in our experiment is in line with observations by Terrón-Sigler (2015) along the Spanish coast for transplanted and translocated colonies of *A. calycularis*, suggesting that fragmentation did not have any detrimental effect in this study. Although long-term experiments are advisable for consistency of results and refinement of technique, our experiment demonstrated that also very shallow exposed reefs may be considered for restoration: this is a key point in that *A. calycularis* is particularly abundant between 0 and 5 m depth in the Central Mediterranean. Given its prevalent shallow distribution, *A. calycularis* is particularly subject to human impacts, both direct (i.e., pollution, anchoring, recreational diving etc.) and indirect (i.e., the increasing power of extreme sea storms as a consequence of climate change) in the Mediterranean Sea (Micheli et al., 2013; Nissen et al., 2014). The fact that corals of opportunity may be collected, fragmented, and directly transplanted without any need for intermediate nursery areas provides fast and cost-effective protocols for restoration purposes. The direct transplant of coral material was among the earliest restoration techniques used, but it was criticized due to possible limitations and pitfalls, including negative impacts on both the donor reefs and the transplanted corals (Rinkevich, 2014). In this study, the impact on the donor reefs was null and the transplanted colonies did not seem to be affected by manipulation, although a long-term experiment on a larger scale would be highly advisable and informative for management purposes.

The used approach suggests that also gardening of fragments of *A. calycularis* within nursery areas is potentially feasible. It may allow the production of novel colonies to be used for several purposes, including research, aquarium trade, and transplant in degraded areas. However, in the latter case the genetic compatibility between gardened colonies and the eventual remnant colonies of the original, depleted population should be tested. In fact *A. calycularis* populations have a high level of connectivity among close localities (ca. thousands of meters apart from each other), while moderate genetic differentiation characterize more distant populations (Casado-Amezúa et al., 2012).

The hypothesis that fragment size may have an effect on their survival was not specifically tested. Nonetheless, fragments made by one polyp had the lowest survival rate, while those made of two polyps had the highest rate and the rest of the fragments had inter-

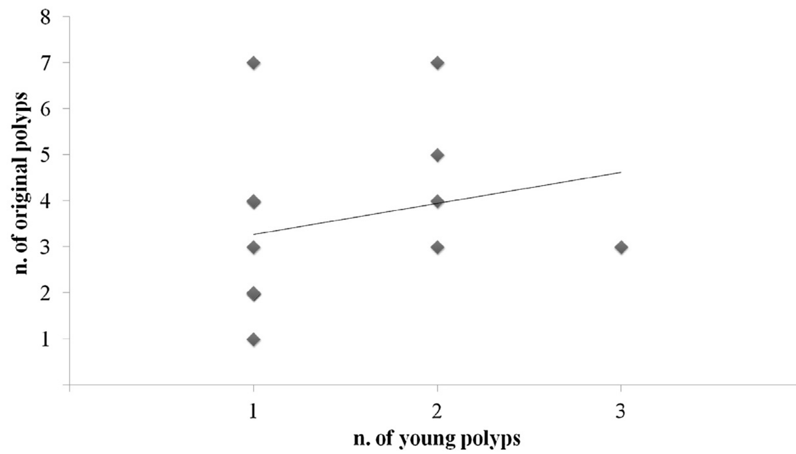

Fig. 6. Scattered plot between the number of original polyps and the number of newly budded polyps in *Astroides calycularis* after a 12-month transplant experiment.

mediate values. This observation suggests no relationship between size and survival in fragments of *A. calycularis*, in line with previous studies on tropical corals (see Lirman, 2000 and literature cited therein). Moreover, the *a posteriori* analysis revealed that the original number of adult polyps, does not influence the capability of budding new polyps. Hence, even small fragments made of two polyps (i.e. nubbins) might allow successful direct transplant or gardening in this species.

The ratio between young (<3 mm) and adult (>3 mm) polyps was significantly higher in the transplanted fragments than in control colonies grown in the same area. Moreover, it is worth noting that such ratio is 2–3 times higher than in Goffredo et al. (2011b) for wild colonies collected from April to September in another Mediterranean location, while Terrón-Sigler (2015) did not observe any difference in the increment of the number of polyps between transplanted and control colonies. It should be however considered that in wild colonies polyp budding occurs preferentially at the edge, where small sized polyps are usually more abundant, possibly as a strategy for space colonization (Goffredo et al., 2011b). We can speculate that in transplanted fragments, polyps benefit more from the higher volume-to-surface ratio compared to larger wild colonies, thus having more space for the budding of new young polyps. Hence, the use of fragments instead of colonies for restoration purposes could result in a more efficient use of the available resources, namely corals of opportunity.

5. Conclusions

The study demonstrates the feasibility of transplanting fragments of the scleractinian coral *A. calycularis*, as a restoration technique in the Mediterranean Sea, encouraging research efforts in this direction. As opposed to using whole colonies, fragmentation, rather than just increasing the number of new colonies it apparently also enhances the budding of new polyps, making it an ideal technique for growing new colonies for restoration purposes in a relatively short period of time. The results are sound enough to suggest that further research should aim at refining this transplant technique. However, besides restoration, integrated protection plans should include the management of human activities, and more specifically, educational programs aimed at increasing attention and care towards *A. calycularis*, also *via* best practices by stakeholders (e.g., diving centres) and end-users (e.g., tourists) in order to reduce the human impact on the species.

Author contributions

LM conceived and designed the research, participated in all phases of the fieldwork from sample preparation, to transplantation, collection and laboratory analyses, performed the data analysis and wrote the first draft of the manuscript. FP contributed to designing the research, to sample preparation and transplantation, provided expertise on coral ecology/biology and reviewed drafts of the manuscript. CP participated to transplantation and collection, helped with data analysis and reviewed the manuscript. GD participated to transplantation and collection, helped with data analysis and reviewed drafts the manuscript. NMG participated to transplantation and collection, helped with data analysis and reviewed drafts the manuscript. TVF contributed to design the research, contributed to the data analysis and to write part of first draft of the manuscript with LM, reviewed drafts of the manuscript. FB funded the research, contributed to design the research and the data analysis, and reviewed drafts of the manuscript. All authors participated in the scientific discussion and approved the final manuscript.

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