Title: Perspectives on working underwater with black coral nubbins (Cnidaria: Antipatharia): the case of *Cirrhipathes anguina* (Dana, 1846)

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Abstract

In order to test the feasibility of transplantation of the whip black coral species *Cirrhipathes anguina* (Dana, 1846) from Madagascar, transplants were installed on cultivation tables in two sites (the North Pass and the Grande Vasque) characterized by distinct environmental conditions. Following transplantation, the transplants were followed for short-term (20 days) healing capacities and medium-term (200 days) mortality and growth rates. Results show a successful transplantation in both sites with 0% mortality (except in the form of missing fragments) and a successful growth over 200 days. Maximum growth rates were 3.4 cm/month and 2.0 cm/month in the North Pass and in the Grande Vasque, respectively. In addition, mean time to total healing was delayed in the Grande Vasque compared to the transplants in the North Pass (10 days). Differences between sites are likely to be related to differences in environmental parameters. Altogether, the promising results obtained with the easy protocol used in this study encourage the use of black coral transplants in further *in situ* experiments and restoration projects.

1. Introduction

Antipatharians, or black corals, are ahermatypic hexacorals characterized by a black spiny flexible skeleton made of chitin and proteins (Opresko et al., 2014). Despite representing a small order within hexacorals (46 genera and around 273 species; Brugler et al., 2013; Molodtsova and Opresko, 2021), they are widely distributed geographically and bathymetrically (Wagner et al., 2012). They have been shown to play important ecological roles as habitat providers or living in close relationships with numerous species (Bo et al., 2014; 2019; De Clippele et al., 2019; Terrana et al., 2019).

In the last decades, over-harvesting of shallow water populations of black corals for commercial purposes in several regions of the world has resulted in the inclusion of all antipatharian species in the IUCN list and Appendix 2 of the CITES (Bruckner, 2016; Grigg, 2001; Todinanahary et al., 2016; Tsounis et al., 2010). This also led to research projects aiming at evaluating the feasibility to transplant black corals in order to replenish the impoverished communities at shallow depths (Bo et al., 2009; Montgomery, 2002). Previous tests to transplant black corals used fragments of colonies. In Hawaii, Montgomery (2002) showed that the transplantation of
two species of branched antipatharians (the formerly named *Antipathes dichotoma* (Pallas, 1766) and *Antipathes ulex* (Ellis & Solander, 1786), now *A. griggi* (Opresko, 2009) and *Myriopathes ulex* (Ellis & Solander, 1786), respectively) negatively affected the health (mean survival of 45%) and growth (shorter fragments after transplantation) of the fragments. The failure was attributed to critical factors such as the species or microhabitat selected for transplant, the transplantation technique used, or even the size of the fragments (Grigg, 1964). Nonetheless, the later study conducted by Bo et al. (2009) in Indonesia revealed that unbranched black corals are more easily handled and transplanted, with high percent of survival and vertical growth rates among the fastest observed for colonial organisms (maximum of 13.3 cm/month for *Cirrhipathes cf. anguina* and 1.3 cm/month for *Stichopathes cf. maldivensis*). They also observed that vertical growth rates were higher after a natural breaking event and in the apical portion of the colony, for *C. anguina*. Nonetheless, none of these studies evaluated the healing capacity over time of small fragments made from whole colonies.

In this study, we cultivated *Cirrhipathes anguina* (Dana, 1846), one of the most abundant whip black coral from the Great Reef of Toliara (Terrana and Eeckhaut, 2019) in two sites characterised with apparent different environmental conditions. To test the feasibility of transplantation of this whip species, we evaluated healing capacities, mortality and growth rates obtained by short-term (20 days) and medium-term (200 days) photo-observations.

### 2. Material and methods

#### 2.1 Cultivation tables

Two submerged tables intended to handle the black coral nubbins were built with 12 mm iron bars (Fig. 1A). The tables measured 90 x 105 cm and were divided by 2 transversal bars (30 cm apart). On each of them, eight 5 cm bars spaced 15 cm apart were welded vertically for attaching the nubbins with plastic tie wraps. The four corners were left free to firmly hammer the table into the substrate by means of four 50 cm long stands. The first row was labelled with plastic letters in order to follow the healing and growth of each specific nubbin. One table was installed within a black coral bed located in the North Pass (NP) of the Great Reef of Toliara at 23 m depth (23° 21.010’ S, 43° 36.837’ E; see description in Terrana et al. 2020), while the other was installed in the lagoon, at the “Grande Vasque” (GV) site at 13 m depth (23° 35.259’ S, 43°
Several environmental features distinguish these two sites. Apart from depth (and light, accordingly), current regimes also differ. The NP is well exposed to the waves and current generated by the dominant south wind, while the GV is a basin of approximately 1.5 km in length and 300 m width situated in the flat of the reef and well protected from the swell. Both sites are exposed to significant terrestrial runoff and sedimentation (Harris et al., 2010; Maina et al., 2012; Sheridan et al., 2014) from nearby rivers (Onilahy and Fiherenana). However, the impact of sedimentation is much stronger in the GV as the substrate is covered by a few centimetres of sediments that prevent the growth of corals (personal observation), likely due to the weak current in this part of the reef. Very few antipatharian colonies were encountered in the GV (and often, covered by epibiotic organisms) as compared to the abundance and diversity of the NP (Terrana et al., 2020).

2.2 Sampling. In total, four colonies of C. anguina (from about 130 to 210 cm in length) were collected at 23 m depth in the black coral bed of the NP site between the 9th and 12th of May 2019 by SCUBA divers. All the colonies were detached from the substrate by hammering the basal anchorage with a chisel. Two colonies were cut underwater, immediately after collection, from the basal anchorage to the growing apical portion into 13 and 15 fragments, respectively. These fragments (nubbins) were installed on the NP table as soon as they were cut from the colony. The two other colonies were transported to the GV site at 13 m depth and the nubbins were installed on the table. For the four colonies, the approximate length of all fragments was 10 cm, except the last one (i.e. basal fragment). For their transportation, we built 2 meters long tubes using PVC pipes with a diameter of 110 mm (Fig. 1B). Each extremity was composed of sealed screw caps allowing the diver to easily open and close it underwater. After detaching from the substrate, the two colonies were inserted into these PVC pipes filled with water from the collection site, brought back to the surface and sailed to the GV site within 30 min. They were pulled out of the pipes underwater, sectioned (17 and 11 fragments, respectively) and the fragments installed on the table as previously described. Each fragment was attached to the table in an ordered way to take into account their position in the colony, with special care to keep the nubbins in their original direction, i.e. with the apical part directed upwards.

2.3 Monitoring. For each fragment, we observed the apical tip of the nubbins and we avoided any manipulation that would have damaged the coral tissues. Every month, photographs of every fragment were
taken with a scale (Fig. 1C) and the length of the growing part (skeleton covered by tissues) was measured (in mm) using the software ImageJ (Schneider et al., 2012). Sometimes, a piece or the whole growing part disappeared and this was noted as a breakage. To calculate the mean and maximum growth rates (cm/month) of the nubbins, values were grouped in three sections: apical, median and basal, each corresponding to one third of the total height of the colony. Every 1-3 day(s), photographs of the apical tip of each nubbin were taken at both sites, for 23 days. The evolution of healing with time was followed based on a semi-quantitative Healing Index (HI) ranging from 0 to 5 (Fig. 1D). At a larger scale, mortality and growth of the fragments were monitored over a period of 200 days.

2.4 Data analysis. The effect of the site (NP, GV) on the occurrence of breakage was evaluated using Kaplan-Meier estimate of survival (survfit, survival, Therneau 2015a) and Cox-proportional hazard model (coxme, Therneau 2015b), due to the binary nature of these data. This statistics estimates the probability that a fragment will not break past a particular day. All analysis and figures were performed using R (R Development Core Team, 2020).
3. Results

3.1 Short-term healing of the fragments and description of a “Healing Index”

The mean HI increased rapidly with time until total healing (HI 4; Fig. 2A). After 10 days, all fragments in the NP were completely healed (mean HI = 4) while two were still healing in the GV (mean HI = 3.93), including one, the most basal fragment of a colony, which remained unhealed (HI 3) even after 20 days. The first signs of vertical growth (HI 5) were observed after 18 days on an apical fragment in the NP. After 23 days, 13 nubbins out of 28 were growing in the NP, while no signs of growth were observed up to 20 days in the GV.
Figure 2. A Mean Healing Index (± sd, n=11-17) by colony and site over time (one line per colony); B Mean growth in height (± sd, n=9-17) by colony and site over time (one line per colony); C Absence of breakage occurrence probability with time, per site. Solid and dashed lines represent data for the North Pass and the Grande Vasque, respectively.

3.2 Medium-term mortality and growth rates of the nubbins

After approximately 200 days, the overall mortality rate is 0%, except for one missing fragment on each table. In the NP, all fragments showed growing apical parts after 190 days while 15 nubbins out of 28 were growing in the GV (Fig. 2B). Mean and maximum growth rates tended to be higher in the apical fragments and to decrease in the intermediate and basal fragments of the colonies, for both sites (Supplementary Table S1). In addition, the highest growth rates were recorded on apical fragments and were 3.4 cm/month in the NP and 2.0 cm/month in the GV. Despite this, all fragments in the NP showed breakage of the growing tip at some point over the 200 days of observations (Fig. 2C). In those cases, the growing part of the fragments increased in length up to a certain maximum (10.7 ± 2.4 mm (mean ± se, n = 27), ranging from 3 to 68 mm) before the breaking event, which either left a small portion of the growing part or completely removed the whole growing tip. In the GV, all growing fragments except three, showed breakage over the 200 days of observations. Their maximum size before breaking was 5.3 ± 0.8 mm (mean ± se, n = 12). Altogether, these results show that the probability of breakage was significantly more important in the NP than in the GV (Cox Proportional Hazards Models, p<0.001; Fig. 2C).
4. Discussion

Our results showed that colonies of the whip black coral *C. anguina* can be easily fragmented and transplanted with no risks of mortality and a successful growth over 200 days. Importantly, small black coral fragments are viable for medium-term following our protocol and showed significant growth, which open the possibility to multiply the number of colonies.

While the success of transplantation in both sites shows the resilience and acclimation capacity of this species, the delayed healing and decreased growth performances in the GV as compared to the NP, supports the need to consider sites with similar environmental conditions as the collection site for transplantation experiments.

Even if the available data prevents us from making statistical comparisons, we suggest that differences between sites may be attributed to differences in environmental parameters such as water temperature, depth, bottom currents, luminosity, substrate availability and/or suspended food availability (Grigg, 1965; Sanchez et al., 1998; 1999; Tazioli et al., 2007). GV is presently deprived of black corals, although dead colonies are frequently observed (Personal observation).

The maximal growth rates observed here (3.4 cm/month) are much lower than those observed by Bo et al. (2009) on the same species (13.3 cm/month). Such difference may be explained by the size of the fragments (11-18 cm) that is much larger in Bo et al. (2009) than in our study (around 10 cm; as previously suggested by Montgomery, 2002). Indeed, longer fragments are associated with a higher number of polyps capturing food, and thus higher energetic inputs from heterotrophic feeding. The higher growth rate may also be attributed to the temporal scale of our study as Bo et al. noted a gradual increase in growth rate over the 20 months of transplantation. In addition, the breaking events that were frequently observed in our study (as well as one missing fragment per table) might prevent the tissue fragments from growing further or even underestimate their maximum growth rates. These breaking events might be caused by strong currents occurring in the NP or the entanglement of fishing nets as the area is visited by fishermen daily. Fragmentation may also occur as a response to the potential stressful conditions of the fragment, that first needs to spend a lot of energy to heal and then to start growing. The lack of energy (that may be related to food availability, especially in the
GV) may bring them to fragment again. Then, breakage may be used as an asexual reproductive strategy by
the coral when facing non-favourable conditions; a hypothesis already reported for *Antipathella subpinnata*
(Coppari et al., 2019). Despite this, breakages also occur naturally on adult colonies (personal observation)
and on other unbranched species from the NP (*Stichopathes cf. maldicensis*, *Stichopathes cf. diversa*; Terrana
et al., 2020), suggesting that fragmentation is part of the life cycle of *C. anguina* and that the experiment itself
may not have triggered the breakage. Finally, we noted that the plastic ties were responsible for local loss of
tissues for a few nubbins, which may also delay growth. We selected this method as it was the most reliable
to face the strong currents in the NP and the less time-consuming to work at these depths. This method also
allowed us to directly work underwater without getting the nubbins out of the water to prevent any stress
due to temporary desiccation. We recommend future experimenters to try other methods such as Epoxy resin
if the local environmental conditions allow it, but the chemical effects of the resin on the black coral tissues
remain unknown. However, we do not recommend to suspend the nubbins with a nylon wire for several
reasons: (i) it is far more time-consuming underwater and therefore it is not efficient when diving air and open-
circuit; (ii) it implies more manipulation of the nubbins, increasing the potential mechanical damages to the
soft tissues; (iii) our previous trials in aquaria showed that the line also impacted the tissues locally (*i.e.* swollen
tissues and impacts on polyp development, see Supplementary Figure S2) and (iv) with this method, the
nubbins would be suspended on a table and the strong currents would lead to the nubbins clinking against
each other, increasing mechanical damages as well.

To improve the transplanting performances, we suggest to exclusively collect apical fragments of the colonies,
likely to show greater growth rates. In addition, this will allow to maximize the number of different individuals
on a single table and therefore make a proper set of replication and to minimize the impact of sampling by
only collecting small fragments of the colonies, thus allowing them to heal. Future developments of the
methods may also be to select favourable sites during the vulnerable stages of the transplants, to further plant
them in less favourable sites when they are more resistant. However, if a site with low current is likely more
favourable as it may cause less breakage of recently fragmented nubbins, less current also implies lower
nutrient income, which in turn will alter the overall fitness of the coral. Fragmentation and re-attachement
ability of antipatharians have already been demonstrated in the branched species *Antipathella subpinnata* (Ellis & Solander, 1786) in rearing conditions (Coppari et al., 2019). Therefore, breaking events could potentially be entirely part of the life cycles of whip black corals as well. This hypothesis is supported by our observations on *C. anguina*. We suggest that the re-attachment capacity of the fragments should be evaluated in future rearing experiments, as this is crucial to evaluate the success of potential future restoration initiatives. It still remains to estimate under which conditions such breakage could occur (naturally or under mechanical or physiological stresses).

Our data also supports the variations in regenerative ability along the colonies (Bo et al., 2009), with the last fragment to heal being the basal fragment of a colony and the first to grow being the most apical fragment. These apical fragments present the advantage to have only one side of exposed skeleton after cutting, while the other side is already the side by which the whole colony is growing. Then, the fragments showing the highest growth rates were located in the apical section of the colony (*i.e.* the apical third of the colony). This is also likely due to a higher concentration of stem cells in apical tissues (younger tissues) relative to the rest of the colony (Bo et al., 2009). In addition, there is probably a relation between the growth rate and the observation of breakage: when growing, the coral creates a thin (few millimetres thick) and fragile skeleton of a few centimetres in length, which shows a high probability of breakage. This is further supported by our results showing that growth rates are more important in the NP than in the GV (Supplementary Table S1), with fragments from the NP showing higher probability of breakage (Fig. 2C).

In conclusion, we suggest that this experimental protocol can be used for future *in situ* studies on black corals with good chances of success. Such studies could be related to feeding experiments or effects of specific stressors on the nubbins. The Healing Index could be used as a standardized endpoint, which does not require any manipulation of the nubbins. Furthermore, our work encourages the use of black coral transplants in restoration projects as we obtained promising results (0% mortality and rapid growth of the fragments) using an easy and straightforward protocol.
5. Acknowledgements

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6. References


Therneau, TM, 2015. coxme: mixed effects cox models. See https://CRAN.R-project.org/package=coxme.


Supplementary Material

Supplementary Table S1. Mean and maximum growth rates (cm/month) of nubbins from the apical, median and basal part of the colonies from the North Pass and from the Grande Vasque sites.

<table>
<thead>
<tr>
<th></th>
<th>North Pass</th>
<th></th>
<th>Grande Vasque</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean ± se</td>
<td>Max ± se</td>
<td>n</td>
</tr>
<tr>
<td>Apical</td>
<td>2.7 ± 1.0</td>
<td>4.7 ± 1.7</td>
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</tr>
<tr>
<td>Median</td>
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<td>4.6 ± 1.8</td>
<td>9</td>
</tr>
<tr>
<td>Basal</td>
<td>2.2 ± 0.9</td>
<td>3.9 ± 1.5</td>
<td>9</td>
</tr>
<tr>
<td>Grande Vasque</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Apical</td>
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<td>2.4 ± 0.9</td>
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<tr>
<td>Median</td>
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<tr>
<td>Basal</td>
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</table>

Supplementary Figure S2. Photographs of nubbins suspended by a line in the experiment carried out in the laboratory prior to the in situ experiment, with local effects (black arrows) such as A growth of “extra” tissue without skeleton on the basal side of the nubbin (here, *Cirrhipathes anguina*); B formation of swollen tissues around the line wrapping the nubbin (here, *Stichopathes maldivensis*); C lack of polyp development and stressed (closed) polyps on the tissues surrounding the line (here, *C. anguina*). Scale bars: 1 cm.