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Running Head

Ecomechanics of Black Corals

Key words: Antipatharians, biomechanics, Young’s modulus, flexural stiffness, Madagascar
ABSTRACT

Mechanical properties of the skeleton of four antipatharians (the whip species Cirrhipathes anguina (Dana, 1846) and Stichopathes cf. maldivensis, and the branched species Cupressopathes abies (Linnaeus, 1758) and Cupressopathes cf. pumila) living in shallow waters off the southwestern coast of Madagascar were investigated using a three-point bending test. The Young’s modulus did not differ according to species but was significantly higher in the distal segment of colonies, compared to the basal and median segments. On the contrary, the flexural stiffness was significantly higher in whip species compared to branched ones and, in the whip species, in the basal segment compared to the other two segments, an observation consistent with a specific adaptation of the species to a strong current environment. While both species cohabit in identical flow conditions, whip species are able to maintain their stalk in vertical position while branched species can readily bend over. This suggests that the specific flexural stiffness is linked to the contrasted feeding strategies of species with different morphs in a similar strong current environment.
1. INTRODUCTION

Antipatharians, also called black corals, are anthozoan colonial cnidarians found from shallow to deep waters in all oceans and from tropical to polar latitudes (Wagner et al., 2012). These hexacorallian suspension feeders are ahermatypic and rheophilic organisms living under varying conditions of hydrodynamics, temperature and light (Yesson et al., 2017).

Although algal cells were observed within the tissues of a few black coral species (Bo et al., 2011; Brook, 1889; van Pech, 1914; Wagner, 2011), antipatharians are considered to be typically heterotrophic. They feed mainly on zooplankton and on suspended particulate organic matter (Carlier et al., 2009; Grigg, 1965; Sokolova, 1997) with direct interception being the dominant mode of food capture (Wagner et al., 2012). Cirrhipathes gut has been observed to contain copepods, polychaetes, amphipods and ostracods (Tazioli et al., 2007).

The order Antipatharia is characterized by a skeleton mostly made of chitin fibrils inside a lipoproteic matrix (Goldberg, 1991; Holl et al., 1992. Juárez-de la Rosa et al., 2012; Kim et al., 1992). Diphenols are also present in the skeleton in concentrations varying with the level and the age of the colony (Holl et al., 1992). Their skeleton, containing a central canal (Fig. 1), is either unbranched (i.e. wire or whip corals) or branched into bush, fan, feather or bottle-brush shapes (Wagner et al., 2012). Contrary to Gorgonians, the soft tissue surrounding the chitin skeleton axis of antipatharians does not contain sclerites and is not believed to contribute to their mechanical properties.

As antipatharians are dependent on suspension feeding, they are predominantly found in habitats showing strong and consistent currents (Tazioli et al., 2007; Warner, 1981). How these organisms cope with flow stress according to their specific morph is expected to be determined to a large extent by mechanical properties of their stalk, allowing it to bend or to resist bending in the current. However, the way the animal shape is modified by currents should impact the exposition and access of polyps to passing food. Therefore, the elastic properties of their skeleton, particularly their flexural stiffness, which is a measure of their ability to resist bending (Koehl, 1977), are expected to be essential traits of their biology.

To date, only two studies have been dedicated to the biomechanics of antipatharians (Juárez-de la Rosa et al., 2012; Kim et al., 1992). In the former study, bending tests showed significant differences of Young’s modulus, i.e. the tensile stiffness, between two branched species. The latter study not only indicated comparable differences of Young’s moduli
between two other branched species with bending tests, but also showed different values of
the Young’s modulus at several measurement points situated between the periphery and the
core of a transverse section of the stalk using nanoindentation tests. However, these studies
were restricted to branched species and tests were conducted on dehydrated-rehydrated or
chemically altered samples. Furthermore, no analysis has been carried out to measure the
Young’s modulus and the resistance to bending of the skeleton at different levels of the stalk
in a colony, while these properties are critical to species presenting different morphologies
and living in currents of variable magnitudes. Other biomechanical studies have been
dedicated to soft corals (Boller et al., 2002; Jeyasuria and Lewis, 1987; Lin and Dai, 1996)
but the different nature of their skeleton makes them difficult to compare with antipatharians.
Therefore, the goal of the present study was to characterize the Young’s modulus and the
flexural stiffness of the skeleton of both branched and whip-shaped antipatharians living
under similar environmental conditions and to assess the impact of different preservation
modes on these characteristics.

2. MATERIAL AND METHODS

2.1. Study area and sampling

Three entire colonies of four antipatharian species, or a total of 12 colonies, were collected in
May 2019 at 22 m depth by SCUBA diving at a first site S1 (S 23° 21.010’ E 43° 36.837’) off the southwestern coast of Madagascar, from a black coral bed located between the
northern part of the Great Reef of Toliara and the coast (Fig. 2): Cirrhipathes anguina (Dana,
1846) and Stichopathes cf. maldivensis (whip corals) and Cupressopathes abies (Linnaeus,
1758) and Cupressopathes cf. pumila (branched corals), which were identified following
Terrana et al. (2020) (Fig. 3). During the same period, three more colonies of C. anguina
were collected at 12 m depth at a second site S2 (S 23° 22.977’ E 43° 38.152’) situated
nearby in a natural basin of the Great Reef of Toliara (Fig. 2). Water temperature in each site
was recorded with HOBO TitbiT V2 sensors (accuracy: ± 0.2°C) every 10 minutes between
May 9th and 23th, 2019.

Although current velocities were not measured, SCUBA diving observations revealed a
stronger flow at the site S1 than at the site S2, due to tidal currents directly affecting the first
site and to the fact that the second site is protected from water movements by its basin
configuration. At both sites, no wave action was observed on the corals. Colonies were
immediately brought back to the laboratory in the "Institut Halieutique et des Sciences
Marines" (University of Toliara) maintained in the water from the collection sites. Colonies of similar size per species were collected and their stalks divided into three major basal, median and distal segments corresponding to thirds of the total length. These three segments were further divided into subsections in order to obtain contrasted samples (ca 1.5 cm to ca 6 cm long) separated from samples of other major segments by unused sections. The samples of each segment were either desiccated (48h, 60°C), kept fresh (alive) or frozen at -20°C. All tested samples had polyps and coenenchyme kept on the skeleton. Pinnules and subpinnules of branched colonies were removed in order to test the main axial stem.

2.2. Mechanical testing

The ability to resist deformation depends upon both shape and material (Koehl, 1977; Wainwright and Koehl, 1976) and provides the physical response of a colony segment as a structure to an external force like a sea current. Three variables used in the static beam theory (Denny, 1988) were measured: E, the Young’s modulus, characterizing the material elasticity; I, the second moment of area of the cross section of the beam, reflecting the way the material is distributed in the structure and EI, the flexural stiffness, accounting for the resistance of a beam to bending when submitted to a load.

In a tensile test, the Young’s modulus E (Pa or Nm²) is defined by the ratio of the stress σ to the strain ε (Wainwright and Koehl, 1976):

\[ E = \frac{\sigma}{\varepsilon} \]

where the stress σ (Pa or Nm²), is the load F (N) per unit of area A (m²), and the strain ε is the ratio of the increase in length ∆L to the initial length L of the sample.

The three-point bending test used to measure the Young’s modulus was performed on a fixed anvil supporting the sample in horizontal position. A load cell applied a vertical force F at the middle of the sample, which produced a bending and a vertical deflection \( w_0 \) of the material (Fig. 4). In this test, the Young’s modulus E can be obtained from the maximal deflection \( w_0 \) observed at the middle of a sample under constraint in the following formula (Gordon, 1978; Vogel, 2003):

\[ w_0 = \frac{FL^3}{48EI} \]
where $F$ is the load (N), $L$ is the effective length of the sample (m), $w_0$ is the maximal deflection (m) and $I$ is the second moment of area (m$^4$) or, using the slope $dF/dw_0$ of the force-deflection curve (Fig. 5):

$$E = \frac{\left(\frac{dF}{dw_0}\right)L^3}{48I}$$

As the typical cross-section of an Antipatharia skeleton is circular and hollow, the second moment of area $I$ of such a cylindrical beam structure, with external and internal radiuses $R$ and $r$ (Fig. 1), is given by (Denny, 1988):

$$I = \frac{\pi}{4}(R^4 - r^4)$$

The flexural stiffness $EI$ (N m$^2$) is obtained from $E$ and $I$.

All samples were tested using an Instron 5543 (1 kN load cell, accuracy 0.25%, Brussels) or a Lloyd L1S1 (10 N load cell, accuracy 0.50%, Toliara) force stand. Both instruments were controlled by software drivers (Bluehill Lite and Nexygen Plus 3, respectively). Each sample was tested by measuring the force-deflection curve (Fig. 5).

When the force $F$ is plotted against the central deflection $w_0$, a straight line is obtained provided that the test remains within the linearly elastic limit of the material, i.e. the beam returns to its original shape after deflection (Wainwright et al., 1982). In order to reduce the measurement error in obtaining the slope of the curve, the elasticity interval was delineated with two limit points on the curve: a) the end of the adjustment artefact observed when the upper flexural accessory (the upper loading anvil) gradually stresses the tissue of the specimen, and b) the start of the asymptotic part of the curve, where the material stops being elastic (Fig. 5). To obtain the Young’s modulus, the slope of the linear part of the curve ($dF/dw_0$) was then computed for each test by linear regression on all the values measured between the limits a) and b). Both external and internal radiuses were measured on the central cross-section of each sample (Fig. 1), cut at half-length after the bending test, with a magnifying lens Wild Heerbrugg M8 equipped with a 10X micrometric ocular calibrated with a 2 mm scaled micrometre (0.04 mm accuracy at 120X magnification).

The load cell was equipped with an accessory fitted with a rounded end to avoid shearing.

Adjacent fresh samples were tested on Lloyd equipment at three different speeds of the load cell displacement (0.1; 0.5 and 5 mm/minute) to verify that the outcome of the test was
independent of the cell displacement speed and that the testing was carried out in static equivalent conditions as required by the static beam theory (Boresi and Schmidt, 2003). As no wave oscillations were observed to impact the collection sites, only the tidal current was responsible for a stress on the colonies, resulting in limited strain rates, comparable to the tested cell displacement speeds. Frozen-thawed and fresh samples were tested on Lloyd equipment to verify that freezing does not alter the mechanical properties. Each fresh sample was localized, in the living colony, between two frozen samples of which the measured average was used in the comparison. The mechanical properties of desiccated and frozen-thawed samples were also tested and compared on Instron equipment. Eventually, the mechanical variables of species and segments were tested on frozen-thawed samples on Instron equipment.

2.3. Data analysis

The significance level was set at $\alpha = 0.05$ for all tests. To limit the collection of samples, the speed effect data was measured on $C.\ anguina$ only and analysed using one-factor repeated ANOVA (with speed as fixed factor and colony as repeated factor). The effect of freezing and of desiccation was studied on the four species and analysed using bivariate linear regression with intercept set on zero. These analyses were carried out on R software 3.4.1 (R Core Team, 2021).

The Young’s modulus $E$, the second moment of area $I$ and the flexural stiffness $EI$ of all samples were analysed on SYSTAT 12 (Systat Software Inc) and compared using two-factor ANOVA mixed model 3 with species (fixed crossed factor), segment (repeated fixed crossed factor) and colony (random factor nested in species) according to the recommendations of Doncaster and Davey (2007). Multiple comparison Tukey tests were carried out on significant effects using the relevant mean square error.

3. RESULTS

The temperature of the water habitat from which the specimens were collected was $25.4 \pm 0.5^\circ C$ (n = 2574) on S1 and $25.5 \pm 0.3^\circ C$ (n = 1581) on S2. Room temperature where the samples were maintained varied between $23^\circ C$ and $25^\circ C$.

3.1. Effect of bending speed
No significant difference was found between Young’s moduli and flexural stiffnesses measured at different speeds of the load cell displacement ($F_{1,5} = 0.133 ; p = 0.73$ and $F_{1,5} = 2.89 ; p = 0.15$, respectively) (Table 1). Consequently, all subsequent tests were conducted at 0.5 mm/minute, a compromise speed which is both fast enough to allow the testing of numerous samples and slow enough to collect high resolution data, in order to easily determine the curve inflection points.

3.2. Effect of sample preservation

Regression of Young’s modulus of adjacent distal segments of 11 colonies from 4 species, tested after freezing-thawing vs fresh preservation, in the 1-10 N force spectrum, had a slope of $1.08 \pm 0.26$ (m ± CI95), not significantly different from 1 ($F_{1,10} = 65 ; p < 0.001$ ; adjusted $R^2 = 0.85$).

The same regression of the flexural stiffness had a slope of $0.84 \pm 0.10$ (m ± CI95), significantly different from 1 ($F_{1,10} = 272 ; p < 0.001$ ; adjusted $R^2 = 0.96$) indicating a slightly lower value for frozen-thawed samples.

Similarly, dried and frozen-thawed samples of adjacent distal segments of 11 colonies from 4 species were compared in the 1-1000 N force spectrum (Instron tester) within the limits of elastic behaviour. The slope of the regression amounted to $1.71 \pm 0.23$ (m ± CI95) for the Young’s modulus, significantly different from 1 ($F_{1,10} = 209 ; p < 0.001$ ; adjusted $R^2 = 0.95$).

The same regression of the flexural stiffness had a slope of $1.72 \pm 0.20$ (m ± CI95), significantly different from 1 ($F_{1,10} = 287 ; p < 0.001$ ; adjusted $R^2 = 0.96$).

3.3. Mechanical properties of four antipatharian species

The Young’s modulus of the antipatharian skeleton did not significantly differ between the four tested species ($P_{ANOVA} = 0.42 ; F = 1.06$) but it did differ between segments in all species ($P_{ANOVA} = 0.001 ; F = 10.79$) (Table 2, Table SI1). The distal segment showed a Young’s modulus significantly higher than that of the other segments, which did not differ from each other.

The second moment of area I, and the flexural stiffness EI, of the same segments differed significantly according to the species, the segments and their interaction ($P_{ANOVA} \leq 0.04$) (Table 3, Tables SI2,3). In whip species, the basal segment had a significantly higher second moment of area and flexural stiffness than the distal segment (both species) and than the
median segment (C. anguina). In branched species, both variables did not differ significantly between segments. Both variables differed significantly between whip and branched species in the basal segment, whip species showing values higher by an order of magnitude (Table 3).

4. DISCUSSION

The Young’s modulus was not affected by the freezing-thawing process but the flexural stiffness measured after thawing was reduced by an average of 16%, a correction factor that can be used if data coming from fresh and frozen-thawed specimens has to be compared. The desiccation more seriously affected both the Young’s modulus and the flexural stiffness as these mechanical properties increased by more than 70% in the process. Although this observation may lead to questioning studies conducted on dehydrated samples, Kim et al. (1992) rehydrated desiccated samples of the two branched species Antipathella fiordensis (Grange, 1990) and Aphanipathes salix (Pourtalès, 1880), formerly known as Anthipathes fiordensis and Antipathes salix, respectively. For both species he measured Young’s moduli values comparable to those of our specimens (1240 N mm$^{-2}$ and 3200 N mm$^{-2}$, respectively). Notwithstanding, if preservation is needed, freezing is recommended.

In the present study, measurements were conducted at the segment level and evidenced that the Young’s modulus of the distal segment was significantly higher than that of the median and of the basal segments and that it did not differ significantly between the two latter segments. This observation was unexpected as the distal segment of a branched species was shown to have a lower diphenol concentration, which was linked to a lower sclerotization (Holl et al., 1992). This should result in a lower stiffness (lower Young's modulus) in the distal segments. Currently, we have no explanation for the observed higher Young's modulus in distal segments.

In the two whip species, the flexural stiffness of the basal segment was higher than that of the distal segment, or even of the median one for C. anguina. This difference is only due to the higher second moment of area of the basal segment (whose Young’s modulus is lower than that of the distal segment in both species). So, the morphology of the basal segment, in particular its diameter, principally determines the relative flexural stiffness of different species.

If a cantilever has a uniform cross-sectional shape, maximum stresses occur near the base of the beam (Koehl, 1977; Wainwright et al., 1982). Therefore, cantilever-like sessile organisms are expected to experience the highest stress from the ambient flow in their basal
Maintaining a whip colony vertically under ambient flow suggests that the relative feeding success of zooids located on the median and distal segments may not be reduced as much as in a bent-over colony (Fig. 6A). Indeed, in the latter configuration, zooids on median and distal segments would be in downstream position and Okamura (1984) showed that upstream colonies of arborescent bryozoan interfere with the feeding success of zooids of colonies downstream. In addition, on a colony held in vertical position, polyps of median and distal segments, being farther from the substratum, are met by a stronger flow, which can contribute to a better feeding success. The high flexural stiffness of the basal segment of whip species probably optimizes food capture.

Branched species do not show significant differences in the flexural stiffness between the segments of a colony, which is in line with the absence of differences in the second moment of area and confirms the importance of the morphology of the skeleton. Furthermore, the flexural stiffness of these species is much lower than that of whip species, which means they readily bend under the current, a movement that is further increased by the larger drag induced by their branched morphology. That flexibility suggests a mechanism allowing these species to avoid being torn off or breaking when the current increases. During bending events, the feeding process may be reduced to a lesser extent than in whip species because a larger food capture area remains functional thanks to the polyps present on the lateral branches and pinnules (Fig. 6B). The more branches the colony produces, the greater feeding capacity it is expected to acquire while, at the same time, it increases its drag. The bottlebrush morphology of the studied species thus represents a compromise between a highly branched colony development and an acceptable friction drag (Tazioli et al., 2007).

In conclusion, the flexural stiffness of black corals, a key character for a sessile organism with a cantilever-like configuration, appeared to be principally determined by the second moment of area, and thus their diameter. The latter is very different between whip and branched species, an observation consistent with different adaptations of each species to a strong current environment. While both types of species cohabit in identical flow conditions, whip species maintain a vertical position while branched species can readily bend over. This suggests that the specific flexural stiffness is linked to the contrasted feeding strategies of species with different morphs in a similar strong current environment. This hypothesis could be tested with cameras and water velocity measuring equipment.

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**FIGURE LEGENDS**

**Figure 1.** Cross section of the skeletal axis of *Cirrhipathes anguina*, showing the central canal with the internal ($r$) and the external ($R$) radiuses.

**Figure 2.** Map of Madagascar (right) and of Toliara bay (left). S1 and S2: collecting sites (arrows); BR: Barrier reef; FR: Fringing reef; IH.SM: Institut Halieutique et des Sciences Marines (modified from Rasolofonirina et al. 2005.)

**Figure 3.** Studied species. Underwater photographs in their natural environment of: (A) *Cirrhipathes anguina*, (B) *Stichopathes* cf. *maldivensis*, (C) *Cupressopathes abies* and (D) *Cupressopathes* cf. *pumila*. Scales: 10 cm (one segment), pictures: (A) (C) and (D) Lucas Terrana, (B) Mathilde Godefroid.

**Figure 4.** A. Three-point bending test configuration, showing the effective sample length (L), vertical deflection at the centre of the sample ($w_0$), and applied force (af). B. Adjustable anvil (aa) and stabilising support (ss). C. Three-point bending test system showing the load cell terminated with a stress accessory in contact with the sample placed horizontally on the anvil during the test.

**Figure 5.** Force-deflection at the centre of the sample curve $F$ (N) vs. $w_0$ (mm) obtained with *Cupressopathes abies*. The slope of the curve ($dF/dw_0$) allows to compute the Young’s modulus. Only the values measured within the elasticity interval defined by the limits $a$ and $b$ have been used to obtain the slope by linear regression.

**Figure 6.** (A) Reduction of food capture by whip coral when the skeleton bends under the current. In vertical position, the skeleton maintains all the polyps in an optimised feeding position, (B) Reduction of food capture by branched coral when the skeleton bends under the
current. The food capture area remains functional thanks to the polyps present on the branches and pinnules.
Table 1. Young’s modulus and flexural stiffness of skeleton distal segments of three fresh colonies of *C. anguina* measured at three load cell displacement speeds (mean ± SD, n = 3; Lloyd tester, 1-10 N).

<table>
<thead>
<tr>
<th>Load cell displacement speed (mm/minute)</th>
<th>Young’s modulus E (MPa)</th>
<th>Flexural stiffness EI (N mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2690 ± 898</td>
<td>19630 ± 13993</td>
</tr>
<tr>
<td>0.5</td>
<td>2560 ± 1842</td>
<td>14155 ± 8694</td>
</tr>
<tr>
<td>5</td>
<td>2801 ± 1209</td>
<td>23020 ± 16109</td>
</tr>
</tbody>
</table>
Table 2. Young’s modulus of the antipatharian skeleton per segment averaged across four species (mean ± SD, n = 12), tested with frozen-thawed samples. Means sharing the same index are not significantly different (Tukey test, α = 0.05).

<table>
<thead>
<tr>
<th>Segment</th>
<th>Young's modulus E (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>distal</td>
<td>2911 ± 953</td>
</tr>
<tr>
<td>median</td>
<td>2207 ± 520</td>
</tr>
<tr>
<td>basal</td>
<td>1661 ± 681</td>
</tr>
</tbody>
</table>
Table 3. Second moment of area and flexural stiffness per species and per segment (mean ± SD, n = 3), tested with frozen-thawed samples. Latin character indices: comparisons between segments of a same species, Greek character indices: comparisons between species of a same segment. Means sharing the same indices for a same segment (Latin character) or for a same species (Greek character) are not significantly different (Tukey test: α = 0.05). Comparisons of the second moment of area and flexural stiffness between species are given only for segments in which significant differences were recorded.

<table>
<thead>
<tr>
<th>Species</th>
<th>Second moment of area I (mm$^4$)</th>
<th>Flexural stiffness EI (kN mm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>distal</td>
<td>median</td>
</tr>
<tr>
<td></td>
<td>Cirrhipathes anguina</td>
<td></td>
</tr>
<tr>
<td></td>
<td>105± 164</td>
<td>134± 135</td>
</tr>
<tr>
<td></td>
<td>Stichopathes cf. maldivensis</td>
<td>15± 5.8</td>
</tr>
<tr>
<td></td>
<td>Cupressopathes cf. pumila</td>
<td>0.08± 0.06</td>
</tr>
<tr>
<td></td>
<td>Cupressopathes abies</td>
<td>0.25± 0.11</td>
</tr>
</tbody>
</table>