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# Tests and jaws' morphological patterns of regular sea urchin *Paracentrotus lividus* (Lamarck, 1816) in relation to environmental conditions

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► **To cite this version:**

Nathalie Caill-Milly, Jérémy Mahias, Noëlle Bru, Marie-Noëlle De Casamajor, Thierry Pigot, et al.. Tests and jaws' morphological patterns of regular sea urchin *Paracentrotus lividus* (Lamarck, 1816) in relation to environmental conditions. *Regional Studies in Marine Science*, 2020, 35, pp.101220. 10.1016/j.rsma.2020.101220 . hal-02515305

**HAL Id: hal-02515305**

**<https://univ-pau.hal.science/hal-02515305v1>**

Submitted on 28 Feb 2023

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1 **Tests and jaws' morphological patterns of regular sea urchin *Paracentrotus lividus* (Lamarck,**  
2 **1816) in relation to environmental conditions**

3

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18 *Submitted to REGIONAL STUDIES IN MARINE SCIENCE*

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24

25 **Abstract:**

26 This paper investigates whether morphological variability of test and Aristotle's lantern can be  
27 detected in sea urchins exposed to domestic pollution and whether this variability can be also related  
28 to tidal level and hydrodynamic features. The study was conducted on four sites along the French  
29 Basque coast. Each site is characterized by its geological layers' orientation, regardless of its proximity  
30 to sewage outlets. Analyses on high-resolution pictures of jaws were performed and three parameters  
31 were retained: length, height and demipyramid dry mass. Five shape descriptors were consequently  
32 defined regarding the test and the jaws. Individuals with heavy test density associated with highly  
33 developed demipyramid regarding test diameter and height were preferentially observed in intertidal  
34 areas, remoteness from treatment plants and in south-oriented geological layers. High test  
35 hemispherical index and demipyramid elongation index were preferentially found far away from  
36 treatment plants. Results contribute to improving knowledge on pressure impact assessment and on  
37 state of the environment conservation in a defined area. It may also be useful to propose management  
38 measures on an appropriate scale for a species that is usually managed on a regional basis.

39

40 *Keywords:* morphometry; body size; Aristotle's lantern; sewage discharge; coastal zone; biological  
41 indices

42

## 43 **1. Introduction**

44 The quality of the marine environment, especially for coastal water, is dependent on driving forces  
45 such as agriculture, industry, population and ports (Uriarte and Borja, 2009). They generate pressures,  
46 for example nutrient discharge and water or sediment pollution, which are the main concerns of local  
47 authorities. In this context, accurately assessing the impact of this pressure alongside ascertaining the  
48 state of environmental conservation in a defined area are a necessity. This is legislatively expressed  
49 within the framework of the European Marine Strategy Directive (MSFD; Directive 2008/56/EC),  
50 which aims to achieve and maintain a good ecological status by 2020. Maintaining the conservation  
51 status of species and habitats is also an objective of the Fauna and Flora Habitat Directive (DFFH,  
52 Directive 1992/43/EC) especially on the Basque coast classified in N2000 zone "rocky Basque coast  
53 and offshore extension". However, meeting those directives and implementing sustainable  
54 management for conservation and/or exploitation purposes present some clear difficulties: firstly,  
55 separating natural processes from anthropogenic impacts; secondly, differentiating anthropogenic  
56 impacts that may operate simultaneously. In this context and at population-scales regarding fish,  
57 bivalves or echinoderms, identification of phenotypic variations in morphological patterns and  
58 clarification of the mechanisms involved contribute to better understand how the populations function.  
59 In particular, it makes it possible to assess the health status of those populations along with other  
60 descriptors based, for instance, on body fat measures (Berkeley et al., 2004). This knowledge  
61 integration is relevant to identify, implement or modify appropriate management measures applied to  
62 those resources from Europe-wide to local conditions (i.e. selection of marine protected areas).

63 Although the Spanish Basque coast already benefits from a lot of available data to address the need of  
64 the eleven MSFD descriptors (Borja et al., 2011), data are scarce on the French side (de Casamajor et  
65 al., 2017). However, the Basque coastal area on both sides of the border is subject to strong human  
66 pressures with a high density of population over the year, and especially during the summer with  
67 tourist activities (Borja et al., 2011).

68 The data collected in this study aims to improve available information to evaluate the impact of human  
69 activities and of physical conditions on a resource. Bivalve molluscs, oysters and mussels are the main  
70 biological indicators used in the marine environment for heavy metals as well as sewage impact.  
71 However, on the Basque coast these species cannot be considered due to unfavourable conditions for  
72 their development. The use of Echinoderms can be an alternative. For example, *P. lividus* is  
73 considered to be a sentinel organism for ecotoxicological studies and is used in monitoring programs  
74 assessing coastal aquatic environments quality (Camacho et al., 2018; Huguenin et al., 2018). More  
75 generally Echinoderms are good candidates for such a study since various parameters have been  
76 identified for their impact on individuals' morphology: hydrodynamic conditions (Jacinto & Cruz,  
77 2012; Jacinto et al., 2013), food resources (defined as food quality and food availability – Ebert, 1996;  
78 Fernandez & Boudouresque, 1997; Guillou et al., 2000), current, ocean acidification (Asnaghi et al.,  
79 2014; Wang et al., 2013), density (Black et al., 1982; Ebert, 1968); pollution (Dinnel and Stober,  
80 1987; Pancucci et al., 1993; Régis, 1986; Rouane-Hacene et al., 2018; Savrima et al., 2015), etc.  
81 Morphological changes concern not only the test but also the dental apparatus and specifically the  
82 demipyramids, calcified constituents of the Aristotle's lantern.

83 Based on a conventional shape analysis using morphometric ratios involving metrics and weight of  
84 calcified structures (test and jaws), the study focuses on whether variability patterns exist in the  
85 morphological characteristics and whether any of the observed morphological patterns can be related  
86 to environmental conditions (natural and/or anthropogenic).

## 87 **2. Material and methods**

### 88 2.1. Study area

89 The French Basque coast is located on the southeastern side of the Bay of Biscay (southwest of  
90 France). The bedrock is mainly composed of a flysch facies intersected in some places by boulder  
91 fields or by sandy beaches and estuaries. This coast is subject to extensive freshwater inflows due to  
92 high rainfall of around 1500 and 2000 mm per year (Usabiaga et al., 2004; Winckel et al., 2004). In  
93 addition, there are several disposal release points from wastewater treatment plants (WWTP) along the

94 shoreline. They contribute to the freshening of the coastal waters. The tidal regime is mesotidal with a  
95 tidal range between 1.85 m and 3.85 m on average. On the south of the Bay of Biscay, the tidal range  
96 resulting with low currents, it has low influence (Idier and Pedreros, 2005; Augris et al., 2009) on  
97 erosion process and benthic colonization. Hydrodynamic conditions are characterized by the presence  
98 of high-energy waves (Abadie et al., 2005) breaking on the shore (mean, 1.8 m height for an average  
99 period of 9.6 s) and on sea urchin habitat.

100 *P. lividus* is well known as a key species for the control of vegetation dynamics (Ruitton et al., 2000;  
101 Boudouresque and Verlaque, 2013). Overexploitation can have an impact on ecosystem stability by  
102 limiting vegetation consumption. For the French Basque coast and in the context of the Water  
103 Framework Directive (WFD), information about macroalgae communities, as well as the dominant  
104 species, is available through two parameters regarding the intertidal and subtidal areas (de Casamajor  
105 and Lissardy., 2018). In algal communities, the main species are Rhodophyta with a high presence of  
106 Corallinales in the intertidal area and Gelidiaceae in the subtidal area. Varying Ochrophyta can also be  
107 observed on this coast, which is highly exposed to swell and high temperatures. Finally, development  
108 of Chlorophyta can be observed in spring and summer. Densities and diversity of algae described for  
109 the Basque coast (de Casamajor et al., 2012) suggest that vegetation is not a limiting factor for sea  
110 urchins. The sampling program was undertaken on the same habitat type (limestone-marl) and during  
111 a short period of time (between May and July 2014).

## 112 2.2. Environmental patterns of the selected sites

113 In the study area, coastal currents are highly subject to highly energetic waves oriented mainly W-NW.  
114 Those currents induce a mixing of water masses and contribute to the harmonization of coastal water  
115 at intertidal and subtidal scales. To consider environmental (natural and anthropogenic) variability  
116 along the French Basque coast, the following conditions were selected:

117 - one concerns the proximity to (less than 250 m), or remoteness from a WWTP, encoded PTP –  
118 proximity of treatment plant; RTP – remote from treatment plant; The treatment plant can be viewed

119 as a potential source of organic matter and nutrients impacting the morphological characteristics of the  
120 individuals.

121 - another concerns bathymetric considerations with two bathymetric levels, intertidal (Intertidal) and  
122 subtidal (Subtidal); Bathymetry induces differences in food resources, hydrodynamic conditions... and  
123 can thus also led to morphological differences among individuals;

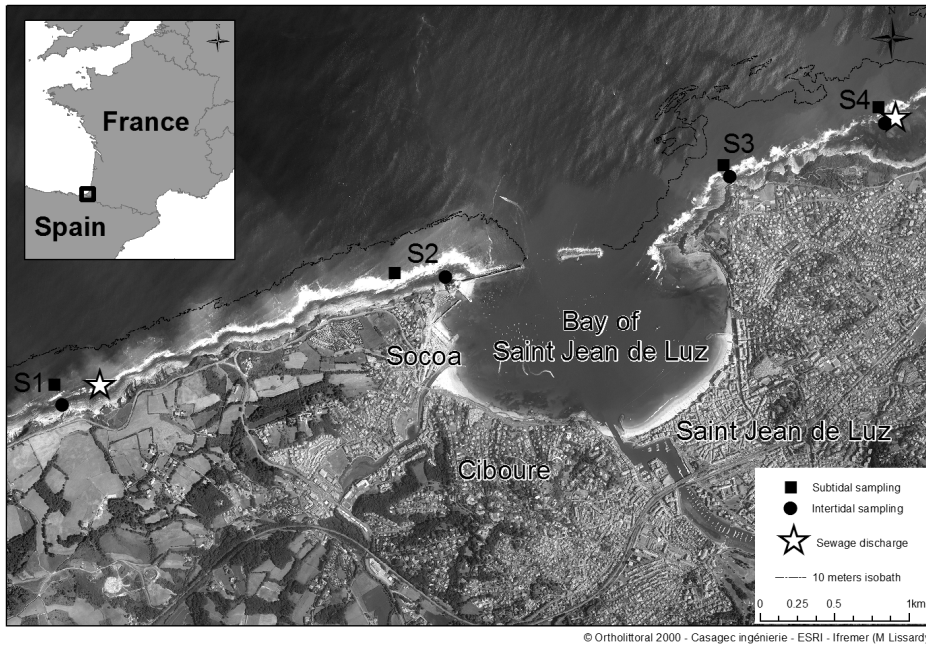
124 - the last concerns, for habitats considered to be similar, variation in geological layers' tilt with south  
125 (Position S) and north (Position N) of the Saint-Jean-de-Luz Bay. These variations are especially  
126 responsible for shelters features differences. The first condition refers to anthropogenic conditions,  
127 whereas the following two refer to environmental ones.

128 Four sites (sampling points) were selected. The sites S1 (south) and S4 (north) are located near  
129 WWTPs. The characteristics of the two WWTPs are the following:

130 - WWTP near S1 : biological filter;  $7\,000\text{ m}^3\cdot\text{d}^{-1}$ ,  $2\,400\text{ kg BOD}_5\cdot\text{d}^{-1}$ ;

131 - WWTP near S4 : activated sludge;  $1\,340\text{ m}^3\cdot\text{d}^{-1}$ ,  $600\text{ kg BOD}_5\cdot\text{d}^{-1}$  .

132 Site S2 (south) and S3 (north) are characterized by mixed waters which are not really influenced by  
133 the direct impact of sewage discharge (marine, continental and sewage). Locations and characteristics  
134 of the sampling points are presented in Figure 1 and Table 1.



135

136 **Fig. 1.** Maps showing the studied area on the French Basque coast (SW France) and localization of the four  
 137 sampled sites (Sources: ESRI, BD Carthage, Ifremer – M. Lissardy).

138 **Table 1**

139 Characteristics of the four sampling points (geographic coordinates, proximity of treatment plant, sampling  
 140 periods, number of sampled individuals and average test diameter (+/- standard deviation) of the sampled  
 141 *Paracentrotus lividus*) with direction of the current mainly from to W-NW.

	S1	S2	S3	S4
Geographic coordinates (WGS84)	43°388' N 01°716' W	43°396' N 01°689' W	43°404' N 01°662' W	43°408' N 01°649' W
Main pressure	Treatment plan	Mixed coastal water	Mixed coastal water	Treatment plan
Sampling period	May–June 2014	June 2014	June 2014	May–July 2014
Number of sampled <i>Paracentrotus lividus</i>	64	64	64	64



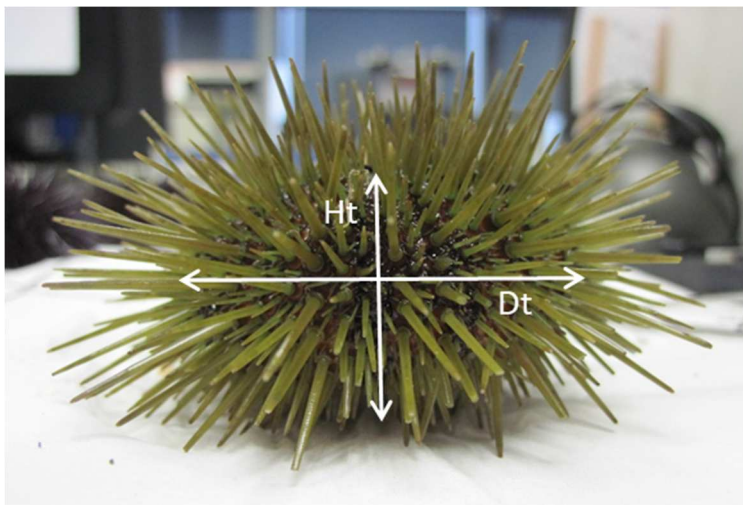
Mean length of sampled <i>Paracentrotus lividus</i>	54.8 mm (SD = 4.8)	53.1 mm (SD = 7.4)	52.0 mm (SD = 4.9)	58.7 mm (SD = 3.5)
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142

143           2.3. Collection and preparation of the biological material, and measurements

144   At each site (S1 to S4) and at each of the two bathymetric levels, 32 sea urchins were hand-collected  
 145   (for a total of 256 individuals). For the intertidal area, *P. lividus* were collected at low tide of high tide  
 146   coefficients; for the subtidal area, they were collected by scuba divers at 5 m depth (reduced to zero on  
 147   the nautical charts). The sampling targeted individuals are those with a test diameter exceeding 35 mm  
 148   in order to consider only adults and to limit variations of sources (i.e. growth-related). Table 1 shows  
 149   the number and average length of individuals for each site sampled. Once collected, samples were  
 150   transported alive in a cooler to the laboratory for processing.

151   Before dissection, each sampled sea urchin was blotted dry on a paper towel. The test diameter at  
 152   ambitus (Dt) and height (Ht) – excluding spines – of each sea urchin were then measured (Figure 2) to  
 153   the nearest millimeter using a digital caliper. Next, urchins were dissected in order to remove  
 154   individuals’ gut and gonads. The test (with spines and the Aristotle’s lantern) was dried at 60°C for  
 155   48 hours in order to obtain its dry mass (DM) with an accuracy of 0.01 g.



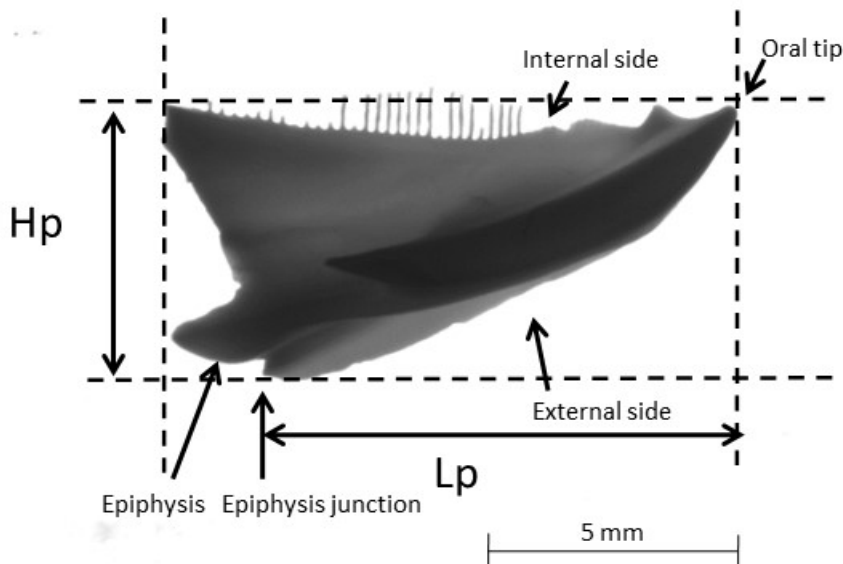
156

157 **Fig. 2.** Measures considered on each sea urchin test: Dt - test diameter at ambitus (mm) and Ht – height (mm).

158 We also focused on the Aristotle's lanterns. They were bleached with sodium hypochlorite (5%) for  
159 48 hours to withdraw ligaments or muscle residues. Once rinsed in tap water and air dried, a randomly  
160 selected demipyramid from each individual was extracted from the lantern components and weighted  
161 to the nearest 0.001 mg. Analyses on high-resolution pictures (from the lateroradial side) were  
162 performed afterwards with TNPC® software (Digital Processing for Calcified Structures,  
163 www.TNPC.fr) on each demipyramid. To describe the morphology of this component, two parameters  
164 were retained (Figure 3):

165 - Length (Lp), defined as the distance from the epiphysis junction to the oral tip of the jaw  
166 (mm);

167 - Height (Hp), as the distance from the distal shelf that articulates with the epiphysis to the  
168 internal side of the demipyramid (mm)



169

170 **Fig. 3.** Measurements retained on demipyramid to describe morphology of the Aristotle's lantern: distance from  
171 the epiphysis junction to the oral tip of the jaw (mm); height (Hp): the distance from the distal shelf that  
172 articulates with the epiphysis to internal side of the demipyramid (mm).

173 Accuracies of almost 0.001 mm is associated with those linear measures. Taken separately, these  
174 parameters do not provide any interesting morphological description of the sea urchin due to a size  
175 effect. Test volume was estimated using the formula of a spherical cap,  $Vol =$   
176  $(\pi * Ht/6) * [3 * (Dt/2)^2 + Ht^2]$  (Pomory and Lares, 2011).

177 Five shape descriptors/ratios were then defined from this set of parameters regarding the test and the  
178 jaws (Table 2):

- 179 - Test hemispherical index (Ht to Dt). Ht to Dt close to 1 reveals a rounded shape;
- 180 - Test density index (DM to Vol). The higher the ratio, the more the sea urchin presents a  
181 heavy test in relation to its volume;
- 182 - Demipyramid length to test diameter ratio (Lp to Dt). This descriptor provides information  
183 on the relative length of the Aristotle lantern regarding test diameter;
- 184 - Demipyramid length to test height ratio (Lp to Ht). The higher the ratio is, the more the  
185 sea urchin has a developed Aristotle lantern related to its height;
- 186 - Demipyramid elongation index (Hp to Lp). The smaller the ratio, the more the lantern has  
187 an elongated shape.

188 Such indices thus enable to describe morphology of the test and the jaws, relative proportion between  
189 test and jaws, as well as test weight considerations. They are supposed to give information on growth  
190 conditions (Ebert et al., 2014) and can be considered in relation to environmental characteristics.

#### 191 2.4. Statistical analysis

192 The coupling between the five ratios defined from measurements on the test and the lantern and  
193 environmental parameters were tested using the Kruskal-Wallis test (with determination of level of  
194 significance) given the lack of normality of the distribution for all the ratios (See Appendix). Box plots  
195 between factors and ratios are presented for significant results (p-value= 5%). In addition, when a  
196 Kruskal-Wallis test value is significant for more than two environmental conditions (i.e. sites), a

197 multiple comparison test after Kruskal-Wallis (kruskalmc) is implemented. Using pairwise  
 198 comparisons, this test helps determining which groups are different.

199 For the five morphometric descriptors, the test diameter was taken into account to detect a potential  
 200 persistent size effect. Normalized Principal Components Analysis (NPCA) was conducted on the 256  
 201 sea urchins sampled to consider the environmental influences on those parameters. Bathymetric level,  
 202 proximity of treatment plant and orientation of geological layers were added as supplementary factors.  
 203 This methodology is widely used for investigations regarding marine invertebrates (Harper and Hart,  
 204 2007; Wildish et al., 1998), even though Berner (2011) recently identified systematic artifacts that can  
 205 disturb the analysis. Significances of qualitative variables according to each principal axis were also  
 206 calculated using correlation tests.

207 Calculations were carried out using R ® software ([http://cran.r-](http://cran.r-project.org/web/packages/Rcmdr/index.html)  
 208 [project.org/web/packages/Rcmdr/index.html](http://cran.r-project.org/web/packages/Rcmdr/index.html)), pgirmess package for kruskalmc and the FactoMineR  
 209 package for NPCA.

### 210 3. Results

211 The five morphological indices present the following mean characteristics (Table 2): a test  
 212 hemispherical index of 0.52 (SD = 0.04); a test density index of 0.47 (SD = 0.07); a demipyramid  
 213 length to test diameter ratio of 0.20 (SD = 0.02); a demipyramid length to test height ratio of 0.39 (SD  
 214 = 0.04) and a demipyramid elongation index of 0.56 (SD = 0.04). One can notice that the associated  
 215 SD are low.

216 **Table 2**

217 Shape descriptors (level and dispersion indicators) regarding the test and the jaws

Descriptor	Formulae	Minimum	Maximum	Standard deviation	
				Median	Interquartile
Test hemispherical index (Ht to Dt)	$= Ht/Dt$	0.41	0.63		0.04

				0.52	0.05
Test density index (DM to Vol)	= $DM/Vol$	0.34	0.72	0.46	0.07 0.08
Demipyramid length to test diameter ratio (Lp to Dt)	= $Lp/Dt$	0.16	0.26	0.20	0.02 0.02
Demipyramid length to test height ratio (Lp to Ht)	= $Lp/Ht$	0.29	0.49	0.39	0.04 0.04
Demipyramid elongation index (Hp to Lp)	= $Hp/Lp$	0.47	0.68	0.56	0.04 0.04

218

219 Whereas test density index (DM to Vol) as well as demipyramid length to test diameter ratio (Lp to  
220 Dt) do not display any links with environmental conditions, the Kruskal-Wallis tests show significant  
221 levels for the three other indices (Table 3).

222 **Table 3**

223 P-value of Kruskal-Wallis tests regarding shape descriptors and environmental conditions

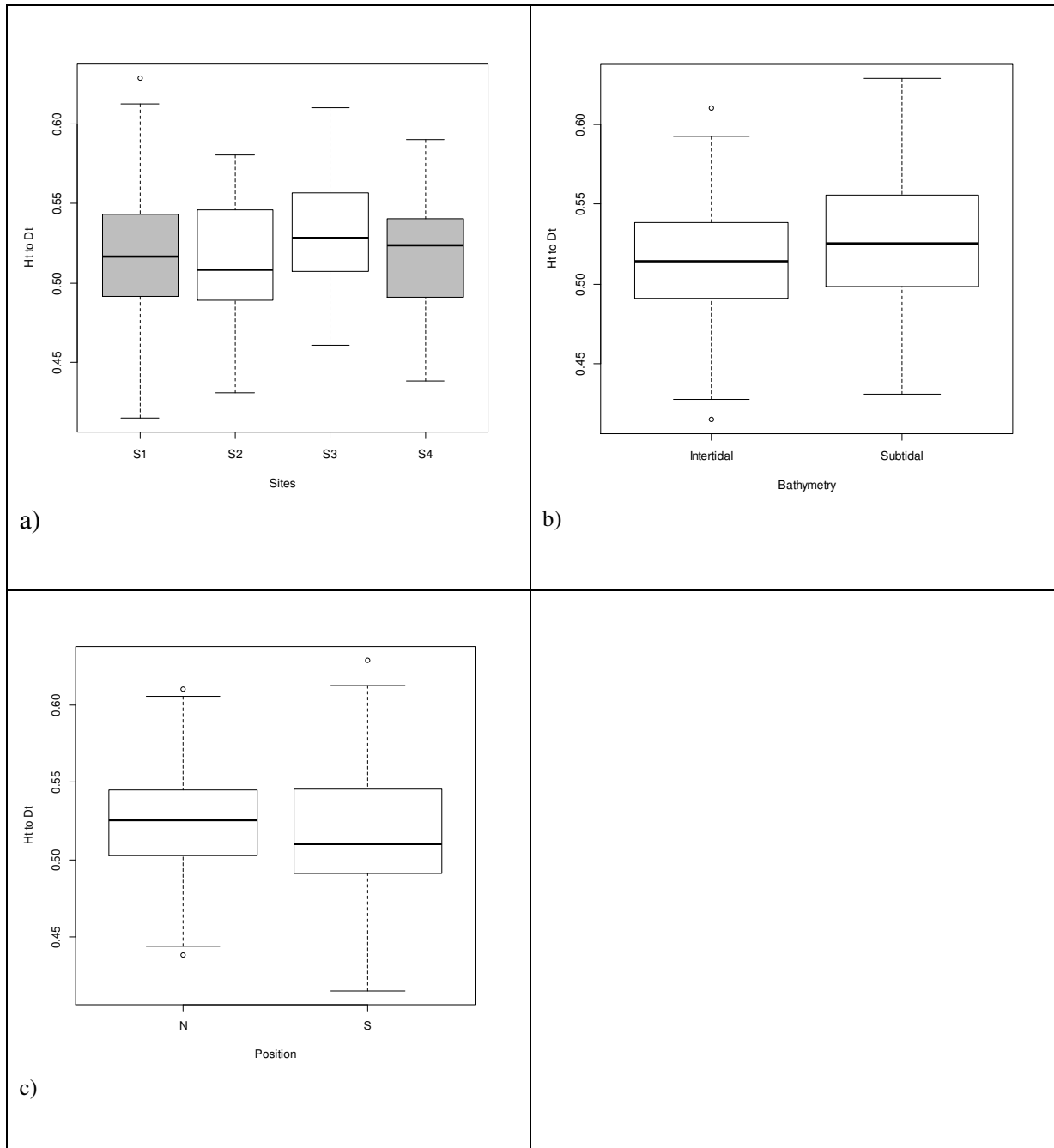
224 (p-value < 0.05 is flagged with “\*”, p-value < 0.01 is flagged with “\*\*”, p-value < 0.001 is flagged with “\*\*\*\*”)

	Sites (S1/S2/S3/S4)	Proximity of treatment plant (PTP/RTP)	Position (N/S)	Tidal level (Inter/Sub)
Test hemispherical index	0.026*	0.352	0.039*	0.010**

(Ht to Dt)				
Test density index (DM to Vol)	0.324	0.067	0.738	0.638
Demipyramid length to test diameter ratio (Lp to Dt)	0.075	0.151	0.406	0.459
Demipyramid length to test height ratio (Lp to Ht)	0.137	0.898	0.019*	0.008**
Demipyramid elongation index (Hp to Lp)	0.705	0.568	0.424	<0.001***

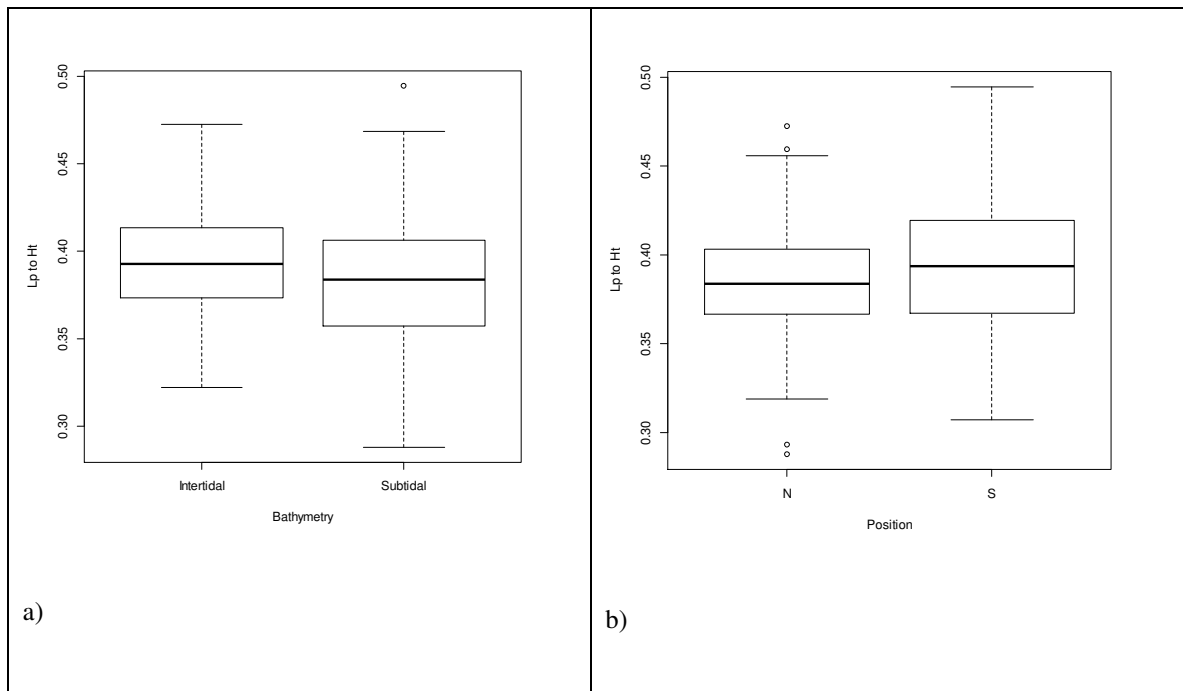
225

226 The test hemispherical index (Ht to Dt) differs from one site to another (Fig 4a) knowing that sites  
227 represent a combination of position and proximity of a treatment plant (in grey, sites closed to the  
228 treatment plant). Using multiple comparison test after Kruskal-Wallis, the pair site S2/S3 observes  
229 difference higher than the critical value. It means that the test hemispherical index from S2 and S3 are  
230 considered statistically different at a given significance level. Site S3 displays sea urchins with higher  
231 value than site S2. Furthermore, this index shows significant differences in terms of tidal level (Fig 4  
232 b) and position (Fig 4c). Higher level of Ht to Dt is obtained for subtidal area and one can notice a  
233 higher variability too; lower level of Ht to Dt is obtained in the southern sites associated also with high  
234 variability. The most significant spatial effect is due to first tidal level (p-value= 0.010), then sites (p-  
235 value= 0.026) and finally position (p-value= 0.039) (Table 3).



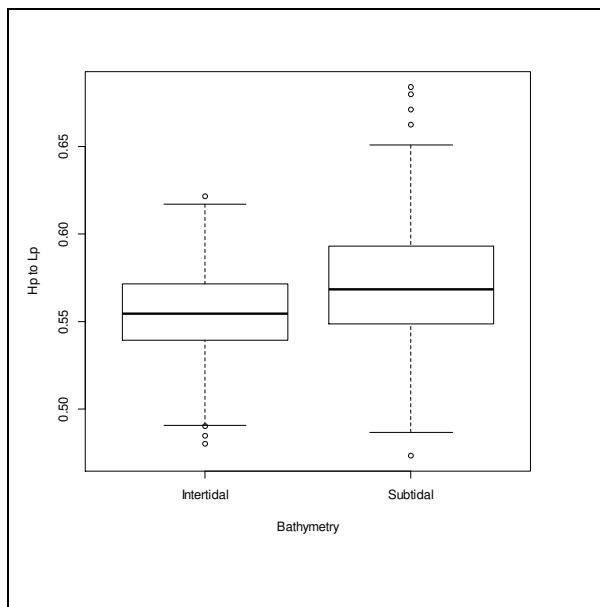
236 **Fig. 4a to c.** Box plots for Ht to Dt and environmental characteristics: a) site (in grey, sites near treatment plant),  
 237 b) bathymetry and c) position. Each characteristic appears significant thanks to the kruskal wallis test,  $\alpha=5\%$ .

238 The demipyramid length to test height ratio ( $L_p$  to Ht) shows significant differences in terms of tidal  
 239 level (Fig 5a) and position (Fig 5b). Lower level of  $L_p$  to Ht is obtained for subtidal area and one can  
 240 notice a higher variability too; higher level of  $L_p$  to Ht is obtained in the southern sites associated also  
 241 with high variability. The most significant spatial effect is due to first tidal level (p-value= 0.008), then  
 242 position (p-value= 0.019) (Table 3).



243 **Fig. 5a to b.** Box plots for Lp to Ht and spatial characteristics: a) position and b) bathymetry. Each characteristic  
 244 appears significant thanks to the kruskal wallis test,  $\alpha = 5\%$ .

245 The demipyramid elongation index (Hp to Lp) shows significant differences in terms of tidal level  
 246 (Fig 6). Higher level of Hp to Lp is obtained for subtidal area and one can notice again a higher  
 247 variability too.



248 **Fig. 6.** Box plots for Hp to Lp and spatial characteristics: bathymetry. This characteristic appears significant  
 249 thanks to the kruskal wallis test,  $\alpha = 5\%$ .



250 Since the tidal level seems to be the most significant effect on the different ratios, we focused on each  
 251 of the bathymetric level to distinguish possible interactions with other environmental conditions  
 252 (Table 4).

253 Table 4

254 P-value of Kruskal-Wallis tests regarding shape descriptors and environmental conditions (p-value < 0.05 is  
 255 flagged with “\*”, p-value < 0.01 is flagged with “\*\*”, p-value < 0.001 is flagged with “\*\*\*\*”)

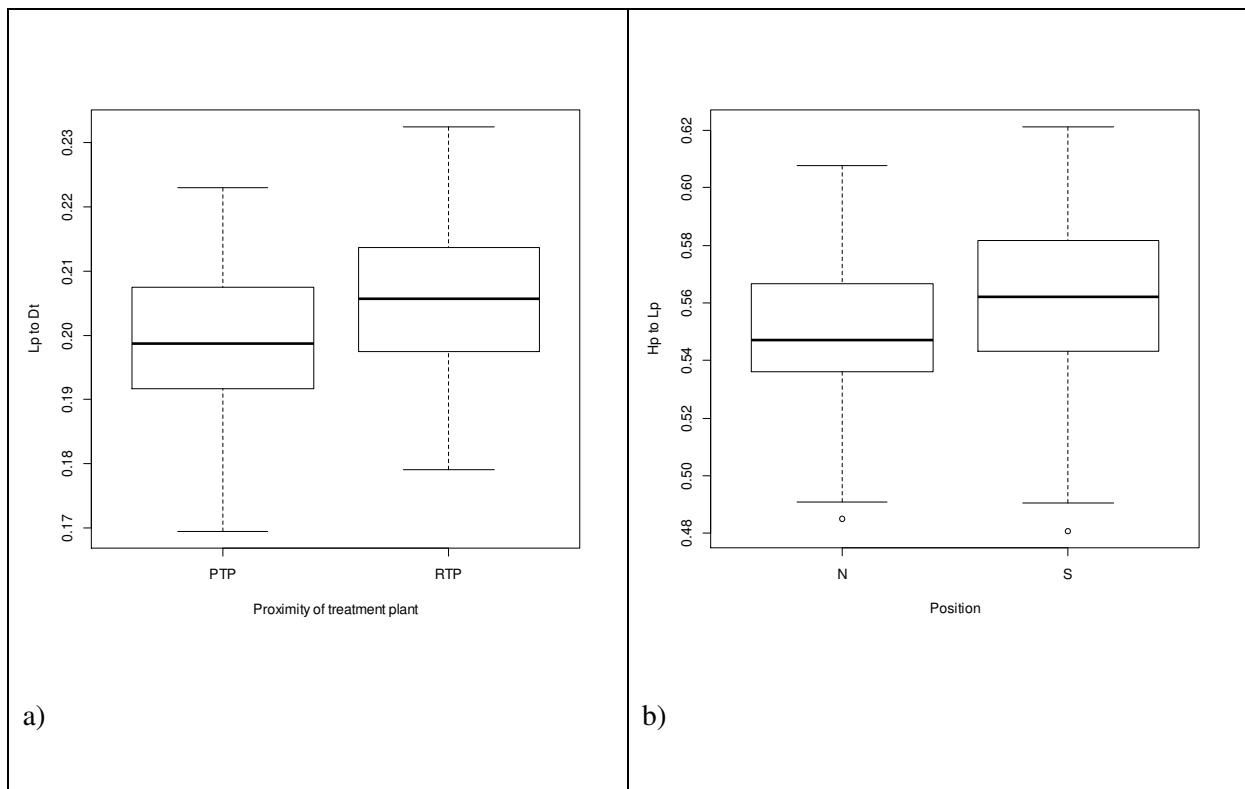
	Intertidal			Subtidal		
	Sites (S1/S2/S3/S4)	Proximity of treatment plant (PTP/RTP)	Position (N/S)	Sites (S1/S2/S3/S4)	Proximity of treatment plant (PTP/RTP)	Position (N/S)
Test hemispherical index  (Ht to Dt)	0.140	0.188	0.069	0.004**	0.928	0.241
Test density index  (DM to Vol)	0.510	0.386	0.970	0.236	0.074	0.710
Demipyramid length to test diameter ratio  (Lp to Dt)	0.044*	0.005**	0.864	0.097	0.790	0.319
Demipyramid length to test height ratio  (Lp to Ht)	0.323	0.545	0.129	0.349	0.739	0.084
Demipyramid elongation index  (Hp to Lp)	0.062	0.386	0.010**	0.491	0.947	0.156

256 For intertidal level:

- 257 - Proximity of treatment plant and sites act on demipyramid length to test diameter ratio (Lp to  
 258 Dt). Lower level is linked to proximity of treatment plant and one can notice a higher  
 259 variability too (Fig 7a). The Kruskal-Wallis test is also significant when considering sites, but

260 the p-value is very closed from the 0.05. In these conditions, we decided not to further analyse  
 261 this last result;

- 262 - Position influences the demipyramid elongation index (Hp to Lp). Higher level of Hp to Lp is  
 263 obtained for the south rather than for the north, but one can notice a higher variability too (Fig  
 264 7b).

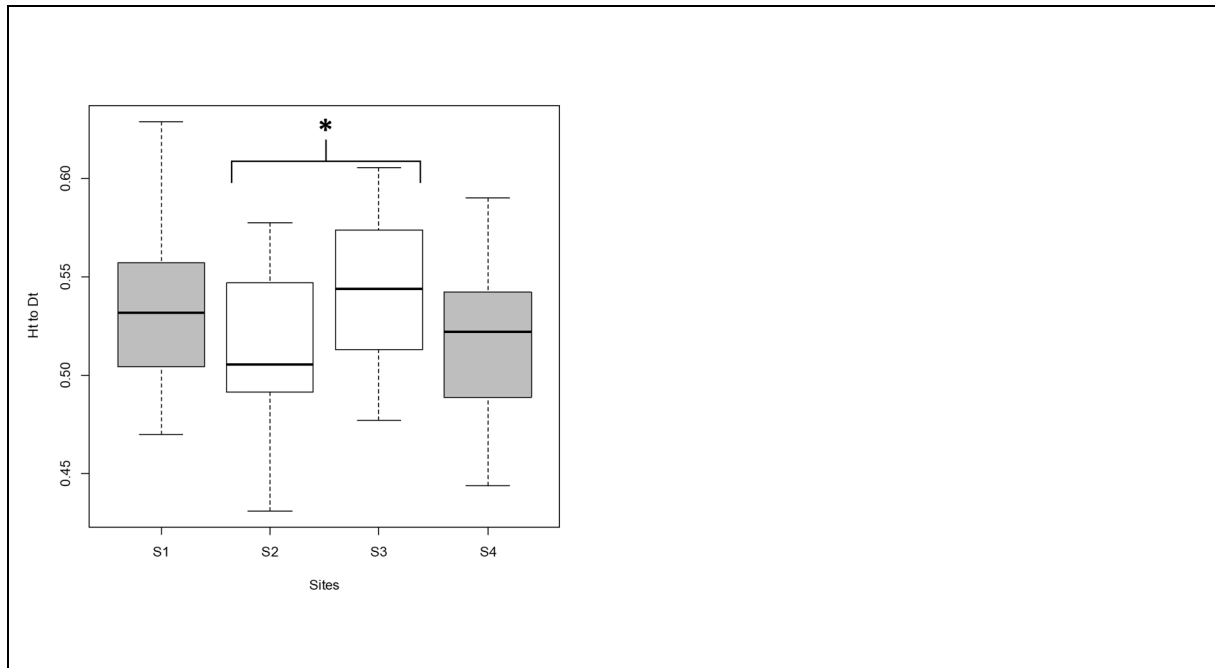


265 **Fig. 7a to b.** Box plots for intertidal: a) Lp to Dt regarding proximity of treatment plant and b) Hp to Lp  
 266 regarding position. Each characteristic appears significant thanks to the kruskal wallis test,  $\alpha= 5\%$ .

267

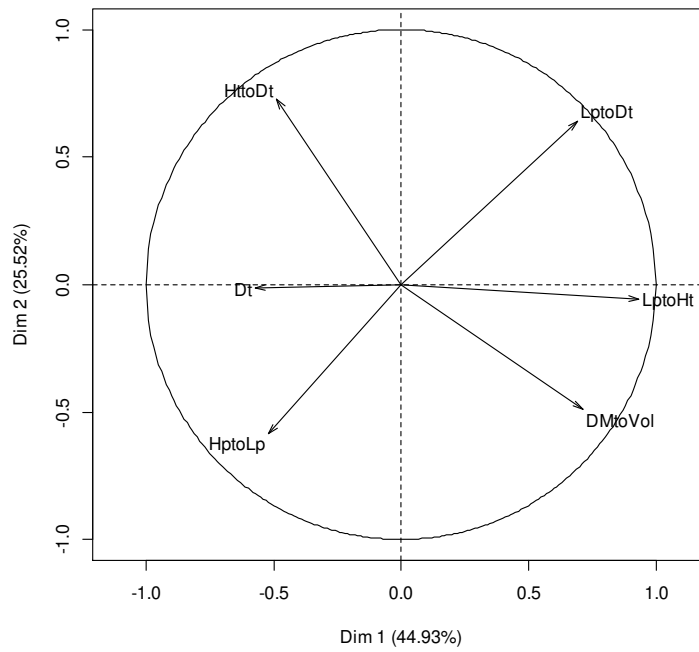
268 For subtidal level, sites act the test hemispherical index (Ht to Dt). Using multiple comparison test  
 269 after Kruskal-Wallis, the pair site S2/S3 observes difference higher than the critical value. It means  
 270 that the test hemispherical index from S2 and S3 are considered statistically different at a given  
 271 significance level. Site S3 displays sea urchins with higher value than site S2 (Fig 8).

272

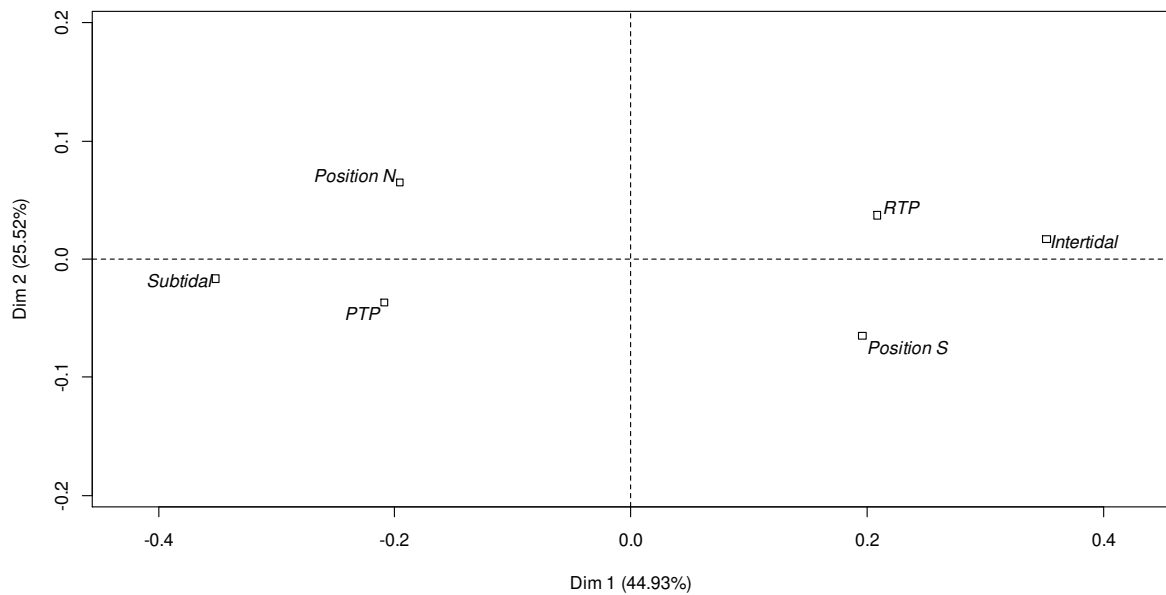


273 **Fig. 8.** Box plots for subtidal: Ht to Dt regarding sites. Significant differences appears thanks to the kruskalmc  
 274 and the concerned pair is marked with “\*”.

275 The NPCA revealed a continuum of variation but no discrete clusters within the considered sample  
 276 (Figure 9). Shape variables were correctly summarized by the first three dimensions of the NPCA  
 277 (89% of the variance). Considering for each principal component the highest values of factor  
 278 coordinates associated with the highest contributions, three variables were identified for each principal  
 279 axis.



a)



b)

280

281 **Fig. 9.** NPCA on morphometric variables with bathymetric level (encoded Intertidal or Subtidal), proximity of  
282 treatment plant (encoded PTP – proximity of treatment plant; RTP – remote from treatment plant) and position  
283 related to the Saint-Jean-de-Luz Bay (encoded Position S and Position N) as supplementary factors: a) Active  
284 variables factor map on the first principal factorial plane; b) Illustrative qualitative variables on the first plane.

285 The first component describes individuals presenting high density of the test ( DM to Vol) associated  
286 with long demipyramid related to the diameter and to the test height (Lp to Dt and Lp to Ht). The  
287 second component is relative to individuals with high test hemispherical index (Ht to Dt) in  
288 conjunction with high demipyramid length related to test diameter (Lp to Dt) and low demipyramid  
289 elongation index (Figure 8). The third component (not illustrated by a figure) describes long sea  
290 urchins (Dt) by opposition with both high test hemispherical index and demipyramid elongation index.  
291 Both test and lantern characteristics showed significant correlations with the three main axes of the  
292 NPCA.

293 Dense tests (regarding volume) with long demipyramid related to the test diameter and height were  
294 found in the intertidal area, far away from a treatment plant and for “S orientation of geological  
295 layers” since those three factors were significantly discriminated on the first axis. P-values of  
296 correlation tests for the bathymetric level and proximity of treatment plant are respectively with axis 1:  
297 0.001 and 0.042.

298 High demipyramid elongation index and low demipyramid length to test diameter were preferentially  
299 situated near treatment plants.

#### 300 **4. Discussion**

##### 301 *General trends*

302 Measurements carried out on sea urchin of the Basque coast were compared with measurements found  
303 in the literature. Ebert (1982) indicated that the hemispherical index tended to 0.5 for several sea  
304 urchin species. Our results are consistent with previous studies undertaken on *P. lividus* in the

305 Mediterranean region (Ballesteros, 1981; Pais et al., 2006) and in Brittany (Allain, 1975). Regarding  
306 the demipyramid length to test diameter ratio, our values are comparable with those obtained by Ebert  
307 (1982, 1988) and Lawrence et al. (1995) for various sea urchin species.

308 Both test and lantern characteristics displayed relationships with tidal level, proximity of treatment  
309 plant and orientation of the geological layers. Whereas previous studies have already shown variability  
310 in the morphometry of the sea urchin test, Aristotle's lantern characteristics have never been used, but  
311 this dental apparatus seems to be sensitive, based on factors identified in this study. Because the  
312 sampling did not focus on one factor but considered a combination of factors that can each introduce a  
313 smoothing effect, some tendencies of interest have been observed.

314 ***Strong influence of bathymetric level, less so of position***

315 Concomitancy between tidal level and morphometry characteristics of the sea urchin is revealed for  
316 ratios involving test and/or jaws measures. The intertidal level is marked by heavier test regarding  
317 diameter and longer demipyramid length regarding test diameter and height. Two hypotheses can  
318 explain this tendency. Firstly, in the intertidal zone, time spent eating is more limited (to a few hours  
319 per day during high tide), while urchins in the subtidal zone can feed without such restriction.  
320 Secondly, despite the fact that the two bathymetric levels are highly exposed on the Basque coast,  
321 intertidal individuals are more exposed to energetic swell and hydrodynamic force than subtidal ones.  
322 Research carried out on the Basque Coast based on the gonadosomatic index and repletion index  
323 shows an influence of the bathymetric level by comparing individuals of the intertidal and subtidal  
324 zone (de Casamajor et al., 2017). Those conditions should promote heavier sea urchins for a given size  
325 (for the stabilization of individuals in a turbulent environment) and individuals with dental apparatus  
326 not only adapted to important grazing (when conditions are favourable) but also to burrowing  
327 behaviour. This hypothesis is furthermore supported by the fact that subtidal individuals in burrows  
328 are less numerous than intertidal ones. Burrowing is less pronounced in sheltered areas (the  
329 Mediterranean, for example). Such behaviour is a better way to provide protection against  
330 hydrodynamic forces (Jacinto and Cruz, 2012), as covering behaviour is more related to protection

331 against predators. Märkel and Meier (1967) demonstrated that *P. lividus* prefer to use the teeth of their  
332 Aristotle's lantern, rather than their spines, to bore rocky shore.

333 Position (South/North) also appears to influence morphological patterns. The geological layers'  
334 orientation changes between stations sampled in the south (S1 and S2) and in the north (S3 and S4) of  
335 Saint-Jean-de-Luz Bay. The dip is parallel to the shore and perpendicular to the orientation of the  
336 dominant waves (NW) in the southern part (Genna et al., 2004), while the northern stations are  
337 characterized by a change in the orientation of geological layers compared to swells impact. In  
338 parallel, the southern part is homogeneous from a geomorphological point of view, with a straight  
339 profile of uninterrupted coastline cliff. In the northern part, the reliefs are much less consistent with  
340 the presence of sandy bays and rocky outcrops. Many alterites are observed, which result in the  
341 presence of blocks of variable size (Alexandre et al., 2003; Genna et al., 2004). A previous study  
342 (Fernandez and Boudouresque, 1997) highlighted that geomorphological differences could affect  
343 morphological characteristics of sea urchins in relation to their phenotypic plasticity. The relationships  
344 described in our work suggest similar links. Even if for now little information is available on this  
345 setting on the studied scale, it appears to be an interesting factor for future investigation as recently  
346 considered by Riquelme et al. (2013). This is in particular the case for the energetic aspect of the  
347 swell, since specific conditions are encountered on the Basque coast, with very strong hydrodynamic  
348 conditions (high waves amplitude) (Abadie et al., 2005) that can favour specific adaptive behaviour,  
349 which in turn affects morphology. All the characteristics of the habitat must be considered in order to  
350 understand correctly the natural sources of variability in the biological characteristics studied.

### 351 *Effect of treatment plants with regard to trophic considerations*

352 Individuals collected near WWTP were generally characterized by a high demipyramid elongation  
353 index and low demipyramid length to test diameter. Those characteristics were also mainly associated  
354 with sea urchin living in the subtidal area. In the intertidal area, proximity of WWTP is related to  
355 smaller demipyramid length to test height.

356 *P. lividus* is mainly herbivorous, but their opportunistic trophic behaviour on different trophic levels is  
357 well known in the absence of trophic-limited conditions (Fernandez and Caltagirone, 1998). The  
358 encountered densities for the French Basque coast were low (the average does not exceed 12 ind.m<sup>2</sup> –  
359 de Casamajor et al., 2014) in comparison with other sites (Black et al., 1982; Boudouresque and  
360 Verlaque, 2013). Under these conditions, it seems reasonable to assume that this factor did not affect  
361 our results. It should be noted that with higher densities, Black et al. (1982) demonstrated a density  
362 effect on jaws allometry.

363 El Jouhari et al. (2011) indicated that the specific algal composition in the sea urchin diet depends on  
364 the size of the individuals but also on their habitat, that is to say where they consume the algae that is  
365 present (Lemée et al., 1996). On rocky shores, a transition between the intertidal and the subtidal area,  
366 resulting in a specific diversification in algal communities, has also been described (Boudouresque and  
367 Verlaque, 2013). For the Basque coast, the main species present on intertidal bedrocks are calcareous  
368 species such as *Lithophyllum incrustans*, *Lithothamnion lenormandii* and *Corallina* spp. We can also  
369 find opportunistic species such as *Ceramium* spp. and chlorophyta (Ulvaceae). In the subtidal area  
370 these species are still present but with a greater diversification of Rhodophyta such as *Gelidium* spp.,  
371 *Plocamium cartilagineum*, *Halurus equisetifolius* and *Pterosiphonia* spp. (de Casamajor and Lissardy,  
372 2018). Trophic availability in the intertidal area consists mainly of calcareous algae (hard prey) and  
373 may explain-combined with the physical conditions described above-the lengthening of lantern size  
374 with regard to test diameter. Such results are consistent with the work of Hagen (2008). Since Ebert  
375 (1996) indicated that sea urchins with a poor diet display longer demipyramid than well-fed ones, we  
376 can raise the question of nutrient quality provided by such calcareous algae for *P. lividus*. A global  
377 increase in the food gathering apparatus was supposed to correspond to low food availability. In such  
378 conditions the observed low demipyramid elongation index suggests that another trophic source may  
379 exist. Proximity of WWTP may contribute to an increase in the available food for sea urchins as  
380 suggested by Harmelin et al. (1981) and Boudouresque and Verlaque (2013).

381 The presence of discharges from WWTP is not only characterized by the addition of organic matter  
382 and nutrients, but also by changes in algal composition (Guinda et al., 2012). Intakes in nutrients



383 favour proliferation of green algae that can get into the diet of the sea urchin (Borja et al., 2013). The  
384 amounts of food as well as the quality of this food are expected to affect the morphology of the sea  
385 urchin. Relationships between Aristotle's lantern and trophic considerations have already been  
386 described in the literature. For *Diadema setosum* and *Strongylocentrotus purpuratus*, Ebert (1980)  
387 suggested that large demipyramid in relation to the test diameter increases the ability to feed in limited  
388 food conditions. This morphofunctional adaptation was later demonstrated for *P. lividus* by Fernandez  
389 (1996) in controlled conditions. Our work suggests that another morphological descriptor, the  
390 demipyramid elongation index may be sensitive to discharges from WWTP but also to others  
391 environmental parameters fluctuation considering variability in oceanic conditions (habitats, algal  
392 cover, sedimentation...). Such facilities potentially diversify the food resources since they are a  
393 source of organic matter and of nutrients. The attractiveness of organic matter sources has already  
394 been demonstrated (Allain, 1975; Delmas, 1992; Harmelin et al., 1981) and mainly concerns small and  
395 medium individuals. The lengthening of spines has been demonstrated to be a morphological  
396 adaptation to efficiently absorb particulate and dissolved organic matter (Régis, 1986). More  
397 generally, spine characteristics differ depending on environmental conditions. Currently our results do  
398 not allow us to go further in this analysis.

399

## 400 **5. Conclusion**

401 We hypothesized that not only the test but also the jaws characteristics of *P. lividus* may respond to  
402 various environmental conditions, sometimes natural ones and sometimes anthropogenic ones. Table 5  
403 summarizes tendencies observed within the present work according to the location of the sea urchin  
404 collected. They complement previous work and, regarding the four sites considered in this work, it  
405 demonstrates that Aristotle's lantern characteristics may be used as indicators to describe local  
406 conditions. It may help to distinguish environmental natural parameters from human pressure.

407 Food resources are not a limiting factor for the development of sea urchins on the Basque coast. They  
408 lead to opportunistic feeding behaviour which includes various algal species and organic matter.

409 Geomorphological conditions and swell energy are environmental parameters to which sea urchins  
 410 must adapt morphologically in order to grow and reproduce.

411 This initial approach studies the influence of sewage WWTP discharges (anthropogenic pressure) on  
 412 morphometric changes in sea urchins (mainly on the demipyramid elongation index and on the index  
 413 involving demipyramid length and test measures). Considering the limitations of these first results,  
 414 and in order to go further in this analysis, it would be interesting to carry out sampling at several  
 415 distances (0 m, 10 m to 50 m for example) at the same bathymetric level to understand the dilution  
 416 effect. It would allow us to characterize the evolution of those indexes according to distance from the  
 417 discharge. In addition, analysis of gut contents would allow us to specify individuals' diet composition.  
 418 Finally, in the context of future work, an increase in the number of sample sites would provide an  
 419 improvement on the evaluation of the quality of this indicator.

420 Such works should contribute to improving knowledge on pressure impact assessment and on state of  
 421 the environment conservation in a defined area. Furthermore, this species is of commercial interest and  
 422 is usually managed on a regional basis. Knowledge derived from such works may also be useful to  
 423 propose management measures on an appropriate scale.

424

425 **Table54**

426 Synthesis of main results, taking into account the bibliographic data considered

Position	Sample site	Bathymetric level	Specific conditions*	**Sea urchin characteristics
South	S1 sewage	Intertidal	Hydrodynamic ++ Calcareous diet ++ Burrowing +++	Demipyramid long Test heavy Diameter small
		Subtidal	Hydrodynamic + Calcareous diet + Burrowing +	Demipyramid short Test light Diameter high

	S2	Intertidal	Hydrodynamic ++ Calcareous diet +++ Burrowing +++	Demipyramid long Test heavy Diameter small
		Subtidal	Hydrodynamic + Calcareous diet ++ Burrowing +	Demipyramid short Test light Diameter high
North	S3	Intertidal	Hydrodynamic +++ Calcareous diet +++ Burrowing +++	Demipyramid long Test heavy Diameter small
		Subtidal	Hydrodynamic ++ Calcareous diet ++ Burrowing ++	Demipyramid short Test light Diameter high
	S4 sewage	Intertidal	Hydrodynamic +++ Calcareous diet ++ Burrowing +++	Demipyramid long Test heavy Diameter small
		Subtidal	Hydrodynamic ++ Calcareous diet + Burrowing ++	Demipyramid long Test heavy Diameter high

427 \* linked with geomorphological

428 \*\*+++ = high - ++ = medium - + = low

429

### 430 **Funding**

431 This work used the data of the ARISTOT project that was carried out under the aegis of CIDPMEM  
432 64/40 and with financial support from the European Union, the French State, the Aquitaine region and  
433 the Pyrénées-Atlantiques department as part of the Axis 4 European Fisheries Fund.

434

### 435 **Acknowledgments**

436 We would like to thank all the people and organizations who contributed to this study, in particular  
437 Muriel Lissardy, Florence Sanchez, Romain Elleboode, Kelig Mahé and Mélanie Brun from Ifremer,  
438 for their help with dissections, image processing and/or realization of location maps. We are also

439 grateful to Mathilde Montperrus and Jean-Claude Salvado for facilitating treatment of the biological  
440 material in the laboratory of the University of Pau & Pays de l'Adour. The authors wish to thank Lorna  
441 Miskelly for English editing.

442 All authors declare no conflicts of interest.

443

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599 **Figure captions**

600 **Fig. 1.** Maps showing the studied area on the French Basque coast (SW France) and localization of the  
601 four sampled sites (Sources: ESRI, BD Carthage, Ifremer – M. Lissardy).

602 **Fig. 2.** Measures considered on each sea urchin test: Dt - test diameter at ambitus (mm) and Ht –  
603 height (mm).

604 **Fig. 3.** Measurements retained on demipyramid to describe morphology of the Aristotle's lantern:  
605 distance from the epiphysis junction to the oral tip of the jaw (mm); height (Hp): the distance from the  
606 distal shelf that articulates with the epiphysis to internal side of the demipyramid (mm).

607 **Fig. 4a to c.** Box plots for Ht to Dt and environmental characteristics: a) site (in grey, sites near  
608 treatment plant), b) bathymetry and c) position. Each characteristic appears significant thanks to the  
609 kruskal wallis test,  $\alpha= 5\%$ .

610 **Fig. 5a to b.** Box plots for Lp to Ht and spatial characteristics: a) position and b) bathymetry. Each  
611 characteristic appears significant thanks to the kruskal wallis test,  $\alpha= 5\%$ .

612 **Fig. 6.** Box plots for Hp to Lp and spatial characteristics: bathymetry. This characteristic appears  
613 significant thanks to the kruskal wallis test,  $\alpha= 5\%$ .

614 **Fig. 7a to b.** Box plots for intertidal: a) Lp to Dt regarding proximity of treatment plant and b) Hp to  
615 Lp regarding position. Each characteristic appears significant thanks to the kruskal wallis test,  $\alpha= 5\%$ .

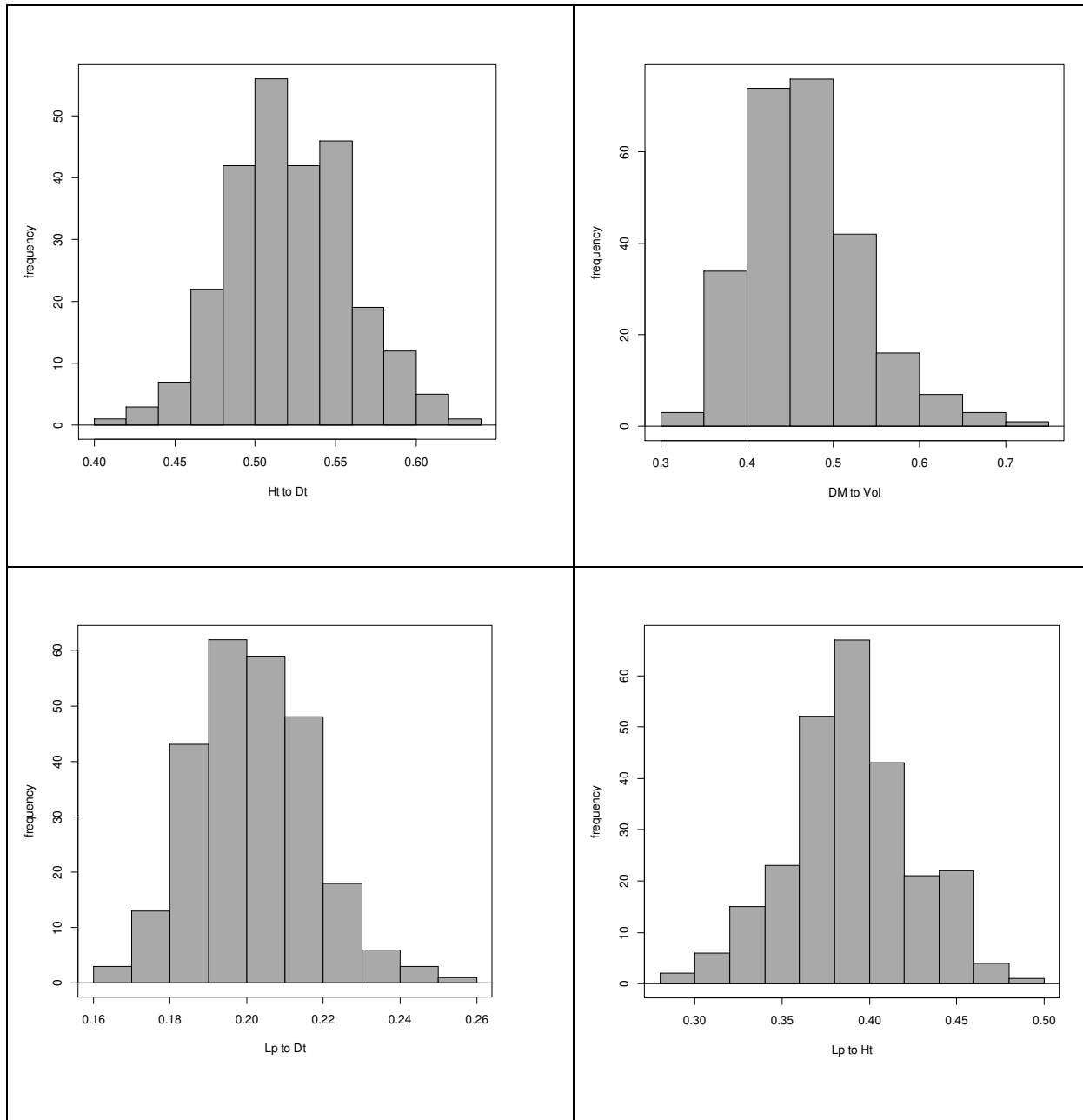
616 **Fig. 8.** Box plots for subtidal: Ht to Dt regarding sites. Significant differences appears thanks to the  
617 kruskalmc and the concerned pair is marked with “\*”.

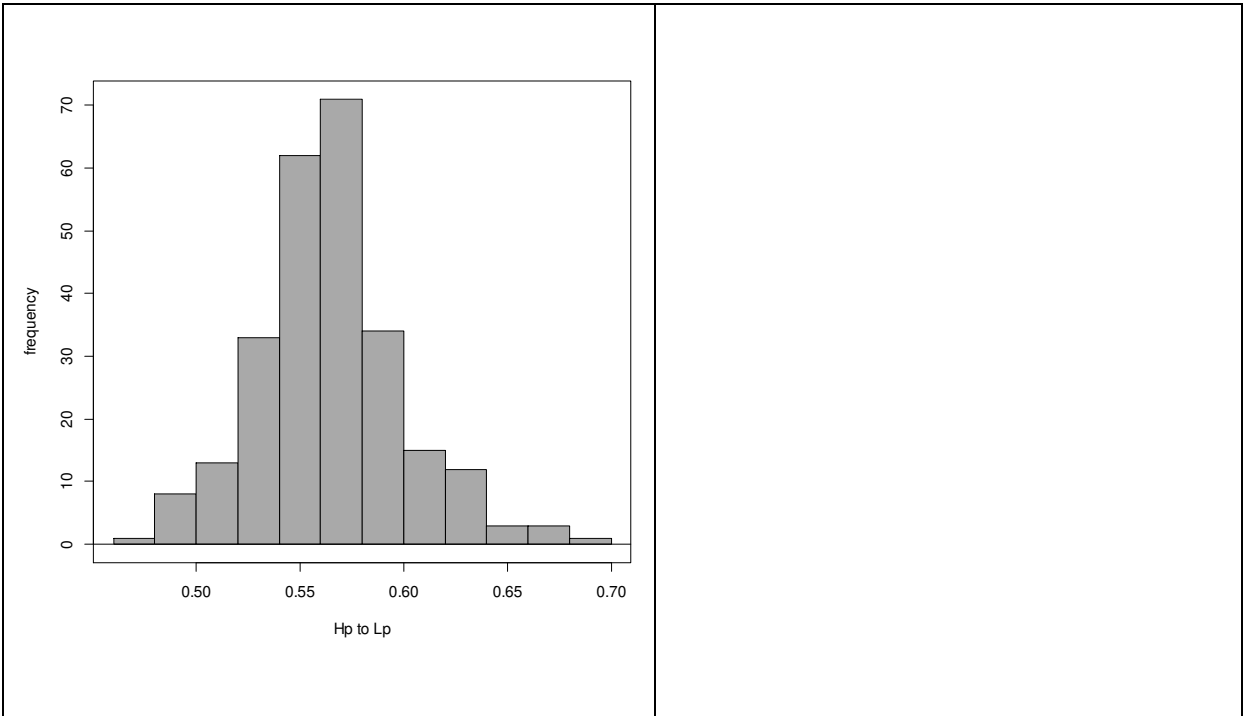
618 **Fig. 9.** NPCA on morphometric variables with bathymetric level (encoded Intertidal or Subtidal),  
619 proximity of treatment plant (encoded PTP – proximity of treatment plant; RTP – remote from  
620 treatment plant) and position related to the Saint-Jean-de-Luz Bay (encoded Position S and Position N)  
621 as supplementary factors: a) Active variables factor map on the first principal factorial plane; b)  
622 Illustrative qualitative variables on the first plane.

623 **Appendix**

624 Frequency histograms of the five ratios (Ht to Dt, DM to Vol, Lp to Dt, Lp to Ht and Hp to Lp)

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