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Abstract:

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

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

1. Introduction

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

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

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

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

2. Material and methods

2.1. Study area

The French Basque coast is located on the southeastern side of the Bay of Biscay (southwest of France). The bedrock is mainly composed of a flysch facies intersected in some places by boulder fields or by sandy beaches and estuaries. This coast is subject to extensive freshwater inflows due to high rainfall of around 1500 and 2000 mm per year (Usabiaga et al., 2004; Winckel et al., 2004). In addition, there are several disposal release points from wastewater treatment plants (WWTP) along the shoreline. They contribute to the freshening of the coastal waters. The tidal regime is mesotidal with a tidal range between 1.85 m and 3.85 m on average. On the south of the Bay of Biscay, the tidal range resulting with low currents, it has low influence (Idier and Pedreros, 2005; Augris et al., 2009) on erosion process and benthic colonization. Hydrodynamic conditions are characterized by the presence of high-energy waves (Abadie et al., 2005) breaking on the shore (mean, 1.8 m height for an average period of 9.6 s) and on sea urchin habitat.

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

2.2. Environmental patterns of the selected sites

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

- one concerns the proximity to (less than 250 m), or remoteness from a WWTP, encoded PTP – proximity of treatment plant; RTP – remote from treatment plant; The treatment plant can be viewed as a potential source of organic matter and nutrients impacting the morphological characteristics of the individuals.

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

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

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

- WWTP near S1 : biological filter; 7 000 $m^3.d^{-1}$, 2 400 kg $BOD_5.d^{-1}$;

- WWTP near S4 : activated sludge; 1 340 $m^3.d^{-1}$, 600 kg $BOD_5.d^{-1}$.

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

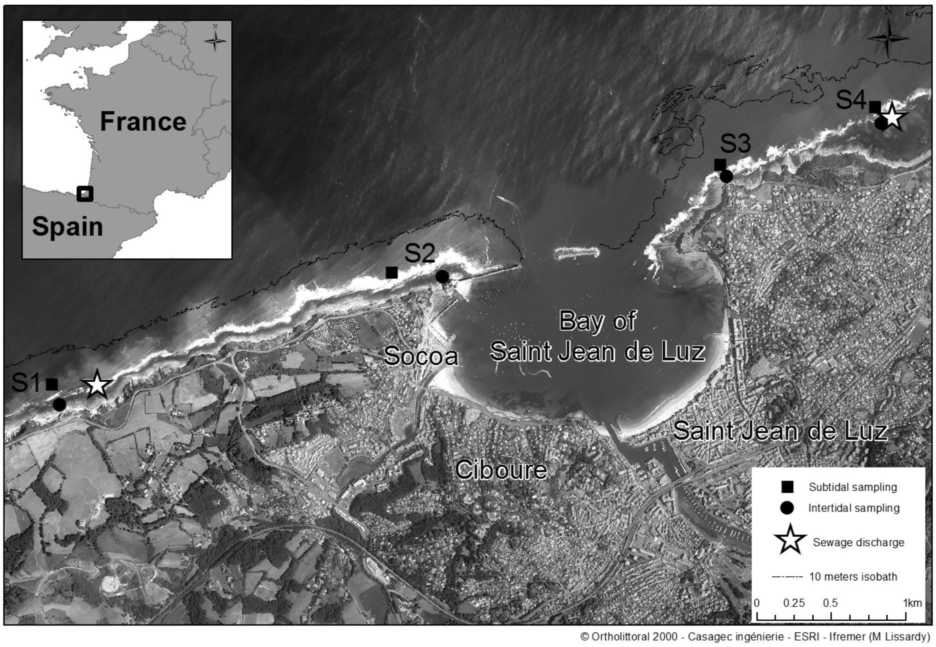


Fig. 1. Maps showing the studied area on the French Basque coast (SW France) and localization of the four sampled sites (Sources: ESRI, BD Carthage, Ifremer – M. Lissardy).

Table 1

Characteristics of the four sampling points (geographic coordinates, proximity of treatment plant, sampling periods, number of sampled individuals and average test diameter (+/- standard deviation) of the sampled *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 *Paracentrotus lividus*	64	64	64	64

Mean length of sampled *Paracentrotus lividus*	54.8 mm (SD = 4.8)	53.1 mm (SD = 7.4)	52.0 mm (SD = 4.9)	58.7 mm (SD = 3.5)

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

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

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

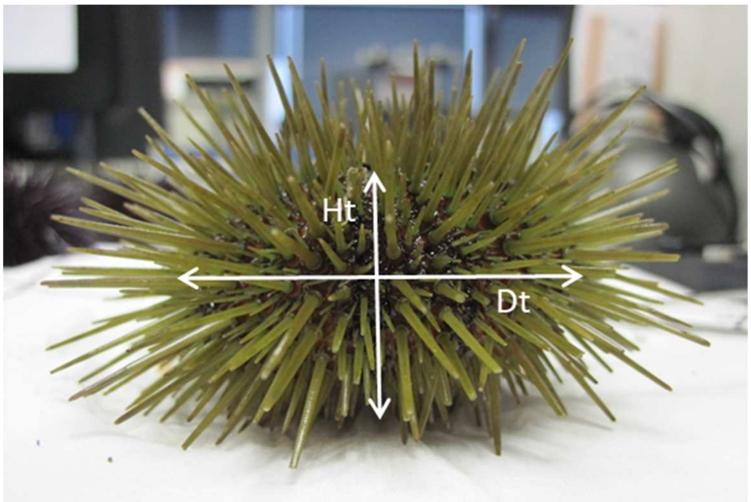


Fig. 2. Measures considered on each sea urchin test: Dt - test diameter at ambitus (mm) and Ht – height (mm).

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

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

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

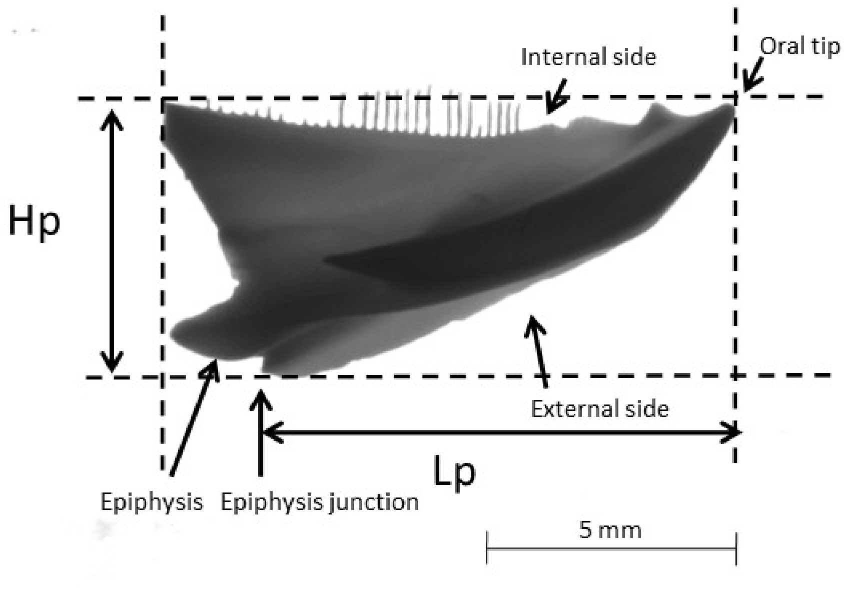


Fig. 3. Measurements retained on demipyramid to describe morphology of the Aristotle's lantern: distance from the epiphysis junction to the oral tip of the jaw (mm); height (Hp): the distance from the distal shelf that articulates with the epiphysis to internal side of the demipyramid (mm).

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

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

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

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

2.4. Statistical analysis

The coupling between the five ratios defined from measurements on the test and the lantern and environmental parameters were tested using the Kruskal-Wallis test (with determination of level of significance) given the lack of normality of the distribution for all the ratios (See Appendix). Box plots between factors and ratios are presented for significant results (p-value= 5%). In addition, when a Kruskal-Wallis test value is significant for more than two environmental conditions (i.e. sites), a multiple comparison test after Kruskal-Wallis (kruskalmc) is implemented. Using pairwise comparisons, this test helps determining which groups are different.

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

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

3. Results

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

Table 2

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

Descriptor	Formulae	Minimum	Maximum	Median	Standard deviation 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		0.07
				0.46	0.08
Demipyramid length to test diameter ratio (Lp to Dt)	= *Lp*/*Dt*	0.16	0.26		0.02
				0.20	0.02
Demipyramid length to test height ratio (Lp to Ht)	= *Lp*/*Ht*	0.29	0.49		0.04
				0.39	0.04
Demipyramid elongation index (Hp to Lp)	= *Hp*/*Lp*	0.47	0.68		0.04
				0.56	0.04

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

Table 3

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

(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***

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

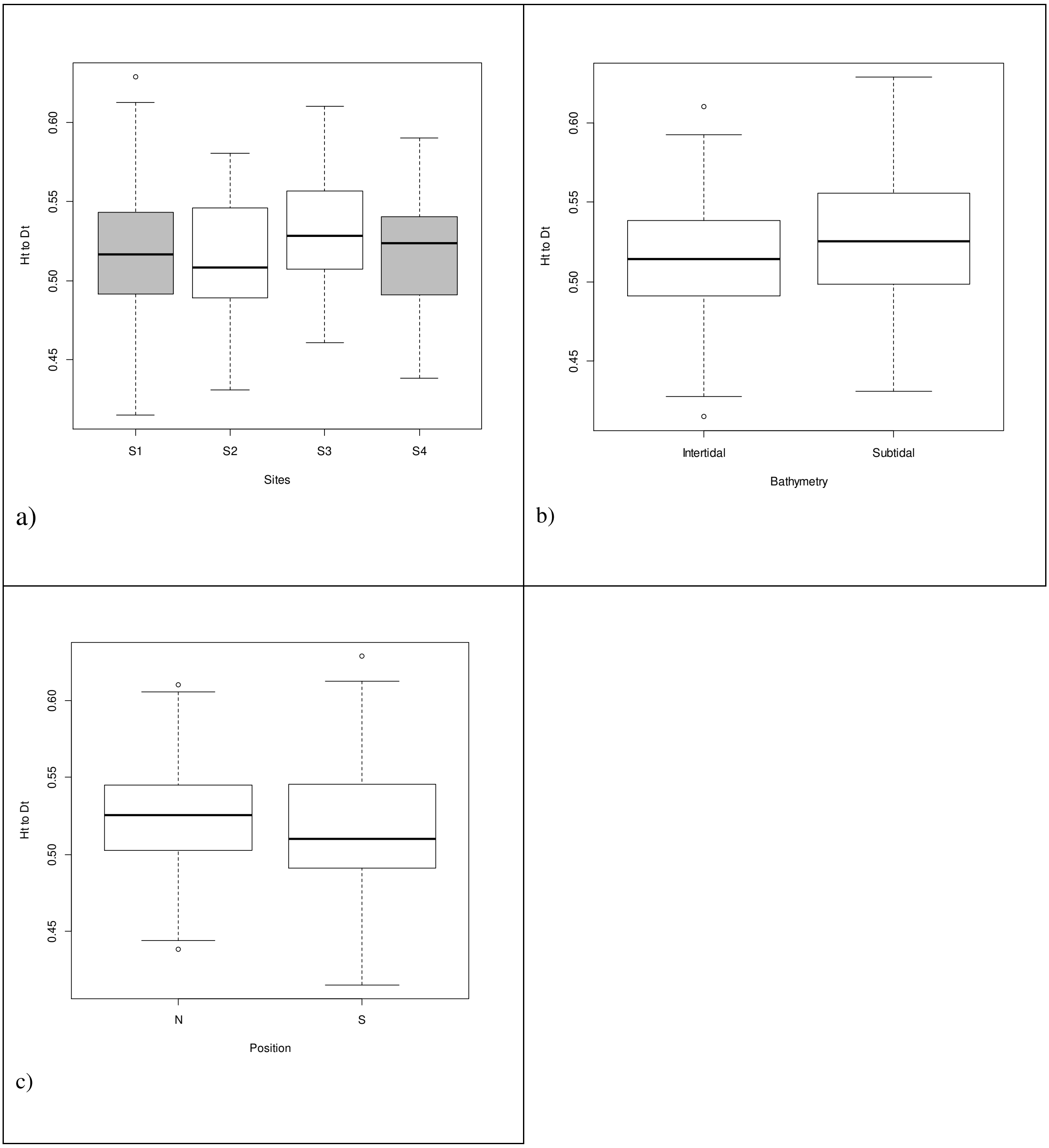


Fig. 4a to c. Box plots for Ht to Dt and environmental characteristics: a) site (in grey, sites near treatment plant), b) bathymetry and c) position. Each characteristic appears significant thanks to the kruskal wallis test, α= 5%.

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

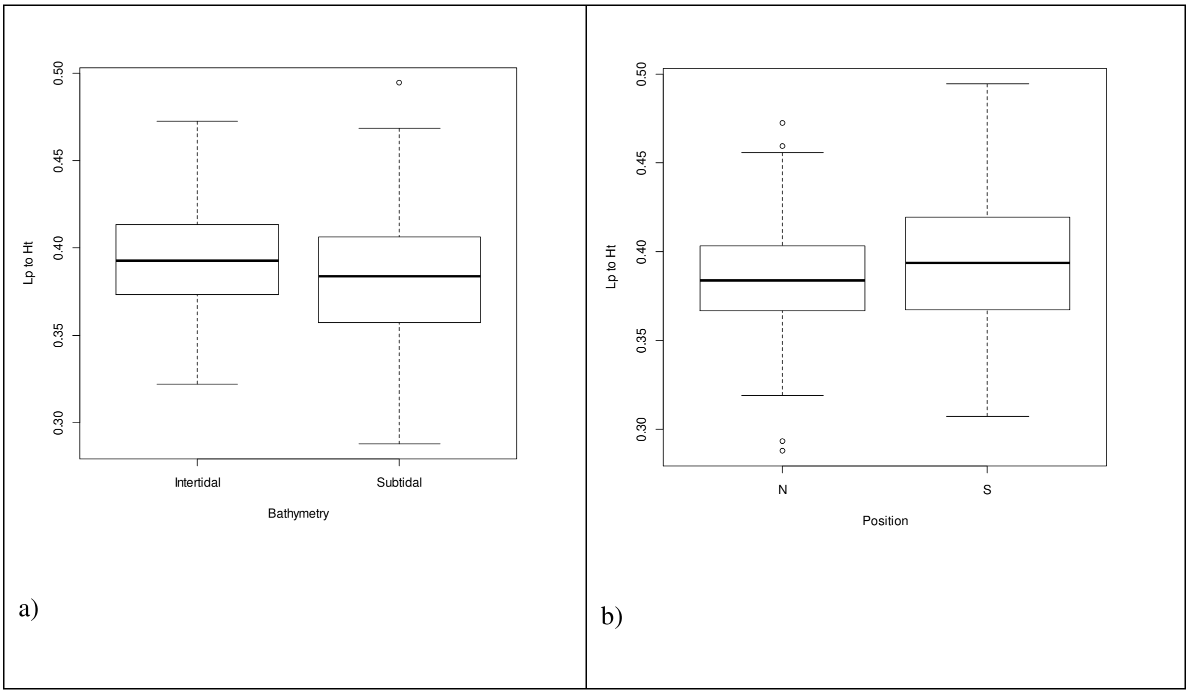


Fig. 5a to b. Box plots for Lp to Ht and spatial characteristics: a) position and b) bathymetry. Each characteristic appears significant thanks to the kruskal wallis test, α= 5%.

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

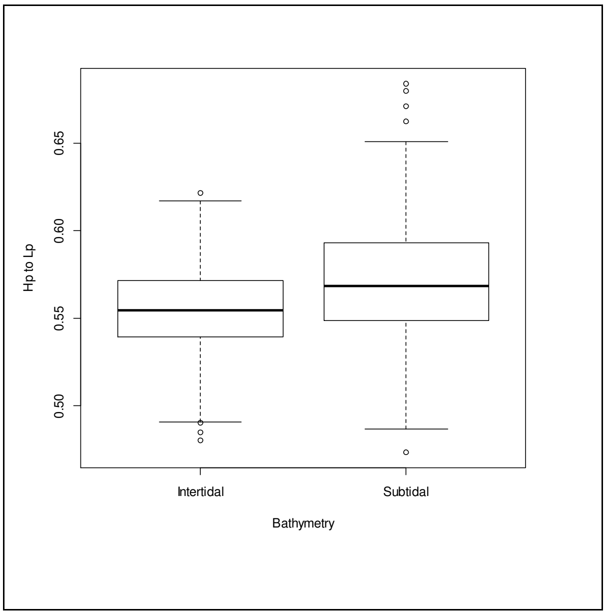


Fig. 6. Box plots for Hp to Lp and spatial characteristics: bathymetry. This characteristic appears significant thanks to the kruskal wallis test, α= 5%.

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

Table 4

P-value of Kruskal-Wallis tests regarding shape descriptors and environmental conditions (p-value < 0.05 is 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

For intertidal level:

- Proximity of treatment plant and sites act on demipyramid length to test diameter ratio (Lp to Dt). Lower level is linked to proximity of treatment plant and one can notice a higher variability too (Fig 7a). The Kruskal-Wallis test is also significant when considering sites, but the p-value is very closed from the 0.05. In these conditions, we decided not to further analyse this last result;

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

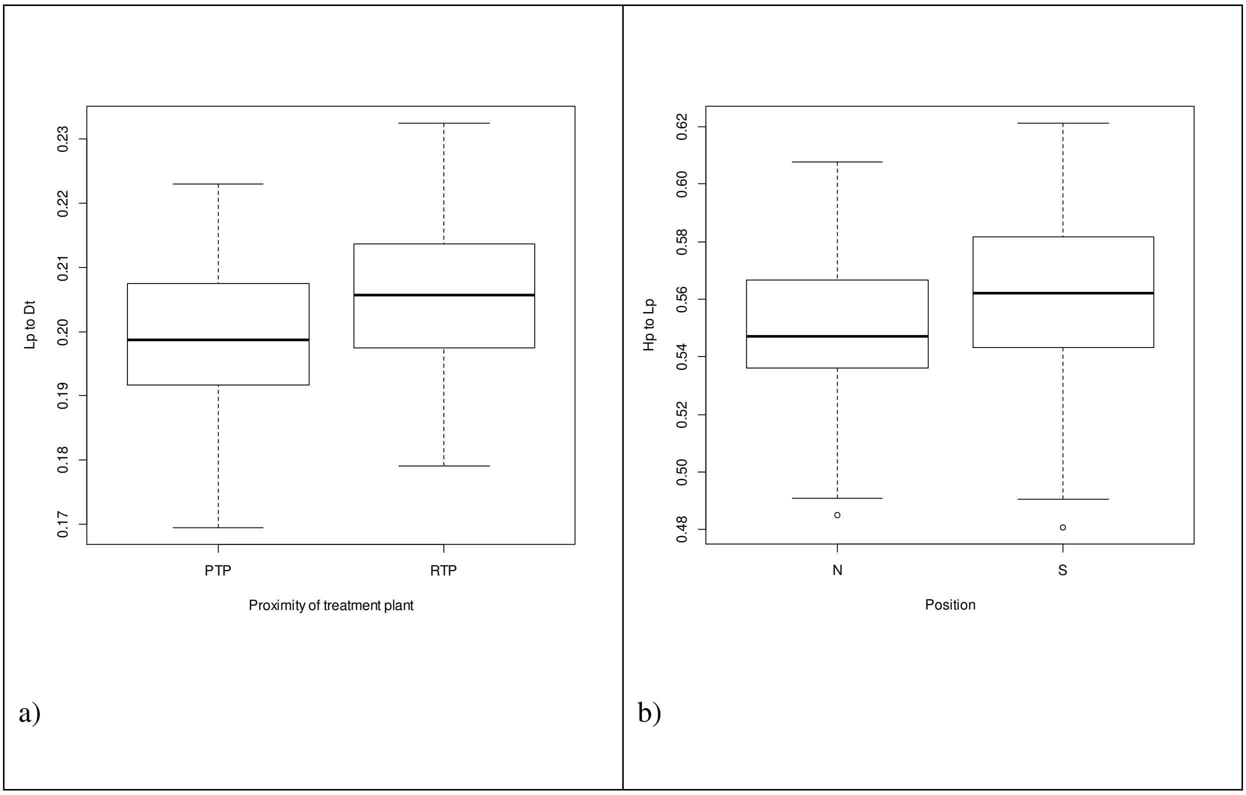


Fig. 7a to b. Box plots for intertidal: a) Lp to Dt regarding proximity of treatment plant and b) Hp to Lp regarding position. Each characteristic appears significant thanks to the kruskal wallis test, α= 5%.

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

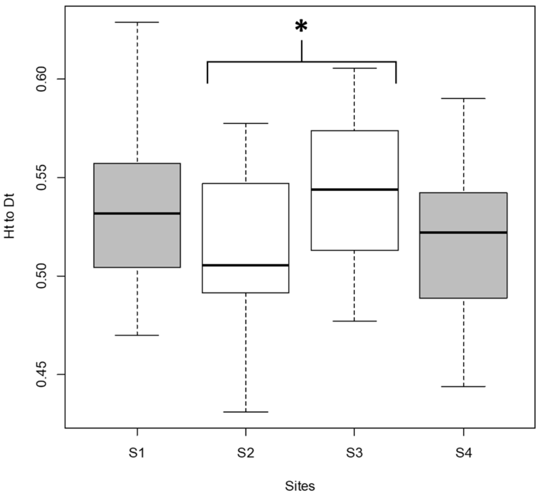
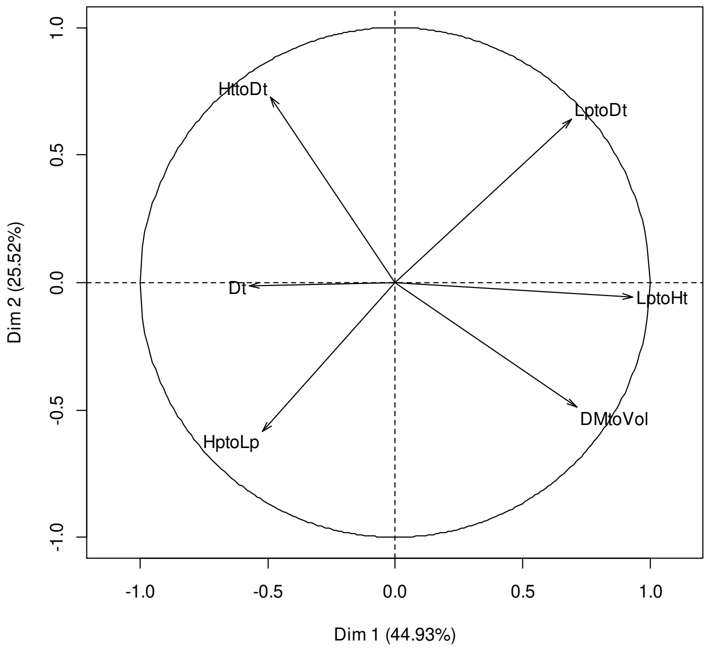


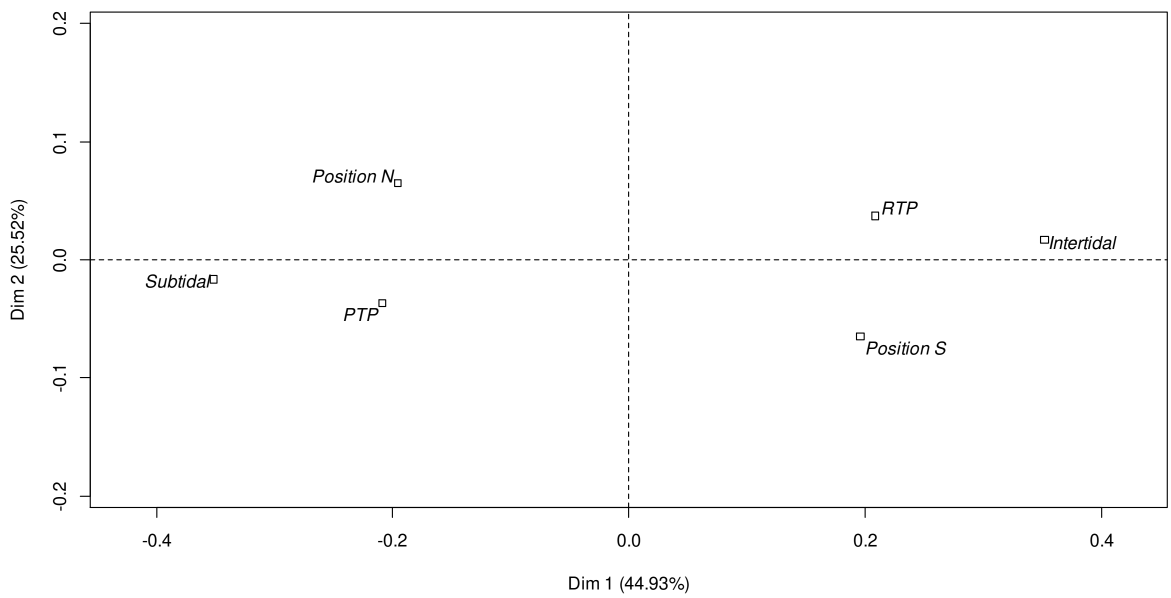
Fig. 8. Box plots for subtidal: Ht to Dt regarding sites. Significant differences appears thanks to the kruskalmc and the concerned pair is marked with "*".

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



HttoDt
LptoDt
Dt
LptoHt
DMtoVol
HptoLp
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
Dim 2 (25.52%)
Dim 1 (44.93%)

a)



Position N
RTP
Intertidal
Subtidal
PTP
Position S
0.2
0.1
0.0
-0.1
-0.2
-0.4
-0.2
0.0
0.2
0.4
Dim 2 (25.52%)
Dim 1 (44.93%)

b)

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

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

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

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

4. Discussion

General trends

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

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

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

Strong influence of bathymetric level, less so of position

Concomitancy between tidal level and morphometry characteristics of the sea urchin is revealed for ratios involving test and/or jaws measures. The intertidal level is marked by heavier test regarding diameter and longer demipyramid length regarding test diameter and height. Two hypotheses can explain this tendency. Firstly, in the intertidal zone, time spent eating is more limited (to a few hours per day during high tide), while urchins in the subtidal zone can feed without such restriction. Secondly, despite the fact that the two bathymetric levels are highly exposed on the Basque coast, intertidal individuals are more exposed to energetic swell and hydrodynamic force than subtidal ones. Research carried out on the Basque Coast based on the gonadosomatic index and repletion index shows an influence of the bathymetric level by comparing individuals of the intertidal and subtidal zone (de Casamajor et al., 2017). Those conditions should promote heavier sea urchins for a given size (for the stabilization of individuals in a turbulent environment) and individuals with dental apparatus not only adapted to important grazing (when conditions are favourable) but also to burrowing behaviour. This hypothesis is furthermore supported by the fact that subtidal individuals in burrows are less numerous than intertidal ones. Burrowing is less pronounced in sheltered areas (the Mediterranean, for example). Such behaviour is a better way to provide protection against hydrodynamic forces (Jacinto and Cruz, 2012), as covering behaviour is more related to protection against predators. Mârkel and Meier (1967) demonstrated that *P. lividus* prefer to use the teeth of their Aristotle's lantern, rather than their spines, to bore rocky shore.

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

Effect of treatment plants with regard to trophic considerations

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

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

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

The presence of discharges from WWTP is not only characterized by the addition of organic matter and nutrients, but also by changes in algal composition (Guinda et al., 2012). Intakes in nutrients favour proliferation of green algae that can get into the diet of the sea urchin (Borja et al., 2013). The amounts of food as well as the quality of this food are expected to affect the morphology of the sea urchin. Relationships between Aristotle's lantern and trophic considerations have already been described in the literature. For *Diadema setosum* and *Strongylocentrotus purpuratus*, Ebert (1980) suggested that large demipyramid in relation to the test diameter increases the ability to feed in limited food conditions. This morphofunctional adaptation was later demonstrated for *P. lividus* by Fernandez (1996) in controlled conditions. Our work suggests that another morphological descriptor, the demipyramid elongation index may be sensitive to discharges from WWTP but also to others environmental parameters fluctuation considering variability in oceanic conditions (habitats, algal cover, sedimentation….). Such facilities potentially diversify the food resources since they are a source of organic matter and of nutrients. The attractiveness of organic matter sources has already been demonstrated (Allain, 1975; Delmas, 1992; Harmelin et al., 1981) and mainly concerns small and medium individuals. The lengthening of spines has been demonstrated to be a morphological adaptation to efficiently absorb particulate and dissolved organic matter (Régis, 1986). More generally, spine characteristics differ depending on environmental conditions. Currently our results do not allow us to go further in this analysis.

5. Conclusion

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

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

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

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

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

Table54

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

* linked with geomorphological

**+++ = high - ++ = medium - + = low

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Figure captions

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

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

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

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

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

Fig. 6. Box plots for Hp to Lp and spatial characteristics: bathymetry. This characteristic appears significant thanks to the kruskal wallis test, α= 5%.

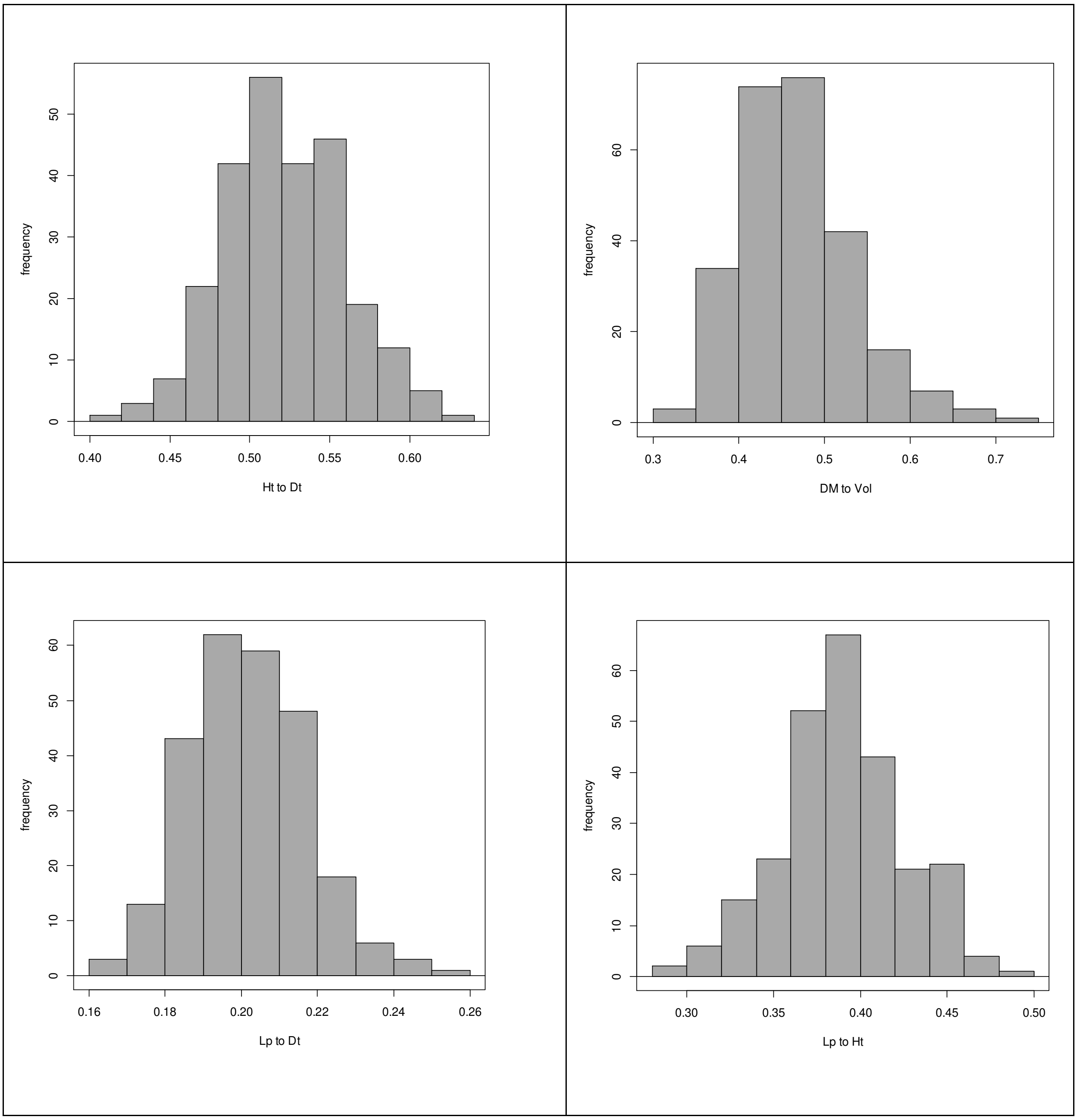
Fig. 7a to b. Box plots for intertidal: a) Lp to Dt regarding proximity of treatment plant and b) Hp to Lp regarding position. Each characteristic appears significant thanks to the kruskal wallis test, α= 5%.

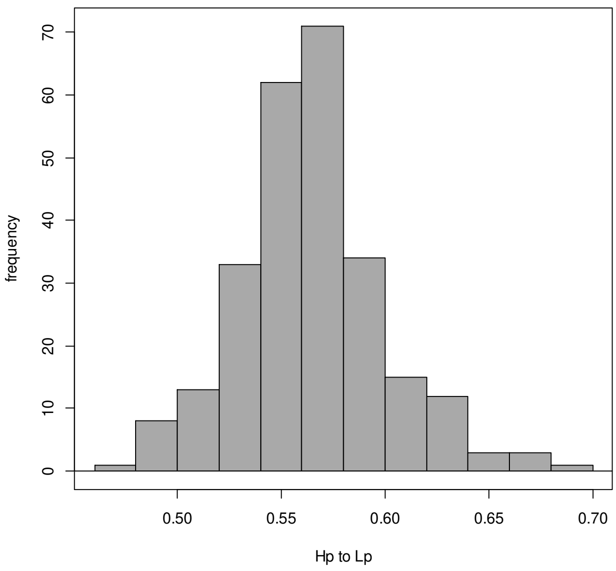
Fig. 8. Box plots for subtidal: Ht to Dt regarding sites. Significant differences appears thanks to the kruskalmc and the concerned pair is marked with "*".

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

Appendix

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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