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Searching for drivers of the patchy distribution of sympatric deposit- feeding sea cucumbers: A multi- scale monitoring study

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Abstract :

Three sea cucumber species, Holothuria atra, Holothuria leucospilota, and Stichopus chloronotus, are patchily distributed within the coral reefs of Reunion Island, exhibiting locally high densities (> 1 ind m−2). Previous studies stated that these sympatric species have thrived for almost four decades; however, factors involved in their distribution and spatiotemporal dynamics are not well understood yet. The aims of this study are to analyze the fine-scale spatiotemporal dynamics of these sympatric species and identify the key sedimentary factors (substrate composition and cover) involved in their distribution. Sea cucumber populations and sedimentary factors were monitored along back-reef and shore-to-ocean gradients during four consecutive seasons. Meanwhile, feeding behavior was assessed by measuring motility and excretion rates. Each species presents its own dynamic between the beginning and the end of the monitoring: increase of density for H. leucospilota, stability for H. atra, and decrease for S. chloronotus. Distribution of H. atra was closely related to substrate organic features (high organic matter, chlorophyll a, and ∂13C), H. leucospilota with the occurrence of seagrass and S. chloronotus with none. Results on feeding behavior showed that S. chloronotus emerges as a specialist while the two others appear to be generalists. Conspecific attraction or recruitment pattern may drive the high-density patchy distribution of both Holothuria species, while the drastic decrease of S. chloronotus could be linked to unstudied factors related to predation or climate change. Water flow regime could also have a significant effect on the patchy distribution of these species, therefore, further studies should pay particular attention to this factor.

Introduction

Deposit-feeding sea cucumbers have been shown to play substantial ecological roles within shallow-water ecosystems and their impact on their associated environments has been reviewed by Purcell et al. (2016). Sea cucumbers also make remarkable practical contributions, such as (1) indicator species for monitoring pollution (Warnau et al. 2006), (2) sources of active substances, with antibacterial and antifungal (Adibpour et al. 2014) or anticancer properties (Janakiram et al. 2015), (3) source of food, traditionally consumed raw, dried or boiled in most of Asian countries (Toral-Granda et al. 2008). To face the high demand of the Asian market (Purcell et al. 2018) and the collapse of most of the high-value species worldwide (Friedman et al. 2011), aquaculture protocols have been developed (Yang et al. 2015; Hamel et al. 2022). As a result of their usefulness, interest in sea cucumbers has greatly increased since the 1990s (Pierrat et al. 2022a). Paradoxically, knowledge on the dynamics of wild populations and the influence of environmental factors on their distribution is still sparse.

Adult sea cucumbers exhibit heterogeneous distribution for some species, with very high-density patches surrounded by extremely low or even zero abundances. Such heterogeneous distribution with high densities of sea cucumbers locally have already been reported for small suspension-feeding sea cucumbers in California (Eckert 2007) or even large deposit-feeding sea cucumbers in Portugal (Domínguez-Godino and González-Wangüemert 2020; Félix et al. 2021) or Brazil (Mendes et al. 2006). Many studies have underlined the influence of environmental factors on sea cucumbers, notably on the feeding or reproduction (Mercier and Hamel 2009a; b) or individual features (Hou et al. 2019; Jobson et al. 2021), mainly using *in vitro* experiments. However, very few have assessed the influence of environmental factors on the patchy distribution of wild sea cucumber populations. Bulteel et al. (1992) evidenced that the depth was an important environmental factor driving the distribution of *Holothuria tubulosa* according to size. Water temperature seems to also influence sea cucumber distribution (Buccheri et al. 2019), as for instance Bonham and Held (1963) showed that the temperature threshold for *Holothuria atra* was 39°C, while *Holothuria leucospilota* was not feeding from 38°C. Azevedo E Silva et al. (2023) stated that the hydrodynamic features are the main drivers of the distribution of *Holothuria arguinensis*. Some studies related sea cucumber distribution to substrate composition, such as high organic matter or chlorophyll *a* loads (Dissanayake and Stefansson 2012; Navarro et al. 2013), while other found correlations with habitat type, such as presence of coral rubbles or seagrasses (Džeroski and Drumm 2003; Eriksson et al. 2012; Asha et al. 2015; Hamamoto et al. 2022), or substrate complexity (Mendes et al. 2006; Félix et al. 2021). All these studies underpin that a large range of environmental factors can influence the distribution of sea cucumbers. In consequence, local monitoring of wild sea cucumber populations, with an understanding of environmental factors shaping their distribution, is needed to fulfil knowledge gaps and feed efficient management plans to preserve natural stocks from collapse, which is essential for the health of reefs and for many human activities.

Almost 40 tropical species of sea cucumbers are found in coral reefs of Reunion Island (Conand et al. 2018). Few studies have assessed their abundance or population metrics since the 1990s. Collectively, they highlighted that three sympatric deposit-feeding species are patchily distributed across reefs with atypical high densities for such large invertebrate: *H. leucospilota*, *H. atra* and *Stichopus chloronotus* (Conand et al. 1998; Jaquemet et al. 1999; Cuvillier 2016), which have the ability to reproduce both sexually and asexually (Dolmatov 2014). At some locations within Reunion Island reefs, two of these sympatric species could be found together at high densities (Conand and Mangion 2002). Unfortunately, those studies did not investigate thoroughly the relation between environmental factors and populational distribution and dynamics.

The aim of this study is to assess the spatio-temporal variability of patchily distributed sympatric sea cucumber species in a small reef complex at Reunion Island and identify the role of sedimentary factors (substrate composition and cover) in the high-density patching.

Materials & Methods

Study area and species

The study was conducted in the volcanic island of Reunion Island, which is a French department, located in the Mascareignes Archipelago in the South West Indian Ocean (21°06′S, 55°31′E). The tropical climate of Reunion Island has two seasons: summer, described as warm and rainy, and winter, considered as cool and dry (Conand et al. 2007). Reunion Island has four fringing reefs on the West (Hermitage/La Saline, Saint-Leu and Étang-Salé reefs) and South (Saint-Pierre reef) coasts. Some locations within the reefs received nutrient inputs from enriched terrestrial waters, and are therefore considered as eutrophic (PAS, PLA, TE and ES sites, see next sections for details; Cuet et al. 1988; Tedetti et al. 2011; Cuvillier 2016). Two monitoring studies were conducted to investigate intra-back-reef and shore-to-ocean variabilities.

Inter-site variability: back-reef gradient

Four sites at the back-reef depression (**Figure 1**) were chosen considering their high densities (> 1 ind.m²) for the three sea cucumber species of this study. From the North to the South in Hermitage/La Saline reef: (1) “MNS” site (Maître-Nageurs-Sauveteurs), is situated in the narrowest part of the reef (150 m), then subject to intense swells; monospecific seagrass meadows of *Syringodium isoetifolium* are present at this site with a high-density population of *H. leucospilota* (Cuvillier 2016); (2) “PAS” site (Passe de l’Hermitage) is in the middle of the reef of Hermitage/La Saline; a high-density population of *S. chloronotus* characterises the site (Cuvillier 2016); (3) “TE” site (Trou d’Eau) is located in the south part, La Saline reef; high-density populations of *H. leucospilota* and *S. chloronotus* have been recorded here (Conand and

Mangion 2002). Finally, (4) “ES” site (Étang-Salé), is the only one located at the Étang-Salé reef, with a high-density population of *S. chloronotus* (Conand et al. 1998).

Intra-site variability: shore-to-ocean gradient

“PLA” site (Planch’Alizés) is located in the south part of Hermitage/La Saline reef (**Figure 1**). High-density populations of *H. leucospilota* and *H. atra* have been documented at this site (Conand and Mangion 2002). However, a different spatial pattern for *H. atra* close to the shore and *H. leucospilota* at the edge of the back-reef depression, near the inner reef flat was observed. The width of the reef here is about 500 m and the back-reef depression where sea cucumbers are very abundant is about 200 m. In this sense, an adapted sampling was carried out at this site, with a shore-to-ocean gradient composed by three sites designed by their distance from the shore: $\mathrm{PLA}_{\mathrm{SHORE}}$ from 0 to 60 m, $\mathrm{PLA}_{\mathrm{MID}}$ between 60 and 120 m and $\mathrm{PLA}_{\mathrm{OCEAN}}$ from 120 to 200 m.

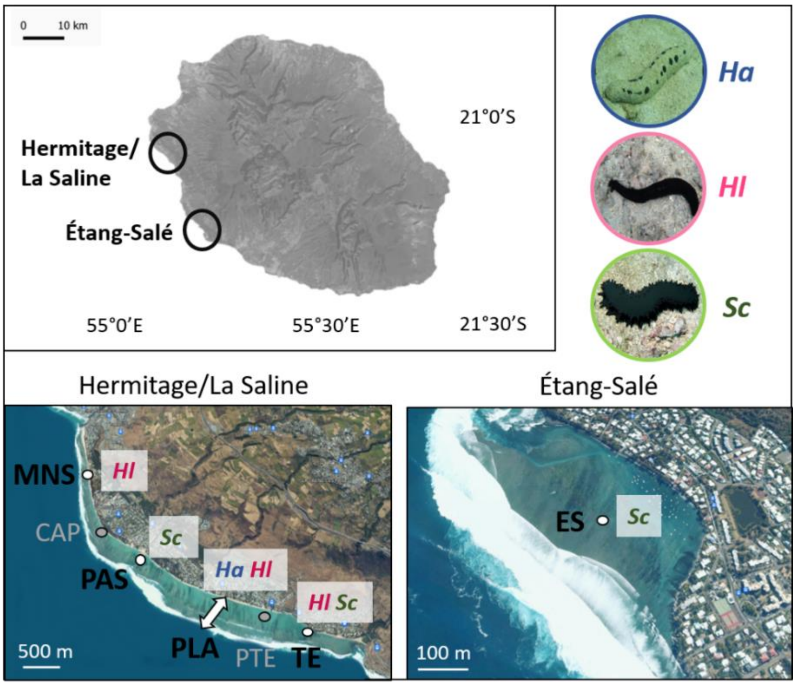

Figure 1: Location of Hermitage/La saline and Étang-Salé reefs on the west coast of Reunion Island, and sampling sites at each reef.
Main sites of monitoring (in black): MNS, PAS, PLA, TE and ES
Low density sites (in grey): CAP and PTE
Dots represent sites that are part of the back-reef gradient and the arrow on PLA represents the shore-to-ocean gradient.
Ha: *Holothuria atra* (in blue); *Hl*: *Holothuria leucospilota* (in pink); *Sc*: *Stichopus chloronotus* (in green)

Sampling design

Population metrics

Abundance at each site were measured in four belt-transects of 50 × 2 m, bimonthly from August 2019 to January 2021 for the back-reef gradient. Regarding the shore-to-ocean gradient at PLA site, three belt-transects of 50 × 2 m were drawn at each of the three stations ($\mathrm{PLA_{SHORE}}$, $\mathrm{PLA_{MID}}$ and $\mathrm{PLA_{OCEAN}}$), from July 2020 to July 2022. Densities were then calculated. Biometric variables (width × length) were measured to the nearest 0.5 centimeter, using a flexible tape measure, on the first 30 individuals of each species in each belt-transect for both gradients. Due to the high constriction behavior of two of the three species studied (*H. atra* and *H. leucospilota*), the tape measure was placed as close as possible to the dorsal side of the individuals without ever touching them. Individuals partially hidden under coral rubbles, living corals or other crevices were counted but not measured.

Sedimentary factors

Quadrats sizing 2 × 2 m were plotted every two meters along the belt-transects in order to estimate substrates cover (%). Ten categories of substrates were observed *in situ*: ‘sand’, ‘seagrasses’, ‘sponges’, ‘cyanobacteria’, ‘red macroalga’, ‘living coral without turf’, ‘living coral with turf’, ‘erect dead coral’, ‘coral rubble without turf’, and ‘coral rubble with turf’. Additionally, sediment samples were cored (core 15.9 cm x 4.5 cm) in triplicates at each site and station for substrate composition analyses: (1) quantification of organic matter load (%), (2) proportion of fine particles (< 63 µm, %), (3) chlorophyll *a* ($\mathrm{mg.m^{-2}}$), and (4) determination of ∂^{15}N and ∂^{13}C (‰). Sediment samples were also collected in triplicates at two low-density reference sites (named “CAP” and “PTE” in the reef of Hermitage, **Figure 1**, < 0.001 $\mathrm{ind.m^{-2}}$), for three consecutive seasons (Winter 2019, Summer 2020 and Winter 2020).

Feeding features

Speed and excretion rate were used as proxies to characterize the feeding behaviour of each species. The moving speed of sea cucumbers was estimated at each site for each species. In the field, 15 individuals were tagged with plastic dermal T-implant into the dorsal tegument. At T= 0 min, locator stakes were placed behind the posterior end of each individual. Every 15 min, during two hours, the distance between the posterior end of the individual and the locator stake was measured. In order to highlight a possible specific active moving period for searching food, this experiment was repeated at 8 and 11 a.m. and 2 p.m. The tags were recovered at the end. The moving speed was calculated for each individual and then averaged. The assessment of the ingestion rate was not possible *in situ*, therefore the excretion rate, which is a proxy of the amount of sediment ingestion rate, was estimated. Faeces were collected inside five quadrats of 1 × 1 m at each site and separated by species. The density of sea cucumbers inside the quadrats was also recorded. This experiment was repeated at 8 and 12 a.m. and 4 p.m. to highlight a possible diel pattern. Night activity was not considered as daily patterns were already described with *S. chloronotus* feeding only at daytime while both *Holothuria* feeding continually (Yamanouchi 1956; Uthicke 1999). Excretion rate is expressed in dry weight of sediment per individual per day. Moving speed and excretion rate were assessed at MNS, PAS, PLA and TE sites.

Analyses of sediment and faeces samples

Calcination method was used to determine the total organic matter load (Byers et al. 1978). First, sediment samples were dried 48 h at 60 °C and weighted to obtain dry weight. Sediment samples were then calcinated 4 h at 450 °C and weighted for ash free dry weight. The difference between the two weights gives the total organic matter load. Dry weight was also quantified for particles analyses. The dried samples were then sieved on 63 µm mesh and dried a second time 48 h at 60 °C to obtain dry weight without fine particles.

Sediment samples for ∂^{15}N and ∂^{13}C analyses were freeze-dried during 48 h. Then ground using a ball mill (3 min at 3,000 rpm). Sediment samples are naturally enriched in carbonates, which can alter isotopic analyses and acidification is often required (Kolasinski et al. 2008). Therefore, they were acidified with 1 ml of hydrochloric acid 1 N until no further CO_2 releases were observed (Jacob et al. 2005). Sediment samples were then frozen (-18°C), freeze-dried again, encapsulated in silver and tin capsules, and sent to the isotopic spectrometry platform of LIENSs (Littoral Environment Societies, La Rochelle, France).

Before 90% acetone extraction overnight at 5°C (Lorenzen 1966), sediment samples were freeze-dried during 48h for chlorophyll *a* analyses as recommended by Hansson (1988). The supernatant was removed and centrifuged 10 min at 3,000 rpm. A first spectrometry read was performed at 665 and 750 nm. Then, 0.45 µl of HCl was added to dissolve chlorophyll *a* pigments during 3 min (Holm-Hansen and Riemann 1978). Finally, a second spectrometry read was realised. Samples were kept away from light with aluminium foil during the experiment. The concentration of chlorophyll *a* was expressed as:

$$[Chla]\ mg.L^{-1} = \frac{26.7\ \times\ [(A_{665}^{na} - A_{750}^{na}) - (A_{665}^{a} - A_{750}^{a})]}{L}$$

with 26.7 the chlorophyll coefficient, $A^{na}$and A^{a} the absorbance before and after acidification, respectively, at 665 and 750 nm, and L the optical path length (cm). Finally, the quantity of chlorophyll *a* was determined as: $Q_{chla}\ mg.m^{-2} = [Chla]\ \times\ \frac{V}{S}$ with V the volume of acetone used for extraction (L) and S the core surface (0.0015 m²).

Faeces were dried 48h at 60°C and weighted. The density recorded in the quadrat, allowed to determine the excretion rate, which is a proxy of the excretion rate (g.DW.ind^{-1}).

Data extraction from previous studies for long term comparison

In order to compare sea cucumber densities from Reunion Island over a large temporal scale and to understand if the current trends existed before, raw abundance data of sea cucumbers were extracted from: (1) four published papers, Conand et al. (1998); Jaquemet et al. (1999); Conand and Mangion (2002) and Conand (2004), (2) three master reports, Mangion (2003); Burgos and Hollinger (2007) and Pierrat (2018), (3) one Ph.D. thesis, Cuvillier (2016) and unpublished data (2013, Bédier). These studies used different sampling methods to record the abundance of sea cucumbers, with quadrats of various sizes or belt-transects. As the result, the total area covered ranged from 40 m² to 1,040 m². Finally, some studies have assessed sea cucumber abundance over Summer and Winter seasons, while others focused only on Summer season. In order to deal with the heterogeneity of the raw abundance data extracted, density (individual per square meter) was calculated for each replicate of each study based on the sampling surface. All the metadata from previous studies and global densities obtained have been merged in **Supporting Information Table S1**. Despite these differences, the compilation and the analysis of all these data is very useful to have a global vision of sea cucumber dynamics in Reunion Island.

Statistical analyses

All numerical analyses were realised using R (R Core Team 2022). Results are expressed as mean ± SE.

Population, sedimentary and feeding parameters

For all the analyses, the monthly replicates were grouped into four seasons, following statistical analyses (PERMANOVAs; p-value > 0.05 for months of each season): winter 2019, summer 2020, winter 2020 and summer 2021. Population variables (density and length of individuals) and feeding parameters (moving speed and excretion rate) were tested for spatial, temporal and specific variability using ANOVAs (when normality and homoscedasticity previously checked were congruent or when the size of sampling was adequate) or PERMANOVAs. Additionally, two-way ANOVAs were used to determine spatio-temporal variabilities of the grouped sedimentary factors investigated during this study (see section below). For all analyses, post-hoc Tukey HSD or pairwise-PERMANOVA tests were used when necessary.

Sedimentary factors driving sea cucumber populations

Multiple linear regressions were built for each species in both back-reef and shore-to-ocean gradients to highlight the variables of substrate composition or cover that could mostly drive the sea cucumber density, over different spatial scales. In order to reduce the number of substrate variables to build more accurate models, some substrate cover variables were clustered together regarding the significant correlation (correlation threshold= 25%, p-value < 0.05, **Supporting Information Table S2**) and the ecological relevance. Therefore, a new variable 'dead coral' includes 'erect dead coral' and 'coral rubble without turf', a variable 'living erect organisms' includes 'sponges', 'living coral without turf' and 'living coral with turf'', and a new variable 'algocyano' includes 'cyanobacteria' and 'red macroalga'. Then, standardized PCA was used and correlation tests between variables were investigated to remove collinear variables in each gradient (mean correlation threshold= 52%, p-value < 0.001, **Supporting Information Table S2**). Selected variables were those with the best normal distribution. Finally, variables were tested with spatial and/or temporal interaction in the models depending on the results of ANOVA shown in **Supporting Information Table S3** for back-reef gradient and **Supporting Information Table S4** for shore-to-ocean gradient.

For the back-reef gradient, the calculated variable is expressed by the following equation for *H. leucospilota* and *S. chloronotus*:

$$Density = \ season + station + chlorophyll\ a + sand + seagrasses + dead\ coral + living\ erect\ organisms + coral\ rubble\ with\ turf + chlorophyll\ a \times season + chlorophyll\ a \times station + sand \times season + sand \times station + seagrasses \times station + living\ erect\ organisms \times station + coral\ rubble\ with\ turf \times season + coral\ rubble\ with\ turf \times station + dead\ coral \times season + dead\ coral \times station.$$

For the shore-to-ocean gradient in PLA, the calculated variable is expressed by the following equation for *H. atra* and *H. leucospilota*:

$$Density = season + station + organic\ matter\ load + sand + fine\ particles + dead\ coral + living\ erect\ organisms + coral\ rubble\ with\ turf + algocyano + \partial^{13}C + sand \times season + fine\ particles \times season + organic\ matter\ load \times station + season \times organic\ matter\ load + coral\ rubble\ with\ turf \times season + \partial^{13}C \times station + \partial^{13}C \times season.$$

The *stepAIC* function in the R package MASS (Venables and Ripley 2002) was used to find out the best models for each species of each gradient, which are presented in **Supporting Information Table S5** with adjusted R^2. Significant variables were tested with ANOVA for each final model.

Density over long-term monitoring

Due to the heterogeneous dataset obtained from previous studies, non-parametric PERMANOVAs were performed to test the temporal evolution of the density for each species at each site. Pairwise-PERMANOVA were used when necessary with a Bonferroni correction.

Results

All details of statistical tests described in the Results section have been combined in **Supporting Information Table S6** for smoother reading.

Population features

Inter-site analyses: back-reef gradient

Two sites had high-density populations for *H. leucospilota*: MNS, with the highest density but the shortest individuals (2.6 ± 0.2 ind.m^{-2}, 19.1 ± 0.3 cm) and TE with the longest individuals (1.2 ± 0.1 ind.m^{-2}, 24.6 ± 0.5 cm). In consequence, significant spatial variabilities for density (ANOVA, p-value < 0.001) and length (ANOVA, p-value < 0.001) of

H. leucospilota were identified. Populations of this species were stable through seasons, while individuals were longest in 2019 than in 2021 at both sites (**Figure 2**).

The density and length of *S. chloronotus* were significantly different among sites (ANOVAs, p-values < 0.001), with the highest density recorded at TE (1.2 ± 0.1 ind.m^{-2}) and longer individuals at PAS and TE (11.6 ± 0.5 and 12.5 ± 0.7 cm, respectively) than at ES (7.8 ± 0.3 cm). A temporal trend shows decreasing densities over the study period, with less individuals in 2021, except for TE site with steady density, while no common trend appeared for length variation of *S. chloronotus* (**Figure 2**). A remarkable pattern can be observed at PAS, where density and length of individuals were inversely correlated over time, with the highest density and the shortest individuals observed in Winter 2019 (1.1 ± 0.2 ind.m^{-2}, 7.9 ± 0.3 cm) and the lowest density with the longest individuals in Summer 2021 (0.5 ± 0.1 ind.m^{-2}, 15.2 ± 1.1 cm), the length/density ratio was therefore four-fold higher in just 18 months.

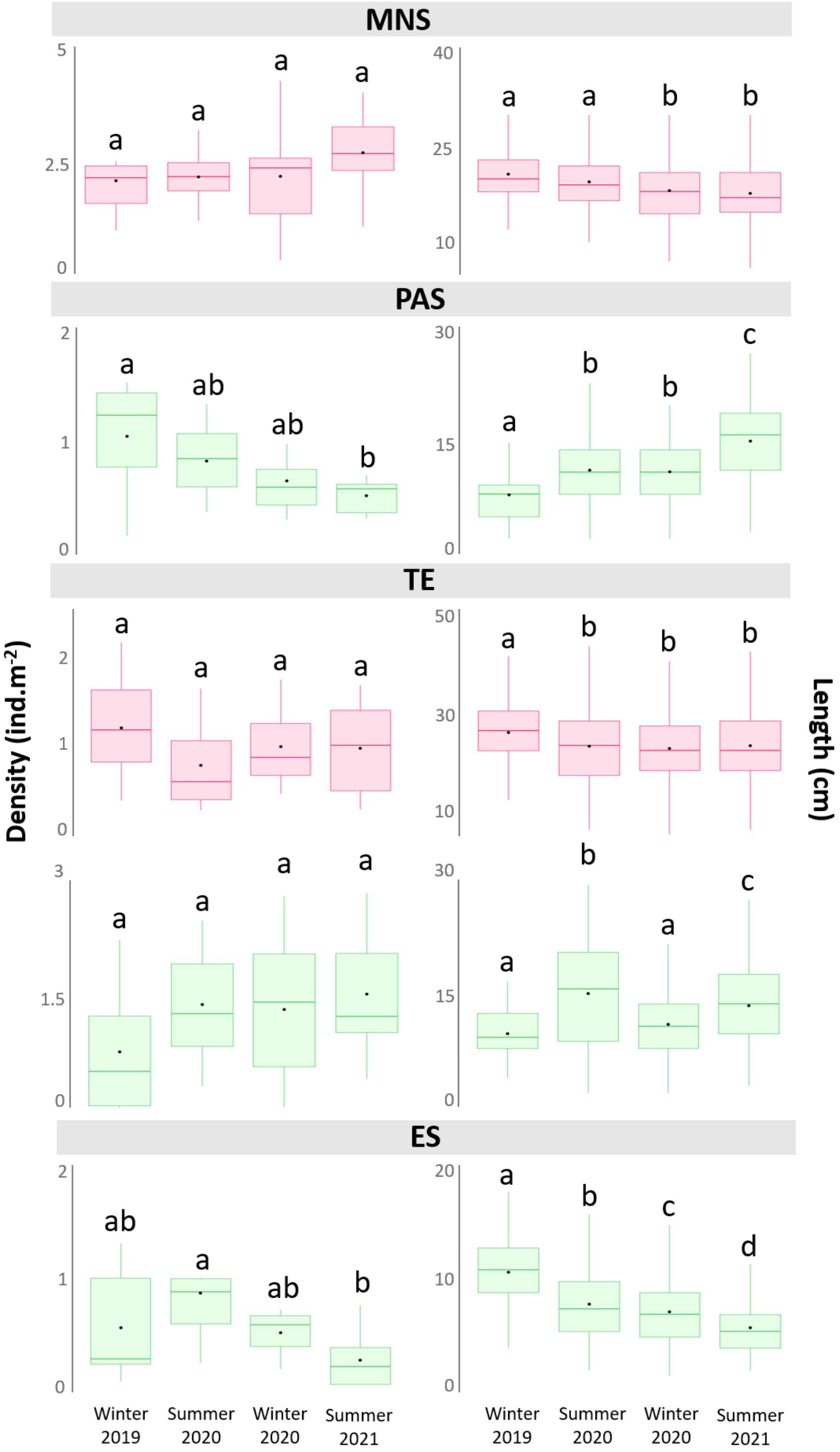

Figure 2: Temporal variability of the density (ind.m^{-2}) and length (cm) of *H. leucospilota* (in pink) and *S. chloronotus* (in green) in back-reefs (MNS, PAS and TE in Hermitage/La Saline reef and ES in Étang-Salé reef) in Reunion Island. Different letters indicate significant differences (Tukey HSD, p-value < 0.05). Black dots represent the mean.

Intra-site variability: shore-to-ocean gradient for community

Significant spatial differences for density and length of *H. leucospilota* (ANOVAs, p-values < 0.001) and also for *H. atra* (ANOVAs, p-values < 0.001) were observed along the shore-to-ocean gradient at PLA site (**Figure 3**). The highest density of *H. atra* was recorded at PLA$_{\text{SHORE}}$ (4.3 ± 0.4 ind.m^{-2}) and the lowest at PLA$_{\text{OCEAN}}$ (0.3 ± 0.0 ind.m^{-2}). The opposite pattern was observed for *H. leucospilota*, with highest densities at PLA$_{\text{MID}}$ and PLA$_{\text{OCEAN}}$ (0.8 ± 0.1 and 1.0 ± 0.1 ind.m^{-2}, respectively) and the lowest at PLA$_{\text{SHORE}}$ (0.3 ± 0.1 ind.m^{-2}). For both species, the longest individuals were found at PLA$_{\text{OCEAN}}$ (30.9 ± 0.3 and 16.8 ± 0.3 cm for *H. leucospilota* and *H. atra*, respectively), and the smallest at PLA$_{\text{SHORE}}$ (24.2 ± 0.4 and 12.3 ± 0.2 cm).

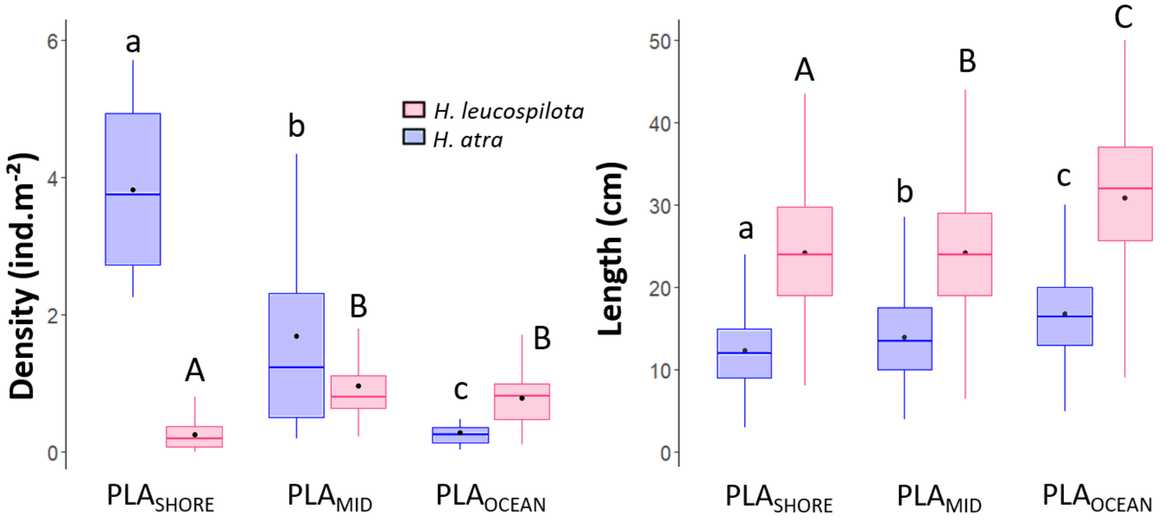

Figure 3: Spatial variability of the density (ind.m^{-2}) and length (cm) of *H. atra* (in blue) and *H. leucospilota* (in pink) over the shore-to-ocean gradient at PLA site (Hermitage/La Saline reef, Reunion Island).
Different lower-case letters indicate significant differences for *H. atra* while different upper-case letters indicate significant differences for *H. leucospilota* (Tukey HSD, p-value < 0.05). Black dots represent the mean.

Distribution of length: evidence of the absence of the early juvenile class-size in the analyses

The distribution of length for the three species are shown in **Supporting Information Fig. S1**. For all sites and seasons combined the mean length reached 23.7 ± 8.1 cm for

H. leucospilota (min= 3; max= 57), 13.8 ± 4.8 for *H. atra* (min= 3; max= 33) and 10.9 ± 5.2 for *S. chloronotus* (min= 1.5; max= 30). **Supporting Information Fig. S1** clearly highlights the absence of early juvenile class sizes, based on length.

Sedimentary patterns

Almost all variables of substrate composition and cover exhibited spatial and/or temporal variability for back-reef and shore-to-ocean gradients (ANOVA results are shown in **Supporting Information Table S7**).

Variability of sedimentary factors between reefs: back-reef gradient

The substrate composition at ES had the significant lowest total organic matter load (2.01 ± 0.03%), chlorophyll *a* concentration (93.5 ± 6.7 mg.m^2), ∂^{15}N and ∂^{13}C (-2.78 ± 0.03‰ and -20.4 ± 0.70‰ respectively), but the highest proportion of fine particles (1.25 ± 0.07%) (**Supporting Information Table S3**). All variable showed temporal variations, except ∂^{15}N, without any common pattern. Additionally, a difference in substrate cover was observed between the two reefs (**Figure 4**). Substrate cover at ES was homogeneous across seasons, mostly dominated by sand, living erect organisms and coral rubble with turf (**Figure 4**). Sites in Hermitage/La Saline reef (MNS, PAS and TE sites) had more diverse substrates, with greater presence of dead corals or seagrasses (at MNS only), presenting significant seasonal variability without particular pattern (**Supporting Information Table S3**).

Variability of sedimentary factors in shore-to-ocean gradient at PLA

Concerning substrate composition, spatial variability was found only for total organic matter load (higher at PLA$_{SHORE}$ with 2.62 ± 0.05%), and ∂^{13}C (higher at PLA$_{SHORE}$, 0.09 ± 0.05‰), meaning organic matter load and quality is heterogeneous there (**Supporting Information Table S3, S7**). Some temporal variabilities were detected, with organic matter load and ∂^{15}N, higher in Summer 2021 (2.62 ± 0.02% and 4.12 ± 0.05‰, respectively), whereas ∂^{13}C was lower (-0.19 ± 0.02‰). Concerning substrate cover, spatial homogeneity among stations in PLA was observed. Temporal variability was recorded for sand and coral rubble with turf on the three stations (PLA_{SHORE}, PLA_{MID} and PLA_{OCEAN}), with higher cover by coral rubble with turf in Summer 2021 while sand cover was higher in winter (**Figure 4**, **Supporting Information Table S4**).

Comparison of substrate composition between low- and high-density sites

Results did not identify a common trend for the different substrate composition variables of low- and high-density sites, monitored from Winter 2019 to Winter 2020. In fact, organic matter load ranged between 1.95 ± 0.05 % at ES and 2.82 ± 0.37 % at PLA_{SHORE}. Significant spatial differences were found between ES and all other sites (pairwise-PERMANOVA, p-values < 0.05) except MNS, but this is likely due to the very low intra-sample variability and all organic matter values can be considered low in our study. Percentage of fine particles, ranging from 0.19 ± 0.05 at PLA_{SHORE} to 1.16 ± 0.29 at PAS, was homogeneous between reefs (PERMANOVA, p-value = 0.213). Chlorophyll *a* concentration reached 75.4 ± 17.2 $mg.m^{-2}$ at ES and was two-fold higher at TE, 156.0 ± 23.9 $mg.m^{-2}$. Differences were found between ES and TE, ES and PTE, TE and CAP (pairwise-PERMANOVA, p-values < 0.05). Almost each site had its own isotopic signature for $\partial^{13}C$ (min. at ES -18.30 ± 2.38 and max. at PLA_{SHORE} 0.14 ± 0.09‰) and $\partial^{15}N$ (min. at ES -2.42 ± 1.42 and max. at TE 5.82 ± 0.29 ‰) (PERMANOVAs, p-values < 0.001). These results indicated that no site had a particular substrate composition related to sea cucumbers density.

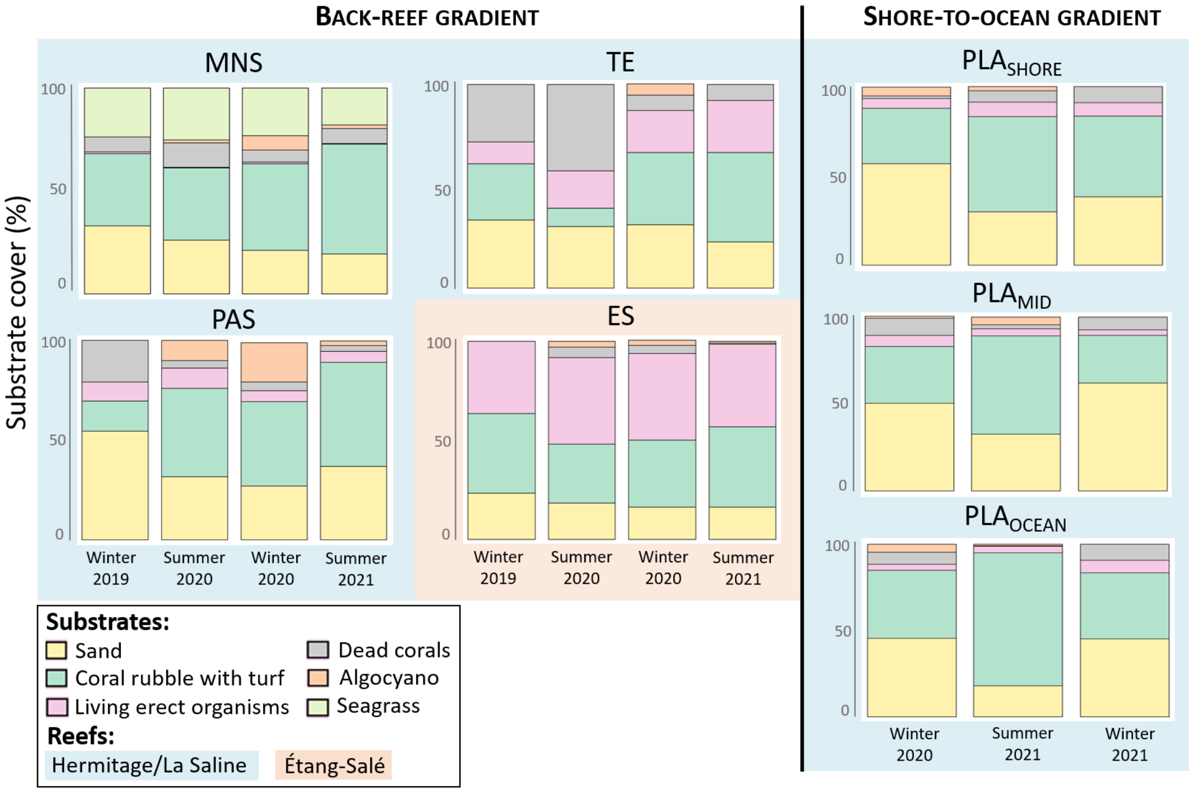

Figure 4: Substrates cover (in %) across the sites of the study (MNS, PAS, TE, PLA and ES) over the seasons of monitoring in Reunion Island.
Algocyano is the contraction of the variables “Algae” and “Cyanobacteria” which have been combined.

Moving and feeding patterns

Concerning the moving speed, no intraday significant difference was found for *H. leucospilota* and *H. atra* at each site (**Figure 5**). Conversely, diurnal variability of the moving speed was observed for *S. chloronotus* at PAS and TE (ANOVAs, p-value < 0.001 and p-value < 0.01), with a lower motion in the early morning, with 10.2 ± 4.7 and 32.5 ± 6.8 cm.h^{-1} respectively. Some spatial variability occurred only for the movement of *H. leucospilota* (ANOVA, p-value < 0.001), which is higher at MNS (18.8 ± 2.4 cm.h^{-1}) than at PLA and TE. Interspecific comparison for high-density species at the same site, gives (1) significantly higher speed for *S. chloronotus* (ANOVA, p-value < 0.001), 41.2 ± 3.4 cm.h^{-1} at TE, than *H. leucospilota* with 8.6 ± 1.1 cm.h^{-1} and (2) similar speeds for *H. atra* and *H. leucospilota* at PLA (12.8 ± 2.1 and 8.9 ± 1.6 cm.h^{-1} respectively) (**Figure 5**).

Globally, the excretion rate exhibited the same diurnal variability pattern as moving speed (**Figure 5**). Excretion rate of *H. leucospilota* was higher at TE compared to other sites (ANOVA, p-value < 0.001). Finally, this species excreted more sediment than *S. chloronotus* on TE (10.8 ± 2.3 and 5.41 ± 1.2 gDW.ind^{-1}.d^{-1} respectively; ANOVA, p-value < 0.05).

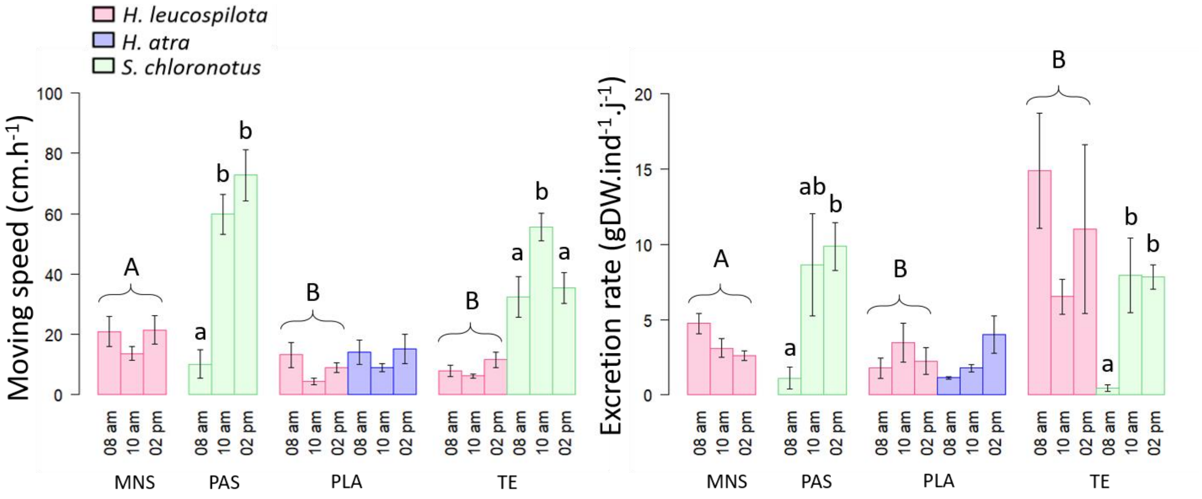

Figure 5: Moving speed (cm.h^{-1}) and excretion rate (gDW.ind^{-1}.d^{-1}) of *H. leucospilota* (in pink), *H. atra* (in blue) and *S. chloronotus* (in green) in different times of the day (08 and 10 am, 02 pm) at different sites (MNS, PAS, PLA and TE) in Hermitage/La Saline reef of Reunion Island (mean ± SE). Different lower-case letters indicate significant intraspecific temporal differences while different upper-case letters indicate significant intraspecific spatial differences (Tukey HSD, p-value < 0.05).

Sedimentary factors influencing sea cucumber distribution

Results of the linear model for each species of the back-reef gradient are shown in **Supporting Information Table S8**. The variance was well explained for both species (adjusted R^2= 0.707 for *H. leucospilota* and 0.423 for *S. chloronotus*). Results of model indicated that density of *H. leucospilota* increased with increasing seagrass and coral rubble with turf cover. The two significant interactions with station had no real meaning as both variable (dead coral and living erect organisms) showed individually no significant effect. The same is for the two significant interactions between season and chlorophyll *a* or dead coral, in the model for *S. chloronotus*. In consequence, no substrate composition or cover variable seemed to have an effect on the distribution of this species.

Regarding the shore-to-ocean gradient at PLA, the distribution of *H. leucospilota* was not explained by any substrate composition or cover variable, the adjusted R^2 for the model of this species was quite low (0.320). Finally, the model seemed to be very accurate for *H. atra*, as the adjusted R^2 reached 0.854. The density of this species increased with the increasing living erect organisms cover while declined with the increasing of algocyano cover. The two significant interactions highlighted by the model cannot be considered here, as organic matter load exhibited no significant effect.

Long-term dynamics for density of sea cucumbers

Stichopus chloronotus: collapsing populations

A decreasing trend was observed for the density of *S. chloronotus* (**Figure 6**). At PAS, even though no significant difference was found, a slight decrease was reported with 0.9 ± 0.4 ind.m^{-2} in 2013 (unpublished data, Bédier) and successively 1.0 ± 0.2, 0.8 ± 0.1, 0.7 ± 0.1 and 0.5 ± 0.0 ind.m^{-2} during our seasonal monitoring. At TE, high density recorded by Conand et al. (1998), 3.7 ± 0.6 ind.m^{-2}, fell to 1.3 ± 0.3 ind.m^{-2} in our monitoring (PERMANOVA, p-value < 0.001). The decrease in density was greatest at ES (PERMANOVA, p-value < 0.01), from 2.0 ± 0.0 ind.m^{-2} (Burgos and Hollinger, 2007) to 0.3 ± 0.1 ind.m^{-2} in Summer 2021 (this study).

Holothuria leucospilota: growing populations

Globally, the density of *H. leucospilota* seemed to increase through time at the three high-density sites, MNS, TE and PLA (**Figure 6**). The density has doubled at MNS between the studies by Cuvillier (2016), with 1.6 ± 0.1 ind.m^{-2} and ours, with 3.1 ± 0.5 ind.m^{-2} in Summer 2021 (PERMANOVA, p-value < 0.01). Moreover, at TE, the density was multiplied by about 10 (PERMANOVA, p-value < 0.05), from 0.2 ± 0.1 and 0.1 ± 0.1 ind.m^{-2} following Conand and Mangion (2002) and Mangion (2003) respectively, to 1.2 ± 0.2 for our study. Population of *H. leucospilota* seems to have increased at PLA from 0.1 ± 0.1 ind.m^{-2} (Mangion 2003) to 0.7 ± 0.1 ind.m^{-2} (mean for all stations of PLA site of this study).

Holothuria atra: loss of population momentum

The density of *H. atra* had decreased between Jaquemet et al. (1999), with 4.8 ± 0.2 ind.m^{-2}, and Mangion (2003) with 3.2 ± 0.6 ind.m^{-2} (**Figure 6**). Such high densities have no longer been observed since Winter 2020 (PERMANOVA, $p\text{-value} < 0.05$), meaning that the general trend is still decreasing, even if these results must be considered with caution because previous studies did not provide precise location of the monitoring of this species on PLA site.

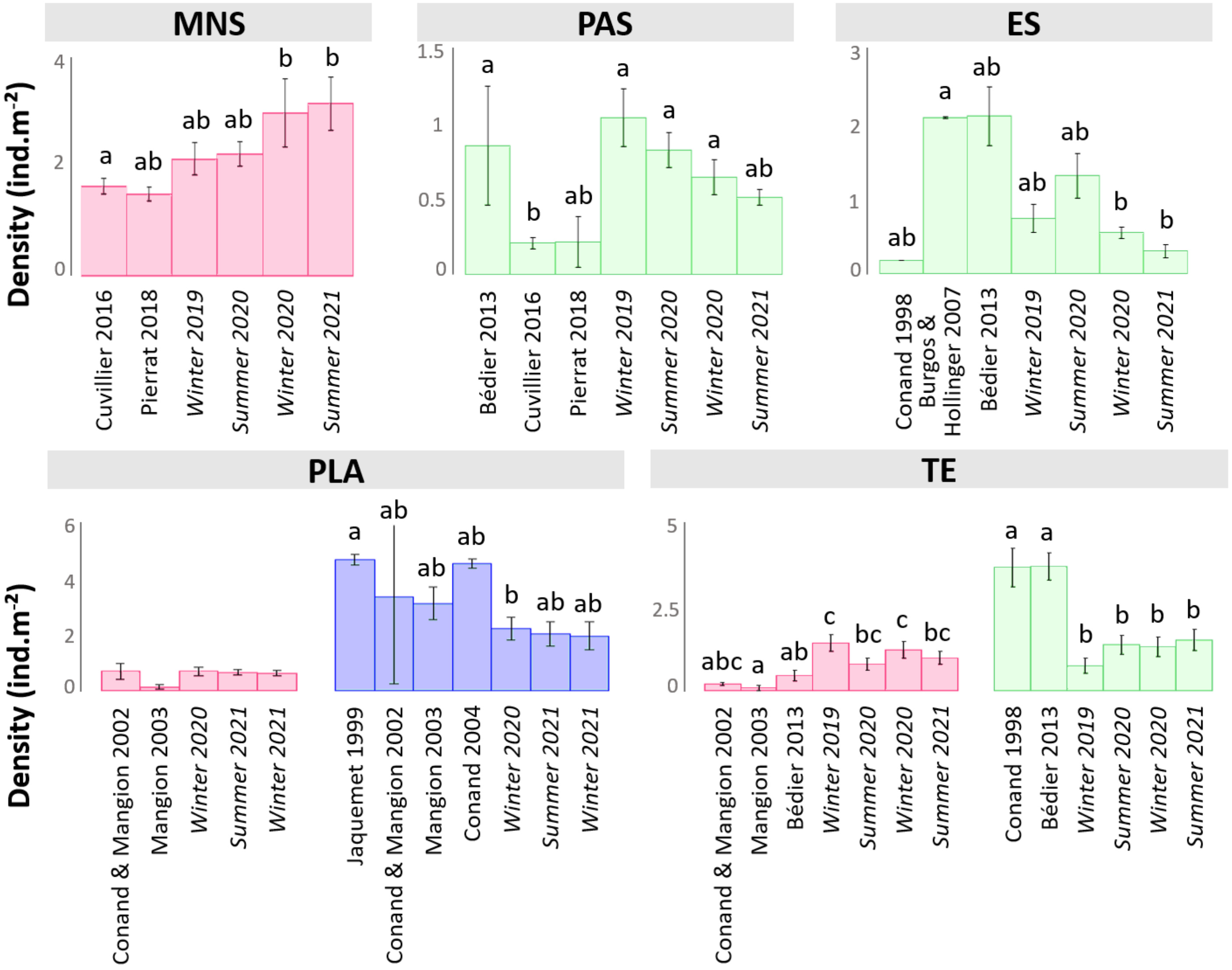

Figure 6: Long term comparison of the densities (ind.m^{-2}) of *H. leucospilota* (in pink), *H. atra* (in blue) and *S. chloronotus* (in green) along the reef of Hermitage/La Saline (MNS, PAS, PLA and TE) and Étang-Salé (ES) in Reunion Island (mean ± SE). Italicized captions indicate the results of this study. Raw density data of our study for the three PLA stations (PLA$_{\text{SHORE}}$, PLA$_{\text{MID}}$ and PLA$_{\text{OCEAN}}$) have been combined. Different letters indicate significant differences (pairwise-PERMANOVA, p-value < 0.05).

Discussion

Patchy distribution of sea cucumbers: links with sedimentary factors

Regarding *H. atra*, only PLA$_{\text{SHORE}}$ showed high-density (about 4 ind.m^{-2}), even though there was no significant difference in substrate cover along the shore-to-ocean gradient. This could indicate that the patchy distribution of *H. atra* at Reunion Island may not to be related to substrate cover. Eriksson et al. (2012) and Asha et al. (2015) have correlated the distribution of this species with the presence of boulders and rubbles, but Tanita and Yamada (2019); Viyakarn et al. (2020) and Hamamoto et al. (2022) with sand. PLA$_{\text{SHORE}}$ is the second station (considering also the sites) where both sand (more than 40%) and coral rubble with turf (almost 45%) dominated but no correlation was highlighted for this study. In consequence, *H. atra* seems to exhibit variability in substrate cover preference depending on the location. Other studies have highlighted the significance of substrate composition for this species, as Dissanayake and Stefansson (2012) and Viyakarn et al. (2020), who respectively demonstrated that distribution of *H. atra* was correlated with organically enriched sediments and high chlorophyll *a* concentrations. Conand and Mangion (2002) also showed that high level of eutrophication, leading to high organic matter availability, was suitable for populations of *H. atra*. The PLA$_{\text{SHORE}}$ substrate composition (highest total organic matter load and ∂^{13}C content among sites and highest chlorophyll *a* concentration among PLA stations) seems to match with the above statements. In consequence, the substrate cover seems to have low impact on the distribution of *H. atra* in Reunion Island compared to the substrate composition.

Correlation was found between the distribution of *H. leucospilota* and the presence of seagrasses, what fits with the results of Hamamoto et al. (2022). However, this has to be considered carefully, as only one reef site hosts seagrasses at Reunion Island. The distribution of *S. chloronotus* was unrelated to sedimentary factors in our study while some links have previously been identified with dead corals, scleractinian corals and coralline algae (Hamamoto et al, 2022). Additionally, as no significant difference was observed between low and high-density sites for substrate composition, and, despite the high number of sedimentary factors monitored during this study (substrate composition and cover variables), they do not explain the high-density patchy distribution of these two species at Reunion Island.

Several hypotheses could then explain this patchy distribution. Firstly, only sedimentary factors have been considered in this study while others have proposed that sea cucumber distribution can be related to hydrodynamic features, such as flow (Sun et al. 2018) and current (Azevedo E Silva et al. 2023), or environmental stability and substrate roughness (Félix et al. 2021) or linked to water column factors, such as salinity (Jobson et al. 2021) or temperature (Bonham and Held 1963). Hints for such hydrodynamic effect can be found in the density vs individual size patterns for *H. atra* at PLA sites, with lower densities and longer individuals when distant from the shore and thus being more exposed to swell effects. This agrees with Chao et al. (1993) who found small abundant individuals of this species in low tidepools and large sparse individuals in deep tidepools and also with Felix et al. (2021), who stated that bigger size classes venture more in unsheltered environment. However, the opposite pattern found at the same location for *H. leucospilota* shows this is not a general rule. Such opposite gradients for these two species could even raise a hypothesis about interspecific competition at PLA sites, but this has to be tested. Secondly, another possible factor to interpret the patchy distribution is conspecific attraction such as known for ophiuroids (Morgan and Jangoux 2004), sea urchins or sea stars (Campbell et al. 2001), and even sea cucumbers for reproductive reasons (Marquet et al. 2018). Claereboudt et al. (2023) recently discovered that sea cucumbers surrounded by good quality and available food secrete a pheromone composed of disaccharide saponins that chemoattract other individuals. Therefore, to reveal the factor that makes the high-density patch regardless of the substrate composition and cover, future studies focusing on such chemical attractant could give some new insights. Thirdly, few studies have demonstrated that some species of sea cucumbers may recruit larva or juvenile gregariously, as for instance *Psolus chitonoides* (Young and Chia 1982) or *Holothuria scabra* (Mercier et al. 2000). However, the recruitment processes of larvae and juveniles of these three species are largely understudied and require more attention.

One sea cucumber species, one temporal dynamic: link with intrinsic characteristics

Populations of *H. leucospilota* have extended over years and, as a consequence this species has now high densities (> 1 ind.m²) at three sites, MNS, PLA and TE, whereas formerly it was only present in high density at MNS (Conand and Mangion 2002) and PLA (P.F. personal observation). Genetic analyses, conducted at the same sites, highlighted that individuals of *H. leucospilota* did not share any multi-locus genes, meaning that the main mode of reproduction is gamete spawning (Pierrat et al. 2023). During this study, no individual resulting from fission had been observed, strengthening this statement. In consequence, the increase of *H. leucospilota* populations in Reunion Island should depend on a high rate of juvenile recruitment and the immigration of adults from outside the high-density patches within the same reef. Observation of early juveniles in Reunion Island reef is very rare (Shiell, 2004; P.F. and J.P. personal observations) due to their cryptic behaviour, size and inconspicuousness. The identification of nursery grounds of *H. leucospilota* could be an essential factor for better understanding the long-term dynamics of this species.

Density of *H. atra* has slightly decreased over the past two decades, even if it can be still considered as very high for such large invertebrates. The fission rate of *H. atra* has been studied in PLA site and has decreased drastically through years, with about 20% in 1995 (Conand 1995), 15% in 2004 (Conand 2004) and no individual underwent transversal fission during our monitoring. Additionally, Pierrat et al. (2022b) evidenced, using genetic tools, that sexual reproduction is mostly used by *H. atra* in Reunion Island. The decrease in density of

H. atra is possibly linked to the diminution of the use of asexual reproduction, combined with a low juvenile recruitment may be due to high larval or juvenile mortality.

Finally, the temporal variability of the density of *S. chloronotus* seems to be the most alarming, with high density currently reported at TE only. The decline of *S. chloronotus* populations is worrying, with now two out of three sites where the density can be considered as low (< 1 ind.m^{-2}). Conversely to the two others species, genetic analyses have indicated the use of asexual reproduction for *S. chloronotus* (Pirog et al. 2017; Pierrat et al. 2023), but with signs of decline. The pattern observed at PAS, where the density decreased while the length increased, can be considered as another evidence of the decrease of asexual reproduction rate by *S. chloronotus*. One observation of illegal harvesting of sea cucumbers in Reunion Island reefs have been reported on social network, what might also contribute to the decrease of *S. chloronotus* populations, a species that has a medium retail price (average of 100 US$ kg^{-1}; Purcell et al. 2018). However, the consumption of sea cucumber is not a tradition in Reunion Island. Hence, the decline of *S. chloronotus* populations is likely due to other unstudied factors which could also constrain populations, such as diseases (Han et al. 2016), global climate change (Buccheri et al. 2019), or predation (Francour 1997; Papafitsoros 2023).

Feeding behaviour distinguishes between generalist and specialist species

The evaluation of feeding features brings useful information concerning the profiles of the species studied. The moving speeds and excretion rates of *H. leucospilota* and *H. atra* did not show any variation during the day, therefore these results confirm that these two species have a continuous diurnal feeding rhythm, as reported previously (Klinger and Johnson 1998; Mangion et al. 2004). Conversely, *S. chloronotus* seems less active in the early morning (8 am), meaning that this species has a diurnal rhythm, which is congruent with other studies (Yamanouchi 1956; Klinger and Johnson 1998). The speed of *S. chloronotus* was higher than these of the two others *Holothuria* species. The speed of sea cucumbers is attributed to foraging activities, and more available the food is, less the sea cucumbers move (Klinger and Johnson 1998; Conand 2004). For instance, Mangion (2003) has demonstrated that mean speeds of *H. atra* and *H. leucospilota* were significantly higher on oligotrophic sediments (120 and 170 $cm.h^{-1}$, respectively) than on eutrophic sediments (40 and 60 $cm.h^{-1}$). Overall, the excretion rate of *S. chloronotus* was much higher than that of *H. atra* and mildly higher than that of *H. leucospilota*. If the nutrient in the sediments decrease, it means that more sediments have to get through the gut of *S. chloronotus* to fulfil its nutrient requirements, and in consequence the excretion rate is higher. The foraging depth of *S. chloronotus* tentacles is only one centimetre, whereas those *H. atra* can reach four centimetre depth (Taddei 2006). Regarding all the features, diurnal pattern, moving speed, excretion rate and depth of foraging, *S. chloronotus* seems to be a specialist species, whereas *H. leucospilota* and *H. atra* are generalist species, as already suggested by Uthicke (1999) based on the selection of sediments with high phycopigment concentrations. As this study highlighted that the substrate composition of Reunion Island reefs is fluctuating across seasons, the specialist behaviour of *S. chloronotus* appears to be a disadvantage. The specialist behaviour of *S. chloronotus* can negatively influence density, as more energy is allocated to foraging to fulfil daily nutrient requirements, compared to the two other species. The behaviour of the species appears therefore to be a key factor explaining the distribution and density of sea cucumber in Reunion Island. Interspecific competition for food could influence the distribution patterns of the three species and their temporal trends, especially for the decreasing populations of *S. chloronotus*. It has to be demonstrated yet. Analysis of food quality, considering spatial and temporal variability in sediments and in sea cucumber diets would be required.

Conclusion

The high-density patches of sea cucumbers that can be found in coral reefs at Reunion island could be explained by environmental factors. This study revealed the correlation between

H. atra distribution and substrate composition but fails to find any relation between sedimentary factors and the distribution of *H. leucospilota* and *S. chloronotus* in Reunion Island. These findings highlight the need for a more inclusive study to fully explain the patchy distribution of sea cucumbers, including factors related to water column and hydrodynamics, physiological features for conspecific attraction purposes, as well as the quality and quantity of food related to sea cucumber diet. Our results and the diversity of driving factors found in the literature show the sea cucumbers have plasticity to thrive in many environments, and local distribution patterns can appear at kilometric scale, linked to species and local conditions. One of the biggest challenges in understanding the distribution of sea cucumbers, is to identify juvenile nursery grounds in order to quantify recruitment in adult high-density patches and investigate gregarious effects. Multiseasonal monitoring shows that the three species displayed three distinct temporal patterns. Populations of *H. leucospilota* have increased since 2002 and are now stable, while the density of *H. atra* has slightly decreased since the 1990’s, but remains very high. The drastic decline of *S. chloronotus* populations is the most alarming. This may be due to a synergy between the diminution of asexual reproduction and the specialist behaviour of this species, confirmed in this study, which could be a limiting factor for the sustainability of these populations in Reunion Island reefs. Other factors such as predation or global climate change may be also involved. The design of one common method for spatio-temporal monitoring of wild sea cucumber populations appears as a necessary tool to understand their long-term dynamics.

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Data Availability Statement

Data are available on request to the authors.

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Supporting Information

Supporting Information Table S1: Metadata from previous studies on the monitoring of sea cucumbers in Reunion Island.
Ha: *Holothuria atra*, Hl: *Holothuria leucospilota*, Sc: *Stichopus chloronotus*.
MNS, PAS, PLA and TE sites are located in Hermitage/La Saline reef and ES in Étang-Salé.

Authors	Season studied	Sampling method	Replicates	Total area sampled m^2	Sites studied	Species studied	Global density (ind.m^2 mean ± se)
Conand et al. 1998	Summer and Winter	Quadrat 1 m^2	60	60	TE	Sc	3.8 ± 0.6
			60	60	ES	Sc	0.2 ± 0.0
Jaquemet et al. 1999	Summer and Winter	Quadrat 80 m^2	8	640	PLA	Ha	4.8 ± 0.2
Conand & Mangion 2002	Summer	Quadrat 20 m^2	2	40	PLA (Pl)	Ha	3.4 ± 3.2
						Hl	0.7 ± 0.3
			2	40	TE (Tr)	Hl	0.2 ± 0.1
Mangion 2003	Summer	Quadrat 100 m^2	3	300	PLA	Ha	3.2 ± 0.6
						Hl	0.1 ± 0.1
			4	400	TE	Hl	0.1 ± 0.1
Conand 2004	Summer	Quadra 10 m^2	24	240	PLA	Ha	4.6 ± 0.2
Burgos & Hollinger 2007	Summer	Belt-transect 100 m^2	3	300	ES	Sc	2.0 ± 0.0
Bédier 2013 (Unpublished data)	Summer	Belt-transect 50 m^2	2	220	PAS	Sc	0.9 ± 0.4
		Belt-transect 60 m^2	2				
		Belt-transect 50 m^2	3	650	TE	Hl	0.5 ± 0.2
		Belt-transect 100 m^2	5			Sc	3.8 ± 0.4
		Belt-transect 100 m^2	5	500	ES	Sc	2.0 ± 0.4
Cuvillier 2016	Summer and Winter	Belt-transect 20 m^2	52	1040	MNS	Hl	1.6 ± 0.1
			49	980	PAS	Sc	0.2 ± 0.0
Pierrat 2018	Summer	Belt-transect 20 m^2	3	60	MNS	Hl	1.5 ± 0.1
			3	60	PAS	Sc	0.2 ± 0.1

Supporting Information Table S2: Pearson's correlation results of substrate composition and cover variables. Variables clustered together are in the same colour. Variables selected (those with the best normal distribution) are in bold.

Variables clustered together for both gradient	*r* value	Df	*P*val
Red macroalga & Cyanobacteria	0.421	243	< 0.001
Erect dead coral & Coral rubble without turf	0.494	243	< 0.001
Sponges & Living coral without turf	0.251	243	0.005
Sponges & Living coral with turf	0.361	243	< 0.001
Living coral without turf & Living coral with turf	0.299	243	0.001
Variables selected or excluded for back-reef gradient	***r* value**	**Df**	***P*val**
Organic matter load & ***Chlorophyll* a**	0.393	37	< 0.001
Organic matter load & $\partial^{15}N$	0.704	37	< 0.001
Organic matter load & ∂^{13C}	0.664	37	< 0.001
***Chlorophyll* a** *&* $\partial^{15}N$	0.595	37	< 0.001
***Chlorophyll* a** *&* $\partial^{13}C$	0.597	37	< 0.001
$\partial^{15}N$ *&* $\partial^{13}C$	0.921	37	< 0.001
Coral rubble with turf *& Fine particles*	0.227	37	< 0.001
Variables selected or excluded for shore to ocean gradient	***r* value**	**Df**	***P*val**
Organic matter load *&* $\partial^{15}N$	0.316	19	0.011
Dead coral *& Chlorophyll* a	0.229	19	0.001

Supporting Information Table S3: Spatio-temporal variations of substrate composition and cover variables from the back-reef depression gradient (MNS, PAS, TE and ES sites) in Reunion Island. Values are mean ±se. Different letters indicate significant difference between seasons or sites (p-value < 0.005). Results of Tukey-HSD tests performed after ANOVA (results presented in **Supporting Information Table S6**) (except for ∂^{13}C seasonal variation where Student Newman-Keuls test was used to reveal the significant difference highlighted by ANOVA). Algocyano is the contraction of the variables "Algae" and "Cyanobacteria" which have been combined.

Sedimentary factors	**Winter 2019**	**Summer 2020**	**Winter 2020**	**Summer 2021**	**MNS**	**PAS**	**TE**	**ES**
Sand %	*a*	*ab*	*b*	*b*	*ac*	*b*	*ab*	*c*
	33.6 ±1.44	26.6 ±1.36	23.9 ±1.13	23.9 ±1.19	24.7 ±1.38	35.8 ±1.69	30.2 ±1.15	19.1 ±0.65
Coral rubble with turf %	*a*	*a*	*ab*	*b*	*a*	*ab*	*b*	*ab*
	31.1 ±2.73	29.6 ±3.88	38.4 ±2.40	47.4 ±2.32	41.3 ±2.74	39.6 ±3.54	29.4 ±3.29	36.3 ±2.06
Living erect organisms %	*a*	*a*	*a*	*a*	*a*	b	c	d
	16.7 ±2.63	18.1 ±3.33	17.6 ±2.79	18.3 ±3.40	0.59 ±0.19	7.42 ±0.83	17.8 ±2.10	40.8 ±2.12
Dead corals %	*ab*	*a*	*b*	*b*	*a*	*a*	*b*	*a*
	13.8 ±3.30	15.9 ±4.18	5.54 ±1.05	4.68 ±1.32	7.89 ±1.44	7.17 ±2.24	21.2 ±4.05	2.34 ±0.59
Algocyano %	a	ab	b	a	*a*	*b*	*a*	*a*
	0 ±0	3.65 ±1.85	8.56 ±2.05	1.29 ±0.45	2.87 ±0.87	9.60 ±2.83	1.54 ±1.06	1.48 ±0.72
Seagrasses %	*a*	*a*	*a*	*a*	*a*	*b*	*b*	*b*
	4.85 ±1.71	6.26 ±2.4	5.82 ±1.67	4.47 ±1.49	22.6 ±1.68	0 ±0	0 ±0	0 ±0
Total organic matter load %	*a*	*ab*	*b*	*ab*	*a*	*a*	*b*	*c*
	2.40 ±0.04	2.30 ±0.06	2.23 ±0.03	2.35 ±0.05	2.27 ±0.03	2.38 ±0.02	2.61 ±0.02	2.01 ±0.03
Fine particles %	*ab*	*a*	*b*	*c*	*a*	*a*	*a*	*a*
	0.90 ±0.05	1.10 ±0.05	0.81 ±0.07	2.04 ±0.07	1.05 ±0.08	1.31 ±0.13	0.99 ±0.10	1.25 ±0.07
Chlorophyll *a* mg.m²	*a*	*b*	*b*	*ab*	*a*	*a*	*a*	*b*
	107 ±5.75	133 ±7.62	131 ±5.86	129 ±6.86	134 ±5.61	128 ±6.07	143 ±4.86	93.5 ±+6.75
∂^{13}C ‰	*a*	*b*	*b*	*ab*	*a*	*bd*	*ad*	*c*
	-8.40 ±1.51	-6.43 ±1.70	-6.52 ±1.12	-7.45 ±1.29	-3.61 ±0.50	-0.86 ±0.12	-2.56 ±0.53	-20.4 ±+0.70
∂^{15}N ‰	*a*	*a*	*a*	*a*	*a*	*b*	*b*	*c*
	2.22 ±0.65	2.95 ±0.66	2.53 ±0.61	2.80 ±0.54	2.52 ±0.44	5.48 ±0.14	5.60 ±0.20	-2.78 ±0.29

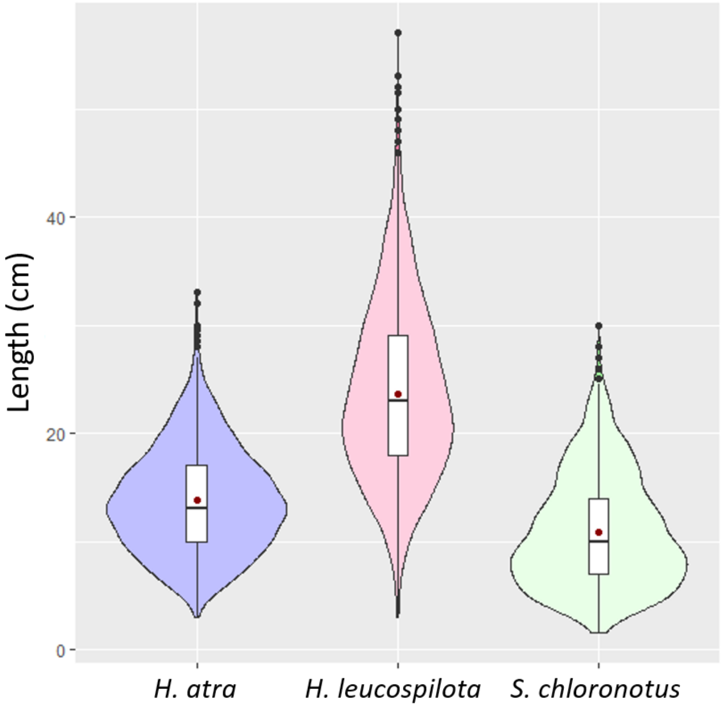
Supporting Information Table S4: Spatio-temporal variations of substrate composition and cover variables from the shore-to-ocean gradient (PLA site) in Reunion Island. Values are mean ±se. Different letters indicate significant difference between seasons or sites (p-value < 0.005). Results of Tukey-HSD tests performed after ANOVA (results presented in **Supporting Information Table S6**).
Algocyano is the contraction of the variables “Algae” and “Cyanobacteria” which have been combined.

Sedimentary factors	**Winter 2020**	**Summer 2021**	**Winter 2021**	**PLASHORE**	**PLAMID**	**PLAOCEAN**
Sand %	*a*	*b*	*a*	*a*	*a*	*a*
	51.0 ±5.84	27.0 ±2.36	48.6 ±2.64	40.1 ±4.16	46.3 ±4.45	33.7 ±4.70
Coral rubble with turf %	*a*	*b*	*a*	*a*	*a*	*a*
	34.3 ±5.96	62.2 ±2.79	36.9 ±4.71	44.6 ±4.24	41.3 ±4.60	55.2 ±6.06
Living erect organisms %	*a*	*a*	*a*	*a*	*a*	*a*
	5.14 ±1.07	5.32 ±0.85	6.01 ±1.02	7.17 ±1.13	4.47 ±0.95	4.76 ±0.65
Dead corals %	*a*	*a*	*a*	*a*	*a*	*a*
	6.03 ±2.39	3.01 ±0.87	8.38 ±2.18	5.71 ±1.67	5.92 ±2.12	4.60 ±1.53
Algocyano %	*a*	*a*	*a*	*a*	*a*	*a*
	3.55 ±1.68	2.49 ±1.05	0.05 ±0.04	2.37 ±1.59	2.22 ±1.03	1.70 ±0.77
Total organic matter load %	*a*	*b*	*a*	*a*	*b*	*b*
	2.47 ±0.05	2.62 ±0.03	2.43 ±0.02	2.62 ±0.05	2.49 ±0.03	2.46 ±0.02
Fine particles %	*a*	*b*	*c*	*a*	*a*	*a*
	0.73 ±0.12	1.92 ±0.10	0.29 ±0.06	1.23 ±0.14	1.46 ±0.14	1.50 ±0.15
Chlorophyll *a* mg.m²	*a*	*a*	*a*	*a*	*a*	*a*
	109 ±4.55	100 ±6.79	94.6 ±10.4	112 ±7.90	99.4 ±8.49	92.2 ±5.56
∂^{13}C ‰	*a*	*b*	*a*	*a*	*b*	*b*
	0.03 ±0.03	-0.19 ±0.02	0.13 ±0.05	0.09 ±0.05	-0.07 ±0.03	-0.12 ±0.03
∂^{15}N ‰	*a*	*b*	*c*	*a*	*a*	*a*
	3.78 ±0.10	4.12 ±0.05	1.93 ±0.09	3.50 ±0.19	3.33 ±0.26	3.37 ±0.21

Supporting Information Table S5: Final linear regression model of each species (*Holothuria atra*, *H. leucospilota* and *Stichopus chloronotus*) for back-reef and shore-to-ocean gradients in Reunion Island used to find out the most significant variables explaining sea cucumbers density. Adjusted R^2 values are presented.

Back-reef gradient	**Final Model**	R^2
H. leucospilota	*= site + chlorophyll* a *+ seagrasses + dead coral + living erect organisms + coral rubble with turf + chlorophyll* a *× site + coral rubble with turf × site + dead coral × site + living erect organisms × site*	0.707
S. chloronotus	*= site + season + chlorophyll* a *+ dead coral + coral rubble with turf + chlorophyll* a *× season + coral rubble with turf × season + dead coral × season*	0.423
Shore-to-ocean gradient	**Final Model**	R^2
H. atra	*= station + season + organic matter load + sand + dead coral + fine particles + coral rubble with turf + living erect organisms + algocyano +* $\partial^{13}C$ + $\partial^{13}C$ *× season + organic matter load × season*	0.854
H. leucospilota	*= station + season + sand + dead coral + fine particles + coral rubble with turf +* $\partial^{13}C$ + $\partial^{13}C$ *× station + fine particles × season*	0.320

Supporting Information Fig. S1: Distribution of all lengths of the three species monitored (*Holothuria atra*, *H. leucospilota* and *Stichopus chloronotus*) during this study (sites, reefs and seasons combined) in Reunion Island.
Dark red dots represent the mean.
Holothuria atra: in blue (*N*= 2012);
Holothuria leucospilota: in pink (*N*= 3876);
Stichopus chloronotus: in green (*N*= 2581)
Violin Plot (using ggplot2 package on R).



Supporting Information Table S6: Details of statistical tests described in Results in chronological order of apparition in the subsections.

	Population features
	Inter-site analyses: back-reef gradient
1.	ANOVA, *N*= 156, df= 3, F= 73.05, p-value < 0.001
2.	ANOVA, *N*= 1920, df= 1, F= 353.7, p-value < 0.001
3.	ANOVA, *N*= 156, df= 3, F= 24.13, p-value < 0.001
4.	ANOVA, *N*= 2580, df= 2, F= 306.7, p-value < 0.001
	Intra-site variability: shore-to-ocean gradient for community
1.	ANOVA, *N*= 63, df= 2, F= 14.99, p-value < 0.001
2.	ANOVA, *N*= 1549, df= 2, F= 132, p-value < 0.001
3.	ANOVA, *N*= 63, df= 2, F= 53.1, p-value < 0.001
4.	ANOVA, N= 1600, df= 2, F= 104.9, p-value < 0.001
	Sedimentary patterns
	Comparison of substrate composition between low- and high-density sites
1.	pairwise-PERMANOVA, *N*= 69, p-values < 0.05
2.	PERMANOVA, *N*= 69, maxT= 2.252, p-values = 0.213
3.	pairwise-PERMANOVA, *N*= 69, p-values < 0.05
4.	PERMANOVA, *N*= 69, maxT= 7.147, p-values < 0.001
5.	PERMANOVA, *N*= 69, maxT= 5.036, p-values < 0.001
	Moving and feeding patterns
1.	ANOVAs, *N*= 45, df= 2, F= 23.72, p-value < 0.001
2.	ANOVA, *N*= 45, df= 2, F= 5.142, p-value < 0.01
3.	ANOVA, *N*= 145, df= 2, F= 10.4, p-value < 0.001
4.	ANOVA, *N*= 90, df= 1, F= 79.03, p-value < 0.001
5.	ANOVA, *N*= 45, df= 2, F= 10.27, p-value < 0.001
6.	ANOVA, *N*= 30, df= 1, F= 4.261, p-value < 0.05
	Long-term dynamics for density of sea cucumbers
	Stichopus chloronotus: collapsing populations
1.	PERMANOVA, *N*= 53, maxT= 4.444, p-values < 0.001
2.	PERMANOVA, *N*= 49, maxT= 3.323, p-values < 0.01
	Holothuria leucospilota: growing populations
1.	PERMANOVA, *N*= 92, maxT= 3.363, p-values < 0.01
2.	PERMANOVA, *N*= 56, maxT= 2.546, p-values < 0.05
	Holothuria atra: loss of population momentum
1.	PERMANOVA, *N*= 79, maxT= 3.084, p-values < 0.05

Supporting Information Table S7: Two-way ANOVA results for substrate composition and cover variables at back-reef (MNS, PAS, TE and ES) and shore-to-ocean (PLA) gradient in Reunion Island, across sites and seasons.

Note that no seagrasses were recorded in PLA site for the shore-to-ocean gradient.

Algocyano is the contraction of the variables “Algae” and “Cyanobacteria” which have been combined.

	Back-reef gradient					**Shore-to-ocean gradient**				
	SS	*Df*	*MS*	*F value*	*p-value*	*SS*	*Df*	*MS*	*F value*	*p-value*
Sand										
Season	2666	3	888.7	5.702	**< 0.001**	8097	2	4048	14.468	**< 0.001**
Site	6576	3	2192.1	14.064	**< 0.001**	1654	2	827	2.955	0.061
Residuals	21821	149	155.9			15110	54	280		
Seagrasses										
Season	72	3	24	0.96	0.414					
Site	14386	3	4795	190.64	**< 0.001**					
Residuals	3521	149	25							
Algocyano										
Season	1923	3	641.1	8.517	**< 0.001**	117.6	2	58.79	2.09	0.134
Site	1421	3	473.7	6.292	**< 0.001**	5.1	2	2.56	0.091	0.913
Residuals	10539	149	75.3			1518.8	54	28.13		
Living erect organisms										
Season	58	3	19	0.188	0.905	7.7	2	3.87	0.213	0.809
Site	37618	3	12539	122.686	**< 0.001**	92.2	2	46.08	2.543	0.088
Residuals	14309	149	102			978.3	54	18.12		
Coral rubble with turf										
Season	6795	3	2265.1	8.549	**< 0.001**	10953	2	5476	15.664	**< 0.001**
Site	2902	3	967.4	3.651	**0.014**	2231	2	1115	3.19	**0.049**
Residuals	37092	149	264.9			18878	54	350		
Dead corals										
Season	3595	3	1198.3	6.08	**< 0.001**	321	2	160.31	2.61	0.083
Site	7962	3	2654	13.467	**< 0.001**	21	2	10.55	0.172	0.843
Residuals	27590	149	197.1			3316	54	61.42		
Organic matter load										
Season	0.766	3	0.2553	15.675	**< 0.001**	0.4698	2	0.23492	10.11	**< 0.001**
Site	7.902	3	2.6339	161.699	**< 0.001**	0.2936	2	0.14678	6.317	**0.003**
Residuals	2.28	149	0.0163			1.2548	54	0.02324		
Fine particles										
Season	33.37	3	11.122	99.587	**< 0.001**	15.631	2	7.815	41.57	**< 0.001**
Site	2.58	3	0.859	7.69	**< 0.001**	0.862	2	0.431	2.293	0.111
Residuals	15.64	149	0.112			10.152	54	0.188		
Chlorophyll **a**										
Season	18756	3	6252	7.142	**< 0.001**	1932	2	966	1.054	0.356
Site	56240	3	18747	21.414	**< 0.001**	4027	2	2013	2.197	0.121
Residuals	122560	149	875			49496	54	917		
$\partial^{13}C$										
Season	107	3	36	5.582	**0.001**	1.2016	2	0.6008	59.294	**< 0.001**
Site	10001	3	3334	521.725	**< 0.001**	0.4847	2	0.2423	23.916	**< 0.001**
Residuals	895	149	6			0.5472	54	0.0101		
$\partial^{15}N$										
Season	11.5	3	3.8	1.586	0.195	55.57	2	27.784	369.044	**< 0.001**
Site	1876.5	3	625.5	257.969	**< 0.001**	0.33	2	0.164	2.177	0.123
Residuals	339.5	149	2.4			4.07	54	0.075		

Supporting Information Table S8: Significance of ecological variables depending on the sea cucumber density from each specific general linear model (ANOVA tests) for the back-reef and shore-to-ocean gradient monitored in Reunion Island reefs.

Back-reef gradient (MNS, PAS, TE and ES)	**SS**	**Df**	**MS**	**F value**	**p-value**
Stichopus chloronotus					
Site	28.034	3	9.345	28.448	**< 0.001**
Season	2.002	3	0.667	2.032	0.113
Chlorophyll *a*	0.295	1	0.295	0.898	0.345
Dead coral	1.046	1	1.046	3.183	0.077
Coral rubble with turf	0.948	1	0.948	2.885	0.092
Season × Chlorophyll *a*	3.814	3	1.271	3.870	**0.011**
Season × Coral rubble with turf	1.78	3	0.593	1.806	0.149
Season × Dead coral	3.501	3	1.167	3.552	**0.016**
Residuals	42.375	129	0.329		
Holothuria leucospilota					
Site	157.671	3	52.557	100.940	**< 0.001**
Chlorophyll *a*	0.492	1	0.492	0.945	0.333
Seagrass	4.509	1	4.509	8.661	**0.004**
Dead coral	1.917	1	1.917	3.682	0.057
Living erect organisms	1.204	1	1.204	2.313	0.131
Coral rubble with turf	4.632	1	4.632	8.895	**0.003**
Station × Chlorophyll *a*	3.944	3	1.315	2.525	0.061
Station × Living erect organisms	6.433	3	2.144	4.118	**0.008**
Station × Coral rubble with turf	3.425	3	1.142	2.192	0.092
Station × Dead coral	10.928	3	3.643	6.996	**< 0.001**
Residuals	66.126	127	0.521		
Shore-to-ocean gradient (PLA)	**SS**	**Df**	**MS**	**F value**	**p-value**
Holothuria atra					
Station	174.186	2	87.093	136.070	**< 0.001**
Season	0.769	2	0.385	0.601	0.553
Organic matter load	0.044	1	0.044	0.069	0.794
Sand	1.397	1	1.397	2.182	0.147
Fine particles	1.049	1	1.049	1.639	0.207
Dead coral	0.061	1	0.061	0.095	0.759
Living erect organisms	5.125	1	5.125	8.007	**0.007**
Coral rubble with turf	0.009	1	0.009	0.014	0.905
Macroalgae	16.489	1	16.489	25.762	**< 0.001**
$\partial^{13}C$	1.304	1	1.304	2.038	0.160
Season × Organic matter load	16.886	2	8.443	13.191	**< 0.001**
Station × Organic matter load	23.588	2	11.794	18.426	**< 0.001**
Station × $\partial^{13}C$	3.518	2	1.759	2.748	0.075
Residuals	28.163	44	0.640		
Holothuria leucospilota					
Station	5.624	2	2.812	15.182	**< 0.001**
Season	0.031	2	0.016	0.085	0.919
Soft substrat	0.019	1	0.019	0.103	0.750
Fine particles	0.022	1	0.022	0.120	0.731
Dead coral	0.012	1	0.012	0.066	0.798
Coral rubble with turf	0.039	1	0.039	0.212	0.647
$\partial^{13}C$	0.075	1	0.075	0.405	0.527
Season × Fine particles	0.810	2	0.405	2.185	0.123
Station × $\partial^{13}C$	1.168	2	0.584	3.154	0.051
Residuals	9.076	49	0.185		