Shallow epifaunal sea cucumber densities and their relationship with the benthic community in the Okinawa Islands

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Sea cucumbers are important ecological engineers in marine ecosystems. However, the fishery demand of some species, especially large-epifaunal and commercially used (LEC) sea cucumbers, has risen drastically, resulting in serious depletion of local populations for many species. Despite this problem, basic ecological data on sea cucumbers, such as population densities and preferred habitats, are often still insufficient. Here, we report on the population densities of multiple LEC sea cucumber species, and their ambient benthic communities at eight sites around Okinawa Islands. Further, we discuss the correspondence between sea cucumber densities and the surrounding coral communities. Our results show two sites within national or quasi-national parks, Aka and Manza, where stricter rules have been placed on fisheries and land reclamation compared to other areas, had the highest and third highest sea cucumber population densities among sites, respectively. *Holothuria atra* was observed at all survey sites and made up the majority of sea cucumber populations at all sites except for Chatan and Sesoko, where *Holothuria leucospilota* and *Stichopus chloronotus* were most abundant, respectively. Regarding relationships between benthic composition and LEC sea cucumber species, *S. chloronotus* was significant correlated with dead corals, scleractinian corals, and coralline algae. As well, *H. leucospilota* had significant correlations with rubble. There were no significant correlations between scleractinian corals and sea cucumber densities, although *S. chloronotus* was marginally insignificant with *Platygyra* and *Psammocora*. Notably, medium- to highly valued species were very sparse in our surveys, and most of them appeared at only one site. Additionally, one site (Odo) had only three LEC sea cucumber individuals observed. Combining these facts with relatively low population densities around the Okinawa Islands compared to densities reported in previous research from the Indo-

West Pacific Ocean region, ~~and~~ we conclude that Okinawan LEC sea cucumber populations have been and are being impacted by high levels of direct (e.g. overexploitation, as well as coastal development) and indirect anthropogenic pressure (e.g. decreasing water quality). To address the current situation, repeated monitoring and more detailed investigation~~s~~ to reveal the drivers that determine LEC sea cucumber species aggregations and population densities are urgently needed, along with more robust management of remaining LEC sea cucumber populations.

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19   ***Abstract***

20   Sea cucumbers are important ecological engineers in marine ecosystems. However, the fishery

21   demand of some species, especially <u>large-epifaunal and commercially used</u> (LEC) sea cucumbers,

22   has risen drastically, resulting in serious depletion of local populations for many species. Despite

23   this problem, basic ecological data on sea cucumbers, such as population densities and preferred

24   habitats, are often still insufficient. Here, we report on the population densities of multiple LEC

25   sea cucumber species, and their ambient benthic communities at eight sites around Okinawa

26   Islands. Further, we discuss the correspondence between sea cucumber densities and the

27   surrounding coral communities. Our results show two sites within national or quasi-national parks,

28   Aka and Manza, where stricter rules have been placed on fisheries and land reclamation compared

29   to other areas, had the highest and third highest sea cucumber population densities among sites,

30   respectively. *Holothuria atra* was observed at all survey sites and made up the majority of sea

31   cucumber populations at all sites except for Chatan and Sesoko, where *Holothuria leucospilota*

32   and *Stichopus chloronotus* were most abundant, respectively. Regarding relationships between

33   benthic composition and LEC sea cucumber species, *S. chloronotus* was significant correlated with

34   dead corals, scleractinian corals, and coralline algae. As well, *H. leucospilota* had significant

35   correlations with rubble. There were no significant correlations between scleractinian corals and

36   sea cucumber densities, although *S. chloronotus* was marginally insignificant with *Platygyra* and

37   *Psammocora*. Notably, medium- to highly valued species were very sparse in our surveys, and

38   most of them appeared at only one site. Additionally, one site (Odo) had only three LEC sea

39   cucumber individuals observed. Combining these facts with relatively low population densities

40   around the Okinawa Islands compared to densities reported in previous research from the Indo-

41   West Pacific Ocean region, and we conclude that Okinawan LEC sea cucumber populations have

42   been and are being impacted by high levels of direct (e.g. overexploitation, as well as coastal

43   development) and indirect anthropogenic pressure (e.g. decreasing water quality). To address the

44   current situation, repeated monitoring and more detailed investigations to reveal the drivers that

45   determine LEC sea cucumber species aggregations and population densities are urgently needed,

46   along with more robust management of remaining LEC sea cucumber populations.

47

48   ***Introduction***

49   Sea cucumbers are ~~common organisms that are~~ abundant in coral-reef coastline benthic

50   communities (Bakus 1973). Most sea cucumber species in these areas feed on small benthic

51   particulate matter and have a significant role as bioturbators (Purcell et al. 2016; Wolkenhauer et

52   al. 2010) and are thus ecologically important. Via their feeding activity, sea cucumbers enhance

53   the productivity of their surrounding ecosystems (Uthicke and Klumpp, 1998; MacTavish et al.

54 2012; Wolkenhauer et al. 2010). Furthermore, sea cucumbers are even suggested to be crucial for

55 buffering local ocean acidification by increasing the pH through ammonia discharge (Uthicke

56 2001a; Schneider et al. 2011, 2013). Finally, sea cucumbers often host many symbiotic species

57 (Purcell et al. 2016; Eeckhaut et al. 2004; Hamamoto and Reimer in press) and hence ~~can be~~

58 ~~considered to~~ contribute to local marine biodiversity.

59 In the past few decades, sea cucumber fisheries have been under intense pressure all over

60 the world following an increase in their commercial demand (Conand and Byrne 1993; Purcell et

61 al. 2011; Conand 2018). Most species targeted for fishery purposes are large-epifaunal and edible

62 sea cucumbers (hereafter called "LEC sea cucumbers"). In the Western Central Pacific region, ~~it~~

63 ~~is known that~~ most of important LEC species inhabit shallow waters (0-30 m, Kinch et al. 2008).

64 As these organisms are easy to catch and their recovery from overharvesting is slow (Uthicke et

65 al. 2004), such heavy exploitation has resulted in severe depletion in many regions of the world

66 (Purcell et al. 2013), and hence management of shallow water LEC sea cucumbers is important to

67 preserve coastal populations. However, basic information such as population densities, species

68 ~~assemblies a~~nd substrate preferences, ~~all of which are essential to set effective and precise~~

69 ~~conservation strategies,~~ are still lacking at the local level for most marine region; Surprisingly,

70 the food sources of most species are largely unknown, with few exceptions (e.g. Uthicke 1999;

71 Mfilinge and Tsuchiya 2016; Yamazaki et al. 2019; Gao et al. 2014, 2022), and thus the main

72 factors that drive sea cucumber distribution patterns are yet to be ~~in~~vestigated.

73 In Okinawa Prefecture, southern Japan, the LEC sea cucumber fishery developed recently

74 and rapidly as in other parts of the world. ~~Sea~~ cucumber fishery in Okinawa is usually done by

75 hand-collection while diving in shallow water, along with collecting other target products

76 (Okinawa General Bureau 2017). The annual harvest reached 225 tons in 2011 with a value of 291

77 million yen (= ca. 2.5 million USD) in 2012 (Okinawa General Bureau 2017). However,

78 overharvesting caused rapid stock depletion and resulted in regulation of the sea cucumber fishery

79 ~~from~~ 2013. This regulation simply prohibits the collection of sea cucumbers for non-fishers

80 regardless of species, size, or season (Okinawa Prefecture HP, February 2022 accessed). Studies

81 collecting basic information such as population density have been conducted in other locations

82 around the world such ~~from~~ La Reunion (Conand and Mangion 2002), Philippines (Dolorosa

83 2015), and Sri Lanka (Dissanayake and Stefansson 2012), but only scant knowledge is available

84 around the Ryukyu Islands (e.g. Yamana et al. 2020 around Amami-oshima Island; Tanita and

85 Yamada 2019 and Nishihama and Tanita 2021 conducted around Ishigakijima Island, Ryukyu

86 Islands). Thus, for Okinawa Prefecture and specifically around Okinawajima Island and

87 surrounding islands, there is a need for basic knowledge such as local population densities, species

88 assemblages, and preferred habitats to make more precise and effective management decisions and

89 rules. To address this need, ~~here~~ we conducted population density and benthic composition surveys

90   of shallow water LEC sea cucumbers at multiple sites around Okinawajima Island and some
91   surrounding islands (hereafter the Okinawa Islands). In addition, we surveyed zooxanthellate
92   scleractinian corals at the same sites to investigate potential relationships between holothurian
93   species and coral communities.
94
95   ***Materials and Methods***
96   Field surveys
97   Eight locations around the Okinawa Islands were surveyed in this study in the summers
98   of 2020 and 2021; Chatan, Kayou, Kin, Manza, Odo, Sesoko, Uruma and Aka (Table 1, Figure 1).
99   Each site's characteristics were as follows: Chatan and Odo are typical lagoons near the reef crest;
100   Kayou and Kin are also lagoons but farther from the reef crest than the previous two sites; Manza,
101   Sesoko and Aka are gradual reef slopes without obvious reef edges; Aka has a large artificial wall
102   made of wave-dissipating blocks (tetrapods; Masucci & Reimer 2019); and finally Uruma is a tidal
103   flat without much reef structure. At each location, two different surveys were performed; (i) line
104   intercept transects of benthos, and (ii) 50-minute timed swims (Figure 2). All surveys were done
105   via snorkeling, and no sea cucumbers were collected, hence no permits were needed. Detailed
106   methodologies of these surveys are as follows.
107   (i)   line intercept transects
108   At each location, six 10 m transect tapes were laid horizontally to the reef crest on the sea
109   bottom, and digital photographs were taken every 50 cm. Time, depth, temperature, and GPS
110   coordinates were recorded at start and end of each transect. After each survey, photographs were
111   analyzed as detailed below.
112   (ii)   50-minute timed swims
113   One observer swam 10 minutes in one direction parallel to shore and took photographs of
114   every LEC sea cucumber individual that appeared with a ruler. Once 10 minutes had passed, the
115   observer swam a few minutes towards the reef crest (away from shore), and then swam 10 minutes
116   parallel to shore in the opposite direction compared to the previous 10-minute swim. When the
117   reef flat was too narrow (i.e. Sesoko) or the reef became too deep so that a 10-minute swim was
118   not possible, the number of replications were reduced (Figure 1). At the start and end of each 50-
119   minute swim, time and GPS coordinates were recorded (Table S1).
120
121   Benthic community investigation and coral identification
122   For benthic community investigation, benthos were assigned into one of 15 categories;
123   scleractinian corals, dead corals, sponges, *Halimeda* algae, soft corals, coralline algae, macroalgae,
124   turf algae, algal assemblage (multiple types of turf, macro-, and/or coralline algae), seagrass
125   meadow, sand, rubble, rocks, others (= other taxa such sea cucumbers, or abiotic materials such as

126   iron bars, etc.), and unidentifiable components (Table S2).

127   Using the photographs taken during the survey, identification of scleractinian corals were

128   performed to the genus level following Nishihira (2020).

129

130   Data analyses

131   All statistical analyses were conducted using R 4.1.2 (R Core Team, 2021). Spearman

132   correlation coefficients were calculated using the "chart.Correlation" function in the

133   "PerformanceAnalytics" package (Peterson et al. 2021) for each LEC sea cucumber species'

134   density, benthic component cover, and coral genera abundance. Subsequently p-values were

135   calculated with "cor.test" with a p=0.05 threshold.

136   To compare benthic components' similarity at each study site, hierarchical clustering was

137   conducted using the "pvclust" package (Suzuki, Terada & Shimodaira 2019). As well, nMDS

138   clustering was carried out to demonstrate how each site are similar each other using "metaMDS",

139   "vegdist", "pam", all in the "vegan" package (Oksanen et al. 2020). To test statistical significance

140   of the groupings, the "labdsv" package was used (Roberts 2019). The Canonical Correspondence

141   Analysis (CCA) was conducted using "cca" function in the "vegan" package (Oksanen et al. 2020),

142   with sea cucumber density as objective and benthic components as explanatory variables. For

143   CCA, we only used species that appeared at more than two sites for sea cucumbers and benthic

144   components in characterizing the nMDS grouping.

145

146   **Results**

147   Surveys were done around Okinawajima Island from September to October 2020 and in

148   November 2021 at Aka Island. Water temperatures ranged from 27℃ to 30℃ during 2020 surveys

149   and 24℃ to 25℃ for the 2021 survey, and the depth range of surveys was 1.0 to 2.5 m (Table 1).

150   During the survey periods, no strong typhoons occurred in the survey area.

151

152   LEC sea cucumber species, density, and relationship with benthic components

153   In the course of the surveys, a total of nine LEC sea cucumber species from three families

154   (Synaptidae, Holothuriidae and Stichopodidae) were observed (Table 2; Figure 3). *Holothuria*

155   *(Halodeima) atra* Jaeger, 1833 was observed at all survey sites, and was the most abundant species

156   at Kayou, Kin, Manza, Odo, Uruma and Aka. *Holothuria (Mertensiothuria) leucospilota* (Brandt,

157   1835) and *Stichopus chloronotus* Brandt, 1835 were the most abundant species for Chatan and

158   Sesoko, respectively. *Stichopus chloronotus* was found only at Sesoko, Manza and Aka, where

159   scleractinian corals were one of the main benthic components. However, at Odo, where

160   scleractinian corals were also present, we observed only three individuals of three different sea

161   cucumber species, but no *S. chloronotus*. Some LEC sea cucumber species were observed at only

162   one site with low numbers of individuals (e.g. *Holothuria (Halodeima) edulis* Lesson, 1830 (n=1)
163   and *Actinopyga echinites* (Jaeger, 1833) (n=3) at Chatan, *Holothuria (Metriatyla) scabra* Jaeger,
164   1833 (n=1) at Uruma, *Bohadschia argus* Jaeger, 1833 (n=1) at Odo and *Synapta maculata*
165   (Chamisso & Eysenhardt, 1821) (n=2) at Aka; Table 2).

166   Total LEC sea cucumber density was highest at Aka Island (=0.0938 ind/m^2), followed
167   by Uruma and Manza (=0.0884 and 0.0801 ind/m^2, respectively). The lowest density was at Odo
168   (=0.0017 ind/m^2). Population densities of species that were observed at more than two survey sites
169   were the highest at Aka for *H. atra*, Chatan for *H. leucospilota*, and Manza for *Holothuria*
170   *(Microthele) whitmaei* Bell, 1887 and *S. chloronotus*.

171   At Chatan, Kin and Aka, most of the benthic surface was covered by sand, whereas at
172   Kayou and Uruma, more than half of the sea bottom consisted of sea grass meadows. On the other
173   hand, Sesoko, Manza, and Odo had substantial hard substrate coverage with low amounts of
174   scleractinian coral cover (=13% to 19%, Table S2). According to the cluster analysis and nMDS
175   results, the substrates of study locations could be divided into three categories; (i) sandy bottoms,
176   (ii) seagrass meadow with macroalgae, and (iii) hard substrate with living scleractinian corals
177   (Figure 4; Figure S1; Table S4). All six transects from the same survey site were assigned into
178   only one nMDS grouping except for Kin, which was assigned into two groups, and Chatan, which
179   was assigned into three groups (Table S5).

180   Based on the CCA plot, *S. chloronotus* preferred scleractinian corals, dead corals and
181   rocky bottoms that were abundantly seen at Sesoko, Manza and Sesoko (Figure. 5). Macroalgae
182   and seagrass meadows that characterized Kin, Kayou, Uruma and Aka had weak relationships with
183   *H. atra*, whereas *H. leucospilota*, which was abundant at Chatan, did not have any strong
184   relationships with components which characterized major nMDS groupings. Correlation tests
185   between abundant sea cucumber species' (*H. atra*, *H. leucospilota* and *S. chloronotus*) densities
186   and benthic components showed significant correlations between *S. chloronotus* with dead corals
187   (p-value = 0.01467), scleractinian corals (p-value = 0.01398), and coralline algae (p-value =
188   0.04455, Figure S2). As well, *H. leucospilota* had significant correlations with rubble and
189   unidentifiable components (p-values = 0.03873 and 0.03002, respectively). *H. atra* showed no
190   significant correlation with any benthic component.

191   In total 16 scleractinian coral genera were identified in the surveys (Table S3). The most
192   abundant genus was *Montipora* at Manza, Odo and Aka, and *Acropora* at Sesoko. *Montipora* and
193   *Porites* were found at all four sites. The greatest number of scleractinian genera was observed at
194   Sesoko (15 genera) followed by Manza (ten genera), Odo (six genera) and Aka (three genera).
195   None of the comparisons between sea cucumber densities and coral genus appearance had
196   significant correlation, although *S. chloronotus* with *Platygyra* and *Psammocora* was marginally
197   insignificant (p-value = 0.05132, Figure S3).

198

199 ***Discussion***

200

201 <u>Species composition and population density</u>

202   Among survey sites, Chatan and Aka had the highest number of LEC sea cucumber

203 species observed (four species), whereas Kin and Kayou had the lowest (one species). The high

204 diversity at Aka may be explained by its inclusion in Keramas National Park. On the other hand,

205 it should be noted that Chatan, which is next to a relatively developed coastal area, also had high

206 species diversity. Chatan was the only site in which the transects were assigned into three different

207 nMDS groups, demonstrating that Chatan had a wider variety of benthic components compared to

208 the other surveyed sites. This characteristic may be one cause of the comparatively high diversity

209 of LEC sea cucumber species at this site.

210   In this study around Okinawajima Island, the population densities of LEC sea cucumber

211 species were generally lower than those observed in previous research conducted in the Indo-

212 Pacific region. For example, *Holothuria atra* at 0.09 ind/m^2 was the highest density observed in

213 the current study, while 0.12 to 3.40 ind/m^2 were observed at Ishigakijima Island (Nishihama and

214 Tanita 2021), and 0.05 to 0.39 ind/m^2 at Mauritius (Lampe 2013) and 10 ind/m^2 at New Caledonia

215 (Purcell, Gossuin & Agudo 2009). The low numbers around Okinawajima Island may reflect the

216 overexploitation of LEC sea cucumbers around Okinawajima Island as previously reported

217 (Okinawa General Bureau 2017) or possible habitat degradation due to coastal armoring (Masucci

218 & Reimer 2019;Masucci, Biondi & Reimer 2021). In particular, some medium- to high-value

219 species such as *H. whitmaei*, *H. scabra*, and *Actinopyga echinites* (Conand and Muthiga 2007;

220 Purcell, Gossuin & Agudo 2009; Purcell 2010) showed extremely low densities in the current

221 study (0.0025, 0.0012 and 0.0036 ind/m^2 maximums, respectively) even though these species were

222 observed under similar reef conditions in previous studies (e.g. Purcell, Gossuin & Agudo 2009;

223 Kohler, Gaudron and Conand 2009; Uneputty et al. 2017). Thus, we conclude the low numbers of

224 these species observed in this study likely indicate ~~impacts of~~ overharvesting. Furthermore, it has

225 been shown that *S. chloronotus* is also overharvested in East Africa and the Pacific (Pirog et al.

226 2019), and around Okinawajima Island ~~b~~ased on extremely low mitochondrial genetic diversity,

227 it has been suggested this species has experienced high anthropogenic pressure (Soliman et al.

228 2016). In the present study, the low population density of *S. chloronotus* compared to previous

229 studies (0.04 ind/m^2 maximum in current study; 0.09 ind/m^2 at Mauritius (Lampe 2013); 1.5-3.6

230 ind/ m^2 at La Réunion (Kohler, Gaudron and Conand 2009)) may also show such impacts.

231 However, the number of individuals of *S. chloronotus* observed in our study is similar to those

232 previously reported from Ishigakijima Island (0.04–0.34 ind/m^{2,} Tanita and Yamada 2019), and

233 hence longer-term monitoring of this species is recommended to better ascertain the status of its

234   populations.

235   Among sites, Odo had the lowest number of individuals (three individuals) and population

236   density (0.0017 total ind/m^2). However, it was reported that 1.3 tons of sea cucumbers were

237   harvested in 2014 from the coasts of Itoman City, which includes the Odo coastal area (Okinawa

238   General Bureau 2017), and therefore we speculate that there were substantial amounts of LEC sea

239   cucumbers present at least in the very recent past around Odo. The gap observed between past

240   reports and the current study may be due to slow population recovery after over-exploitation,

241   which has been ~~frequently~~ reported in previous research (Uthicke et al. 2004; Friedman et al. 2011;

242   Rehm et al. 2014; Ramírez-González et al. 2020). The majority of sea cucumbers are gonochoric

243   broadcast spawners (Babcock et al. 1992; Mercier and Hamel 2009) with low mobility, and thus

244   considered vulnerable to drastic population size declines due to the allele effect (Purcell et al.

245   2011). In other words, once a population size becomes too low, the population can no longer

246   maintain itself and recruitment from elsewhere is needed. However, it has been suggested that

247   there may be no sexual recruitment in sea cucumbers as juveniles are typically not observed in

248   coastal reef areas (e.g. *H. atra* at Taiwan, Chao, Shen & Alexander 1994; *H.atra* and *S. chloronotus*

249   at Great Barrier Reef, Uthicke 2001b; *H. nobilis* at Great Barrier Reef, Uthicke and Benzie 2002),

250   therefore local population recovery may take much longer than ~~has been~~ expected (Uthicke et al.

251   2004; Ramírez-González et al. 2020).

252   *Holothuria atra* was observed at all locations and comprised the majority of individuals

253   at five out of eight locations. Similar patterns have been reported in other studies across the Indo-

254   West Pacific Ocean (Purcell, Gossuin & Agudo 2009; Lampe 2013; Setyastuti 2015; Tanita and

255   Yamada 2019). However, it should be noted that the relatively high population density of *H. atra*

256   reported in the current study, and perhaps in some previous studies, might reflect its low economic

257   value compared to other more highly-valued species. Among sites, Aka showed the highest

258   number of individuals (131 individuals) and density (0.0903 ind/m^2) of *H. atra*, and this site is

259   within Keramashoto National Park (see Figure 1 for its location), which was reported recently to

260   harbor the highest genetic diversity of this species (Hamamoto et al. 2021). As well, Manza, which

261   showed the second highest COI genetic diversity (Hamamoto et al. 2021) and had the second

262   highest total individual numbers following Aka, and third highest total population density among

263   sites in the current study, is within a quasi-national park. Combined, these facts may indicate this

264   species is comparatively better conserved within parks. In Japanese national parks, although

265   fisheries are not completely prohibited, stricter rules are put on the capture of some organisms such

266   as fish, anemones, and scleractinian coral species, and also the rules on coastal landfilling (land

267   reclamation), sewage discharge, and construction are stricter (Ministry of Environment 1957), thus

268   all of these local stressors can potentially impact local populations of benthic organisms such as

269   sea cucumbers. Such higher population densities of holothurian species in protected areas

270   compared to unprotected areas have been reported previously (Uthicke and Benzie 2001; Rehm et
271   al. 2014; Asha et al. 2015).

272

273   <u>Benthic composition and sea cucumbers' microbiota</u>

274   Benthic components were divided into three major types: sandy substrate, seagrass
275   meadow with macroalgae, and hard substrate with living scleractinian corals (Figure 4). The sites
276   characterized by sandy and seagrass substrates were mostly occupied by *H. atra* both in terms of
277   number of individuals and population density, in accordance with previous reports (Bakus 1973;
278   Purcell, Gossuin & Agudo 2009; Tanita and Yamada 2019; Nishihama and Tanita 2021).
279   However, *H. atra* did not have significant correlation with sand or seagrass meadows. On the other
280   hand, *S. chloronotus* showed significant correlations with living scleractinian corals and dead coral
281   cover (p-values = 0.01398 and 0.01467, respectively). It has previously been confirmed that *S.*
282   *chloronotus* has a stronger preference than *H. atra* and *H. edulis* for, and are selectively feeding
283   on, sediments with higher microalgal organic content (Uthicke & Karaz 1999), and therefore the
284   correlation observed here may indicate that there is appropriate organic content around corals and
285   on dead coral rubble. However, no pairs of any coral genus and abundant LEC sea cucumber
286   species had significant correlations, and thus this theory remains to be confirmed. According to
287   our results, only *S. chloronotus* with *Platygyra* and *Psammocora* were ~~even~~ marginally
288   insignificant, but as we observed *S. chloronotus* at only three sites, we believe that future studies
289   including additional sites and data such as organic content, other abiotic indices measurement, and
290   microbe metabarcoding may provide further insights to better understand these relationships.
291   It is widely known that sea cucumber intestinal biota shares components with ambient sediment
292   (Yamazaki et al. 2019, Gao et al. 2022). Moreover, Cleary et al. (2019) found that sea cucumbers,
293   algae, and stony corals' bacterial communities had similar taxonomic composition and diversity,
294   and members of phylum Planctomycetes were usually abundant. Planctomycetes are commonly
295   found on healthy individuals of the stony coral *Acropora cytherea*, ~~while~~ are scarce in coral
296   colonies affected by skeletal growth anomalies (SGA) (Rajasabapathy et al. 2020). As sea
297   cucumbers discharge distinctive microbes that have metabolic capabilities specific to host food
298   digestion (Yamazaki et al. 2019; Jaengkhao, Maeroh and Wanna 2022), their intestinal biota is
299   thought to influence the surrounding bacterial community, both in the water column and sediment
300   (Enomoto, Nakagawa & Sawabe 2012; Bogatyrenko and Buzoleva 2016; Yamazaki et al. 2019).
301   Therefore, given the importance of sea cucumbers in enhancing ambient productivity and in
302   buffering ocean acidification via their feeding activity, the interaction between these organisms
303   with the ambient microbial community, and their ecological roles, need to be further explored.

304

305 Conservation recommendations and conclusions

306   In this study, relatively low population densities of LEC sea cucumbers were observed
307 around Okinawajima Island and we assume this is likely caused by persistent anthropic impacts
308 such as overexploitation or coastal reclamation. In particular, the Odo site showed extremely low
309 densities, possibly due to past or present high fishing pressure, and slow recovery after depletion
310 (Uthicke et al. 2004; Friedman et al. 2011). On the other hand, Aka and Manza, both in national
311 or quasi-national parks, had higher population densities, and therefore protection might have
312 successfully affected local benthic conservation. Currently, in Okinawa Prefecture, there are
313 regulations on the harvesting of all sea cucumber species, and therefore capture of any sea
314 cucumber is prohibited for non-licensed citizens. However, there are no rules on fishing activities
315 such as minimum sizes and/or seasonal management. In addition, the sea cucumber fishery does
316 not require expensive equipment as they are slow sedentary organisms. Due to these conditions,
317 sea cucumbers are often sold without ~~the~~ fishery association supervision, a situation called
318 "*hamauri*" (="beach-selling") in Okinawa Prefecture. This makes sea cucumber~~s~~ management
319 more difficult, as setting harvest restriction amounts is nearly impossible, and there are no data on
320 what species are actually harvested and from ~~what~~ location. Therefore, the establishment of
321 additional regulations such as requiring the trade of sea cucumbers to be conducted via fishing
322 associations would allow the establishment of more effective additional restrictions. Even with
323 such measures, however, basic reproductive information including spawning season and
324 maturation sizes are yet unknown for most tropical LEC sea cucumbers. Therefore, future
325 ecological investigations such as repeated monitoring at both high-density and low-density sites
326 and deeper water (<30 m) habitat surveys to confirm if Okinawan population are in accordance
327 with the patterns found in other parts of the Ryukyu Islands (e.g. Amamioshima Island, Yamana
328 et al. 2020) will provide important knowledge to help develop protection strategies for LEC sea
329 cucumbers in Okinawa Prefecture. As well, to estimate the factor(s) driving each sea cucumber
330 species' density, DNA metabarcoding of bacteria or microalgae in ambient sediments may also be
331 ~~of utility~~.

332

333

334 ***Acknowledgements***

341 manuscript.

342

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Figure 1

Map showing the Ryukyu Islands.

Survey sites are shown with red stars in the higher resolution map. Light yellow shows quasi-national park and dark yellow shows national park areas.
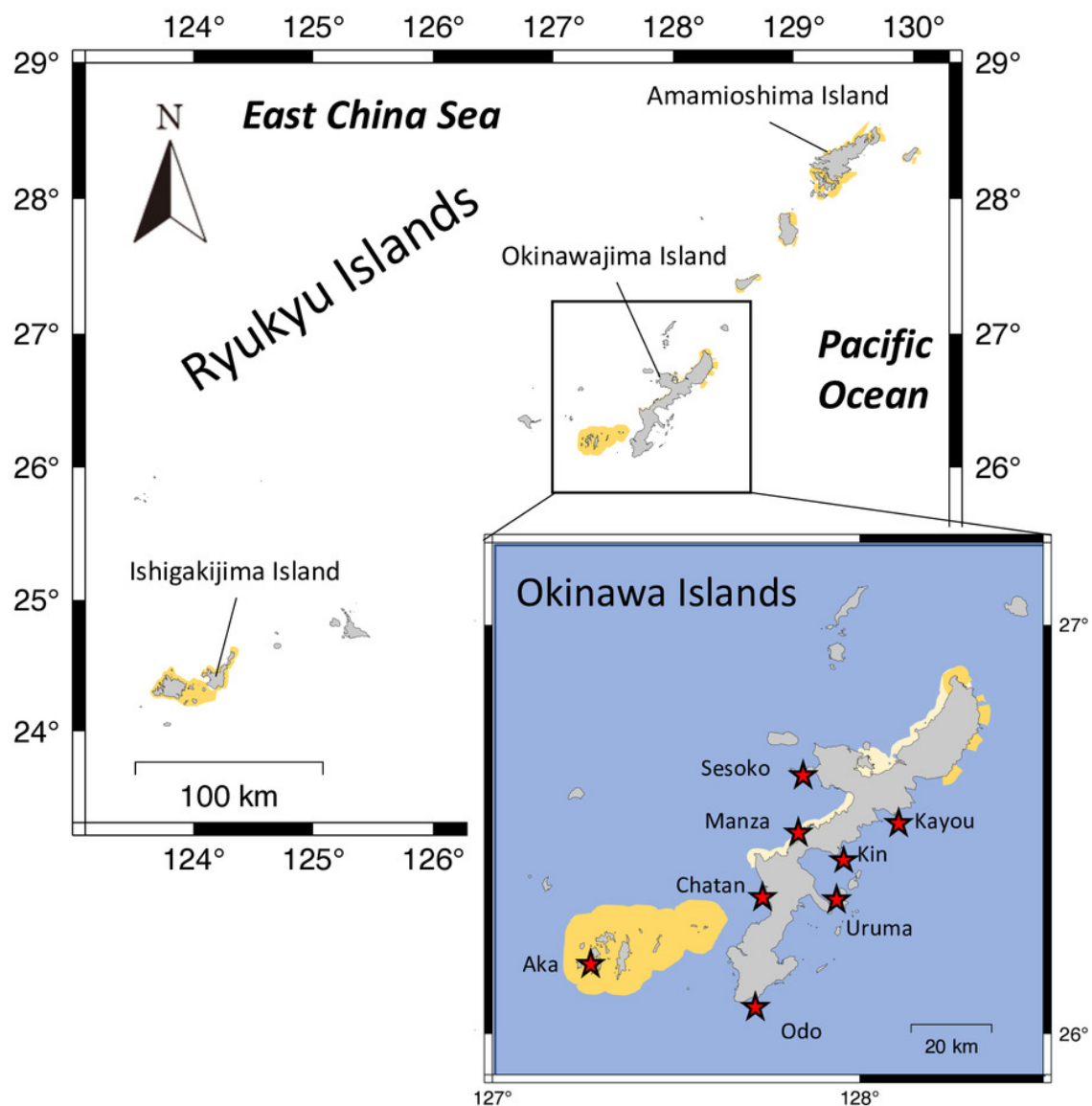
Figure 2

Aerial photograph of each survey site.

A = Sesoko, B = Kayou, C = Manza, D = Kin, E = Uruma, F = Chatan, G = Odo, and H = Aka.
Scale is shown in each photograph. Transect track (green lines) and areas surveyed (red
mesh) based on GPS coordinates recorded during the survey are projected in each
photograph. Gray points indicate locations calculated from field notes and not GPS. Map data
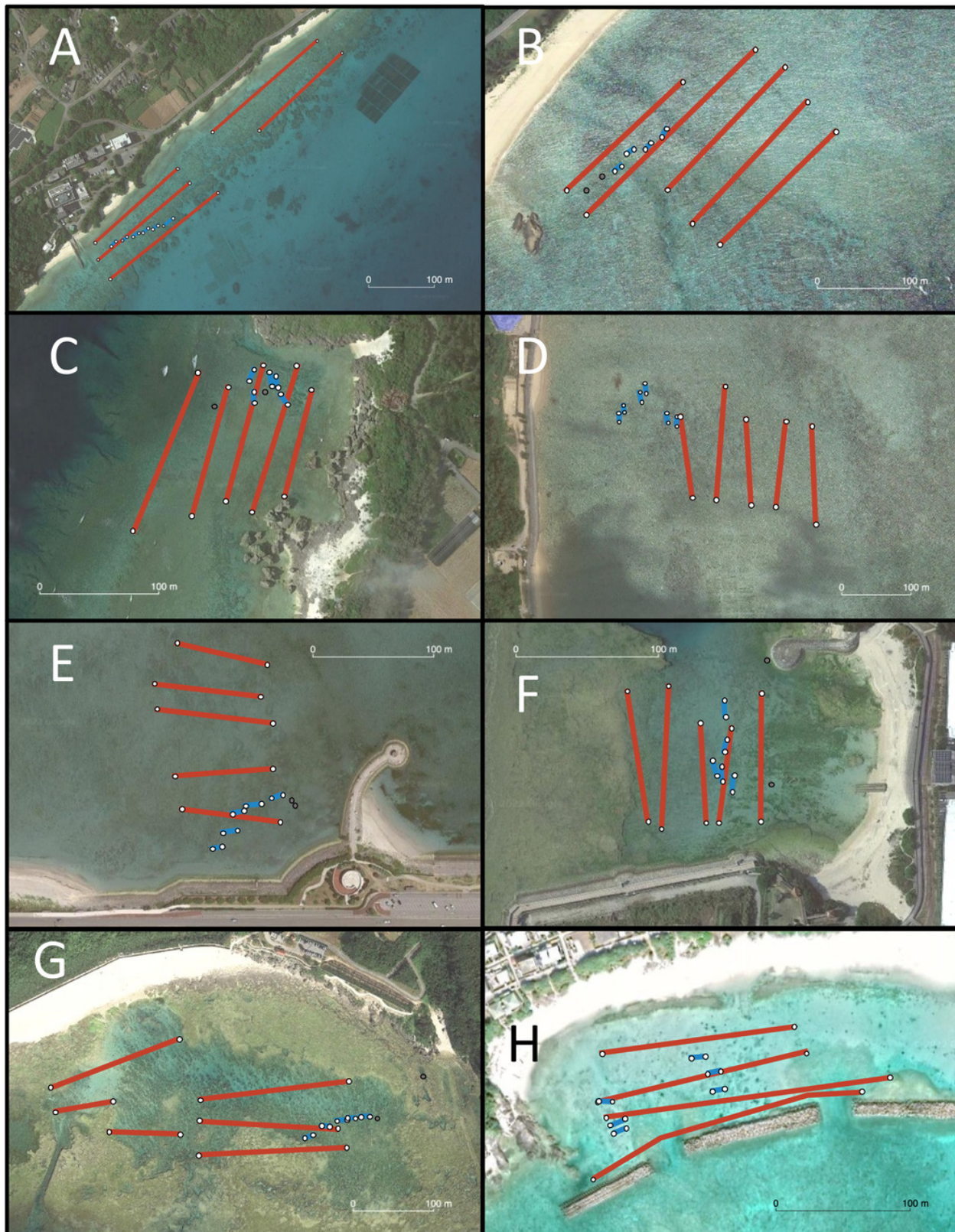© 2022 Google

Figure 3

Number of individuals of sea cucumber species at each site.

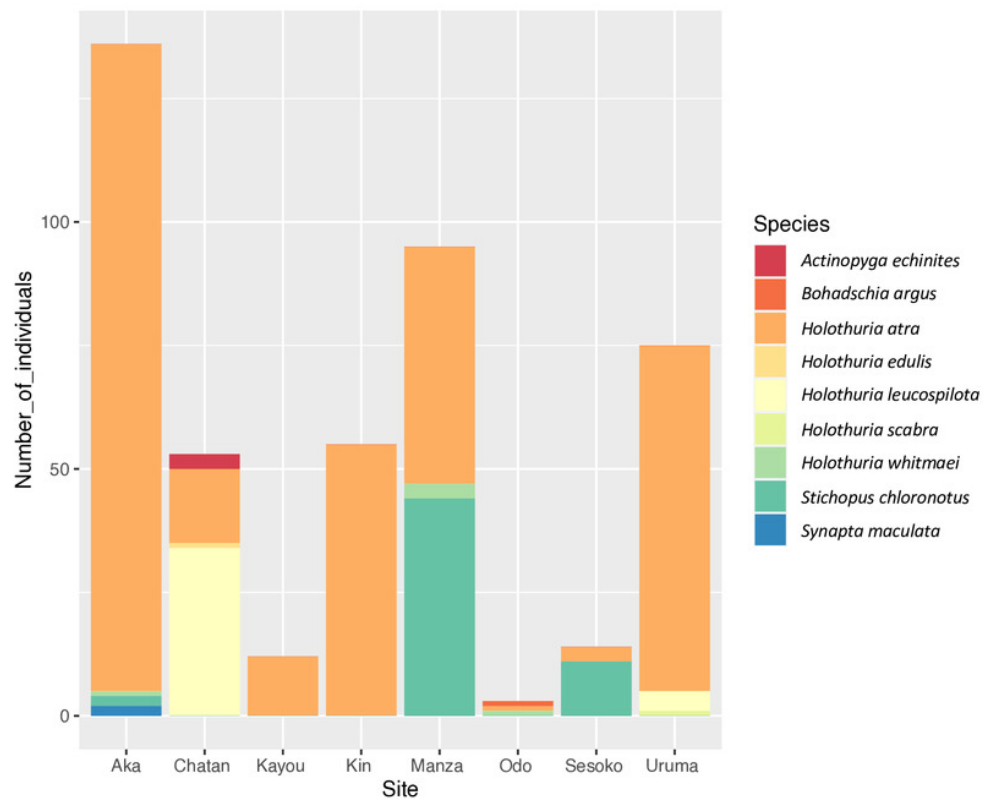Color shows each sea cucumber species, and the y-axis shows the number of individuals.

Figure 4

Result of nMDS analysis.

(A) sandy bottoms, (B) seagrass meadow with macroalgae, and (C) hard substrate with living scleractinian corals. Colors designate major groups observed.
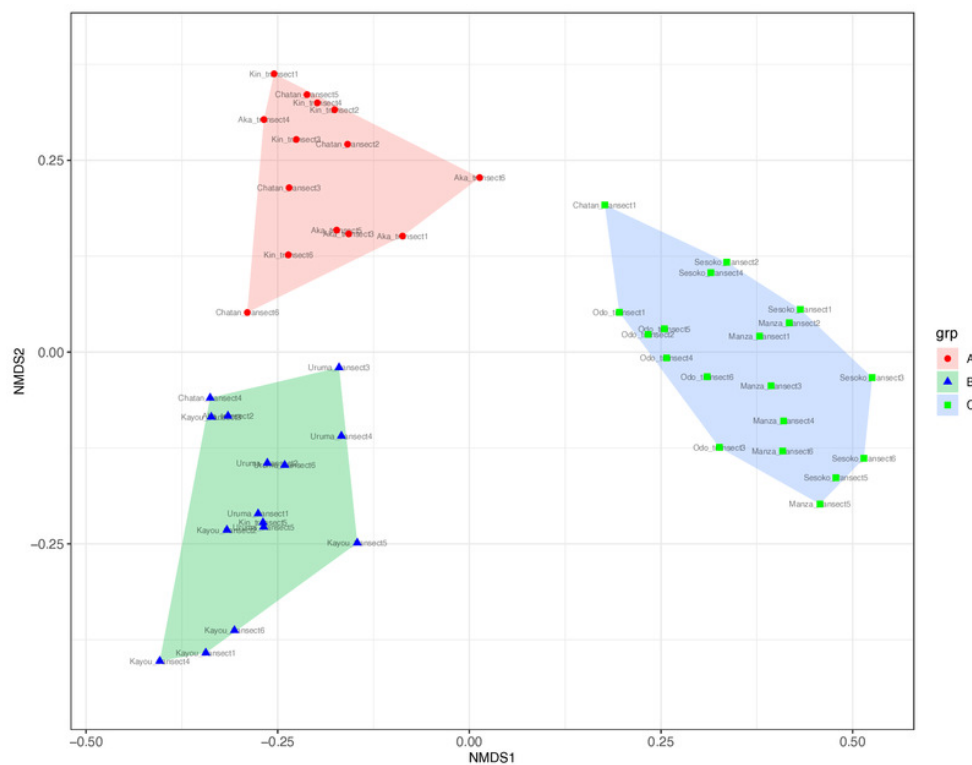
Figure 5

Result of Canonical correspondence analysis with each sea cucumber species' density as objective variables and sediment and biota types as explanatory variables.

Only sea cucumber species that appeared at more than two sites and benthic components that characterize nMDS grouping were considered as variables.
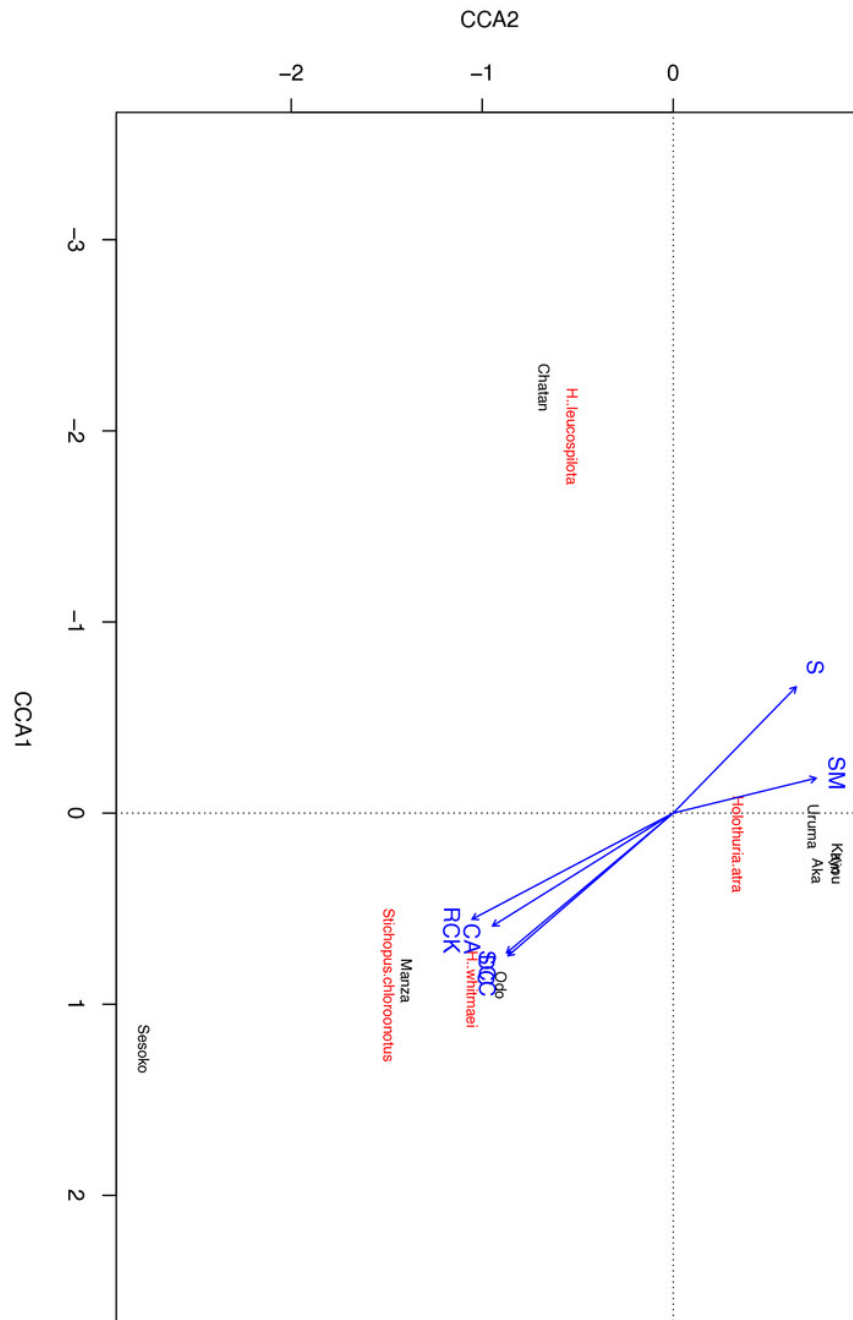
Table 1(on next page)

General information of survey sites.

1

Location	Site	Area Investigated (㎡)	Total ind. No.	Total pop. den. (ind/m^2)	Water temp. (℃)	Depth (m)
Okinawa Island	Chatan	840	53	0.0631	29	1.0-1.8
	Uruma	848	75	0.0884	30	1.7-1.9
	Kin	1352	55	0.0407	29	1.0-1.5
	Odo	1748	3	0.0017	28-29	1.6-2.1
	Kayou	1942	12	0.0062	29	1.7-2.3
	Sesoko	1892	14	0.0074	27	1.7-2.5
	Manza	1186	95	0.0801	27	1.4-1.7
Kerama Islands	Aka	1450	136	0.0938	24-25	1.2-1.6

Table 2(on next page)

Number of individuals of each species observed at survey sites.

Population density is shown in parentheses (ind/m^2).

1

	Holothuria atra	*Holothuria leucospilota*	*Holothuria edulis*	*Holothuria whitmaei*	*Holothuria scabra*	*Actinopyga echinites*	*Bohadschia argus*	*Stichopus chloronotus*	*Synapta maculata*	Total
Chatan	15 (0.0179)	34 (0.0405)	1(0.0012)	-	-	3 (0.0036)	-	-	-	0.0631
Uruma	70 (0.0825)	4 (0.0047)	-	-	1 (0.0012)	-	-	-	-	0.0884
Kin	55 (0.0407)	-	-	-	-	-	-	-	-	0.0407
Odo	1 (0.0006)	-	-	1 (0.0006)	-	-	1 (0.0006)	-	-	0.0017
Kayou	12 (0.0062)	-	-	-	-	-	-	-	-	0.0062
Sesoko	3 (0.0016)	-	-	-	-	-	-	11 (0.0058)	-	0.0074
Manza	48 (0.0405)	-	-	3 (0.0025)	-	-	-	44 (0.0371)	-	0.0801
Aka	131 (0.0903)	-	-	1 (0.0007)	-	-	-	2 (0.0014)	2 (0.0014)	0.0938