Persistent dominance of non-indigenous species in the inner part of a marina highlighted by multi-year photographic monitoring

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1 **Persistence of dominance of non-indigenous species in the inner part of a marina**

2 **highlighted by multi-year photographic monitoring**

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11

12 **Abstract.** As a result of urbanization, the coastal environment is being disturbed by various anthropogenic
13 pressures. These are concentrated in harbor areas where the addition of artificial structures and the presence
14 of pollutants seems to favor the settlement of non-indigenous species. Today, most of the studies working
15 on these organisms are often carried-out in a single time window without integrating temporal variability.
16 Our work consisted in analyzing multi-year photographic data of marina communities taken from three
17 experiments held between 2016 and 2019 in the same marina. These photographs were taken from
18 recruitment plates placed at the inner, middle and entrance locations of the marina, permitting us to discern
19 the community differences and the distribution of non-indigenous taxa between these 3 locations. Over all
20 the studied periods, the communities that grew at the entrance and the inner locations of the marina were
21 always different. Non-indigenous taxa also appeared to be more prevalent in the inner location of the marina.
22 Our results suggest that the presence of different environmental filters between the entrance and the inner
23 location could explain these observations. We suggest this could be due to a pollution gradient with high
24 pollution at the inner location of the marina and to a competitive pressure exerted by the tunicate *Ciona
25 intestinalis* at the marina entrance.

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27 **Keywords:** *Non-Indigenous species, Ciona intestinalis, Image analysis, Fouling communities, Marina*

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Introduction

The urbanization of coastal environments has dramatically increased over the last few decades. Over 2,8 billion people, almost 40% of the world's population, live in coastal areas (< 100 km from the sea) and this figure is projected to increase to 3.2 billion before 2035 (Maul & Duedall, 2019). Presently, nearly 50% of European and Asian coasts have been modified with artificial structures (Dafforn et al., 2015). This growing urbanization is a major source of disturbance for coastal ecosystems (Lee et al., 2006; Burt, 2014), such as seagrass beds and rocky shores that support high biodiversity (Bowen & Valiela, 2001; Lee et al., 2006; Burt, 2014). Apart from destroying habitats, artificial structures also do not act as a substitute for natural substrata, but rather facilitate a differential recruitment of organisms, depending on the wide variety of materials they are composed of, e.g. plastics, wood, metals, stones and concrete (Connell & Glasby 1999, Bulleri & Chapman 2010, Mineur et al. 2012). Furthermore, all these structures are also likely to transform the coastal hydrodynamics and larval dispersion (Burcharth & Lamberti 2005, Moschella et al. 2005). Coastal urbanization may, thus, impact the structure and diversity of communities along coastal environments (Inglis & Kross 2000, Deegan 2002, Rosa et al. 2003, Bulleri & Chapman 2010, Scherner et al. 2013).

Urbanization of the coastal environment is concentrated near harbor areas, which are a major source of pollutants for coastal ecosystems. Lubricating oils, exhaust gases and fuel spills are frequently released in the environment which cause various types of hydrocarbon pollution (Voudrias & Smith 1986). In addition, harbor areas often collect runoff, sewage and industrial waters, facilitating the input of large amounts of heavy metals and pesticides (Bryan & Langston 1992, Kennish 2002, Rivero et al. 2013). Compounds used to prevent biofouling on ship hulls also spread highly toxic copper and zinc molecules into the water (Voulvoulis et al. 1999, Karlsson & Eklund 2004, Lagerström et al. 2018). The distribution of these contaminants frequently appears structured with spatial heterogeneity. Indeed, the entrance of harbor areas are often less disturbed than the inner parts (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b). This difference may be explained by the location of pollutant sources in the harbor and the hydrodynamics within, resulting in higher water retention in the inner areas (Floerl & Inglis 2003, Schiff et al. 2007). Water retention in the innermost parts of the harbor might alter environmental parameters of natural (such as temperature; Menniti et al. 2020) or anthropogenic origin (such as heavy metals) impacting organisms in harbor environments (Owen & Sandhu 2000, Schiff et al. 2007, Aly et al. 2013). The combined effects of these stressors may act in an additive manner or result in synergistic impacts

70   (Sokolova & Lannig 2008, Saloni & Crowe 2015, Kinsella & Crowe 2016), which may constitute a

71   selective filter. Moreover, the loss of biodiversity induced by these disturbances is considered as

72   one of the factors that may enhance the settling of non-indigenous species (NIS) in harbor areas

73   (Elton 1958, Piola & Johnston 2008, Crooks et al. 2011).

74   NIS have mainly been dispersed in the marine environment through the development of maritime

75   transport (Hewitt et al. 2009, Clarke Murray et al. 2012). As a result, they are found in high

76   concentrations in harbor areas (Simkanin et al. 2012, Ferrario et al. 2017) where numerous species

77   appear tolerant to disturbance (Lenz et al. 2011, Lejeusne et al. 2014, Marie et al. 2017). These

78   include resistance to heating events (Kelley 2014, Kenworthy et al. 2018a) and heavy metals (Piola

79   & Johnston 2006a b, Crooks et al. 2011). While these parameters can disturb the native harbor

80   biodiversity, NIS often appear more resistant than native organisms in these environments (Arenas

81   et al. 2006, Piola & Johnston 2008). This tolerance, together with the presence of new artificial

82   substrata, could then facilitate their settlement and prevalence in harbors (Glasby et al. 2007,

83   Dafforn et al. 2012). NIS thus represent a fundamental issue for research and management of

84   coastal ecosystems. It therefore appears essential to study the distribution of NIS populations in

85   harbor environments and to understand the processes structuring these communities.

86   In this context, marinas represent a study environment that can contain a greater concentration of

87   NIS than other areas, such as commercial harbors and they even support NIS exclusive to marinas

88   (Marins et al. 2010, Ferrario et al. 2017). Although more studies are now focusing on the inventory

89   of these organisms, they are often carried out for a single period without integrating the seasonal

90   or interannual dynamics of the studied communities (Webb & Keough 2000, Ashton et al. 2006,

91   Ferrario et al. 2017, Kenworthy et al. 2018b, Spagnolo et al. 2019). The study of these NIS then only

92   occurs within a small-time window without knowing whether it is variable on a larger time scale. In

93   the present study, we assess data from three community-scale experiments carried out in 2016,

94   2017 and 2019 in the Marina du Château (Brest, France) conducted independently but with similar

95   overall methods. Each experiment collected photographic data of the evolution of communities

96   recruited over a few months each year. Consequently, the photographs collected over these three

97   years provide an opportunity to study the temporal variability of recruitment and the evolution of

98   the macrofauna in a marina context. The present work therefore aims to combine the photographic

99   data from these three experiments to study the structure of fouling communities and the presence

100 of NIS at the entrance, middle and inner parts of a temperate marina at a multi-year scale. We

101 hypothesized that (1) the fouling communities are spatially heterogeneous among the marina's

102 locations; (2) the inner location is characterized by a greater concentration of NIS; (3) and that the

103 temporal variations of community structure in our study area are less important than spatial

104 variations within the marina.

Material and Methods

106 Study site

107 The study site, the "Marina du Château" is located in the Bay of Brest, France (48°22'44"N,

108 4°29'21.0"W). This marina is nested within a larger harbor complex with commercial and military

109 activities. Three locations within the marina have been equipped with macrobenthic settlement

110 plates in 2016, 2017 and 2019 (Fig. 1; Fig. 2). This marina seems to have negligible variability of

111 temperature among locations (< 0.1°C; Gauff et al. 2022), but significant variation of several

112 important contaminants with higher contamination at the inner location compared to the entrance

113 (Tab. 1; Kenworthy et al. 2018b, Gauff et al. 2022). The contaminant levels seem to be relatively

114 stable for the duration of our study (2016 – 2019; Tab. 1), however some pollutants, like copper

115 seem to have a slight increase compared to earlier measures in the marina (REPOM 2013).

116 Sampling Methods

117 Photographic data were compiled from three different experiments, which all had the aim to

118 characterize the spatial variability of the macrobenthic communities present at three different

119 locations of the study area: the entrance, the middle and the inner locations of the marina (Fig. 2).

120 Settlement plates made of polyethylene or PE (20 cm x 20 cm), were installed horizontally at the

121 dark underside of the pontoons (to mimic the pontoon habitat) at approx. 1 m depth, > 3 m away

122 from the seafloor. In 2016, these plates were placed on the lower part of a triangular structure,

123 whereas in 2017 and 2019 they were positioned on a grid frame.

124 Panels were deployed in May for 5 months (October) in 2016. In 2017 and 2019 intermediate dates

125 were added as panels were deployed in May 2017 and early April 2019, photographed in August

126 2017 and June 2019 and then photographed again in October 2017 and end of August 2019 (Fig. 1).

127 Because we used photographic data from three independent experiments, which slightly varied in

128 scientific aim between the years, some disparities are present. Even if panels were handled in a

129 manner that would minimize the stress experienced by the community, these precautions were

130 different between years (ex: June 2019 panels photographed underwater, October 2017 panels

131 photographed in a water container). Furthermore, panels were sampled between the two periods

132 of 2017 and 2019 reducing their number (ex. June 2019: 20 panels per location; August 2019: 5

133 panels per location). Because of theses disparities and because longer time series are needed to

134 effectively study interannual fluctuations, we choose to analyze each period independently, thus

135 avoiding biases due to protocol dissimilarities.

136 Photographic processing

137 To avoid a border effect, a smaller quadrat (18 cm x 18 cm) has been digitally projected on each

138 photo. These quadrats were analyzed with the photo point method in a random stratified design,

139 with a total of 144 randomly generated points. This number is above the threshold of 0.4 points per

140 cm², which allows to reliably assess the cover of species with more than 5% cover with a confidence

141 interval above 95% (Taormina et al. 2020). Most of these organisms were identified using

142 morphological criteria. Identified species (Tab. 2) were grouped within one of the three categories:

143 native species (IS), non-indigenous species (NIS), and cryptogenic species (CS) (i.e., species whose

144 native or introduced status in the study area has not been clearly established). Unidentified species

145 were assigned to the bare space cover, as they were too small to be identified and cover less than

146 1% of the plates.

147 Because it can be difficult to identify species even with detailed morphological analyses (Newcomer

148 et al. 2019), we maximized the precision of this photo analysis by training the photo observer on life

149 communities sampled and analyzed in the laboratory. We conducted a quality assessment of our

150 photo analysis by comparing data from the panels sampled in 2019, that were analyzed with image-

151 analysis and taxonomic analysis in the laboratory. The complete procedure of this quality

152 assessment is described in the supplementary material. In brief, image-analysis underestimated the

153 cover of many species and had a lower taxonomic resolution (Sup. Tab. 1). However, on the

154 community level, the results from both analyses were very similar (Sup. Fig. 1) showing that the

155 image-analysis method appears robust enough to study the overall structure of communities over

156 the multi-year monitoring.

157 Data analysis

158 Due to disparities in methods used, each year was assessed separately. Community analyses were

159 conducted using R (R core Team 2020, version 3.5.1) with the "vegan" package (version 2.5-6;

160 Oksanen et al. 2018). The cover of all species was used to construct several Bray-Curtis dissimilarity

161 matrices which were graphically represented by non-metric multidimensional scaling (nMDS). For

162 each year, the homogeneity of sample dispersions from the three study locations was tested via the

163   "betadisper" function from "vegan". While homogeneity was not systematically respected over all

164   the studied periods, the PERMANOVA test has been shown to be robust to deviations from this

165   homogeneity if the number of replicates is balanced between the different groups (Anderson et al.,

166   2013), as it was in these experiments. We conducted a PERMANOVA analysis (10^4 permutations)

167   testing for the effect of marina location on community structure. When it showed significant

168   differences, we continued with a pairwise PERMANOVA (10^4 permutations) testing which locations

169   were significantly different from each other in terms of community structure. This was performed

170   using the "pairwiseAdonis" package (version 0.3; Martinez Arbizu 2019), applying a Benjamini-

171   Hochberg adjustment method (Benjamini & Hochberg 1995). Lastly, we tested if the temporal

172   variations of community structure were smaller than the spatial variations within the marina, by

173   conducting a two-factor PERMANOVA using year, location and their interaction as explanatory

174   variables for community structure (10^4 permutations).

175   SIMPER tests were carried out for each period to characterize the taxa most contributing to the

176   contrast between the communities of the different locations (Clarke 1993). Indicator species for

177   each location and period were identified using the multipattern analysis provided by the

178   "indicspecies" package (version 1.7.7; Cáceres et al. 2011). Histograms comparing the cover of

179   native, NIS, and cryptogenic species as well as unoccupied space were also created for each location

180   and each period. The significant differences in percent cover between these different categories

181   were identified using Kruskal-Wallis tests and Wilcoxon Mann-Whitney multiple comparison tests

182   using the Benjamini-Hochberg adjustment method.

183

Results

185   Comparison of the overall structure of the recruited communities

186   At the level of the community structure, it is possible to observe a systematic difference between

187   the entrance and the inner location of the marina (Tab. 3; PERMANOVA, df=1, $p < 0.025$, $R^2 > 0.46$).

188   This is the case for all the study periods for the total community (Fig. 3), for the native community

189   (Fig. 4), and for the non-indigenous community (Fig. 5). Furthermore, in October 2016, August 2017,

190   October 2017 and June 2019, communities from the middle location were also different from both

191   other locations for the total and native community (Fig. 4; Fig. 5; Tab. 3). In many cases however,

192   the middle community may either be similar to the inner location (ex.: total community in August

193   2019; PERMANOVA, df=1, $p = 0.182$, $R^2 = 0.184$; Fig. 3; Tab. 3) or to the entrance location (ex.: non-

194   indigenous community in October 2016 and 2017; PERMANOVA, df=1, p < 0.01, R^2 > 0.28; Fig. 5;

195   Tab. 3). The PERMANOVA testing the effect of year, location and their interaction on community

196   structure revealed a significant effect of all three (respectively: R^2 = 0.16, p < 0.001; R^2 = 0.22, p <

197   0.001; R^2 = 0.14, p < 0.001), but annual variations of community structure seem less important than

198   spatial variations within the marina.

199   Species cover

200   For all the studied periods, NIS cover was higher at the inner location compared to the entrance

201   (Fig. 6; Tab. 4; Wilcoxon's test, p < 0.045), while native species cover was lower at the inner location

202   compared to the entrance (Fig. 6; Tab. 4; Wilcoxon's test, p < 0.032). The only exception is August

203   2019, where native cover was higher at the inner location compared to the entrance (Wilcoxon's

204   test, p = 0.02). In all these cases (except August 2019) their cover was significantly different from

205   each other with more NIS than natives in the inner location and more native cover than NIS at the

206   entrance (Fig. 6; Tab. 4). Cryptogenic species and bare space had variable cover among the study

207   periods (Sup. Fig. 2).

208   Identification of indicator species

209   The simper analysis revealed that for all inter-location comparisons of any study period, five or less

210   species contribute to more than 75% of the contrast between communities (Fig. 7). In 8 out of 15

211   cases, *Ciona intestinalis* (native) (Linnaeus 1767) is the most important contributor to community

212   contrasts, with up to 62% (Fig. 7; June 2019 Middle *vs* Entrance). The bryozoan *Bugula neritina* (NIS)

213   (Linnaeus, 1758) is the second most frequent main contributor, appearing at first place 6 times out

214   of 15 and 5 times second (Fig. 7). Three more important contributors are the bryozoan *Tricellaria*

215   *inopinata* (NIS) d'Hondt & Occhipinti Ambrogi, 1985 (First in June 2019 Inner vs Middle), the colonial

216   sea squirt *Diplosoma listeranum* (NIS) (Milne Edwards, 1841) and the bryozoan *Watersipora subatra*

217   (NIS) (Ortmann, 1890). These important contributors were often associated to one of the three

218   locations in the multipattern analysis (Tab. 5). The native *C. intestinalis* was associated to the

219   entrance location three times, while the non-indigenous *B. neritina* and *W. subatra* were indicator

220   species of the inner location in respectively 3 and 4 of the study periods (Tab. 5). All study periods

221   confounded, a total of 5 NIS were identified as indicator species for the inner location, while only

222   one, *T. inopinata*, has been identified twice as indicator species of the entrance (Tab. 5).

223   **Discussion**

224 The work presented in this paper aimed to characterize the macrobenthic communities settled

225 under three pontoons of the Château marina (Brest), respectively at the inner, middle and entrance

226 location of the marina. The main objective was to study the heterogeneity of communities as well

227 as the distribution of NIS among these locations over a multi-year period. As assumed in our

228 hypothesis, the results obtained illustrate a systematic difference between the communities settled

229 at the entrance and at the inner part of the marina for all dates considered over three years. Such a

230 distinction at this small spatial scale (< 100 m between locations) is consistent with results previously

231 obtained by Kenworthy et al. (2018b) in the same marina. It thus appears that communities remain

232 spatially discriminated over a multi-year scale. Furthermore, temporal variations of community

233 structure appeared less important than spatial variations within the marina, however longer time

234 series are required to support this observation. The observed differences between these

235 communities might be related to ambient pollution, since pollution levels measured in sediments

236 of the inner location were often higher compared to the entrance (Tab. 1; Kenworthy et al. 2018b,

237 Gauff et al. 2022). While differences between the entrance and inner communities appeared to be

238 conserved, their differences with respect to the community settled in the middle of the marina were

239 much more variable. A distinction between communities of the three locations has sometimes been

240 observed, but at other periods it has also been observed that no differentiation of the middle

241 community with either the inner or the entrance communities of the marina was present. This may

242 indicate that the middle location consists in a variable environment, intermediate to both other

243 locations.

244 Literature on spatial variability of communities within harbor areas shows rather divergent results.

245 In some study areas, spatial variability is observed between communities at the entrance and inner

246 locations of a harbor (Webb & Keough 2000, Ryu et al. 2011, Kenworthy et al. 2018b) but for others

247 they appear relatively homogeneous (Lam & Todd 2013). The authors put forward different

248 hypotheses to explain these results. Some highlight a presence of pollutants like copper, as we did

249 here, but spatiotemporal variability in other abiotic parameters may also highly influence

250 community structure. It is difficult to identify which factor may be responsible for the variation of

251 community structures due to the great diversity of biotic (e.g. competition, predation) and abiotic

252 (e.g. salinity, hydrodynamics, pollution) factors that can participate in the structure of these

253 communities (Floerl & Inglis 2003, Blum et al. 2007, Piola & Johnston 2008, Crooks et al. 2011,

254 Kinsella & Crowe 2016). While the work presented here is not able to formally specify the causes of

255 the differences observed at the study site, the details of our results suggest a plausible link to
256 pollution levels.

257 Firstly, as assumed, a systematic distinction between the inner and entrance communities is
258 accompanied by a difference in the distribution of native and non-indigenous species. Over all the
259 studied periods, the cover of NIS was greater towards the inner location of the marina compared to
260 the entrance, and greater than the cover of native species at the inner location. These results differ
261 from those of Kenwothy et al. 2018 which didn't reveal any differences in NIS persistence between
262 different parts of the marina. However, the habitats considered, and the studied community differ
263 in several points from ours and it is likely that the processes observed on mature pillar communities
264 may be different from those observed on settlement plates deployed under pontoons like here.
265 Conversely, the cover of native species is almost always maximal towards the entrance of the
266 marina. Moreover, the nMDS, established from respectively native and NIS data, also highlighted
267 distinct communities at the entrance and the inner locations of the marina throughout the studied
268 periods. These results suggest different environmental filters between the inner location and the
269 entrance of the marina that impact the cover and the community composition, which is observable
270 for NIS and natives. It has already been observed that NIS are often more resistant to the presence
271 of copper than native taxa (Piola et al. 2009, Crooks et al. 2011). Therefore, the inverse distribution
272 between native and NIS species in our results could be related to the present copper pollution
273 gradient (Tab. 1). The structure of marinas is known to often facilitate the formation of eddies that
274 can lead to greater water retention in the inner parts of marina (Floerl & Inglis 2003), which may be
275 the reason for this increased pollution at the inner parts of the marina (Schiff et al. 2007). This lower
276 water mixing with the external environment may, however, also result in a greater variability in
277 temperature, pH or turbidity in the most enclosed areas (Rivero et al. 2013, Camp et al. 2017). All
278 these factors could induce heterogeneous environmental conditions within the marina and may
279 induce the recruitment of distinct communities between the entrance and the inner locations, as
280 observed in other marinas (Rivero et al. 2013).

281 Secondly, biotic factors may also play a role in the appearance of these differences. All our results
282 highlighted the ascidian *Ciona intestinalis* as the species frequently explaining most of the contrast
283 between communities among locations. Various works have already shown the importance of sea
284 squirts in the structure and composition of fouling communities (Dijkstra et al. 2007, Lindeyer &
285 Gittenberger 2011). In particular, *C. intestinalis* can lead to a decrease in species richness within the
286 communities when present (Dijkstra et al. 2007, Blum et al. 2007). This may be explained by the

287 highly competitive nature of *C. intestinalis* that tends to monopolize free space through rapid

288 development/growth and massive arrival of larvae (Koechlin 1977, Paetzold et al. 2012). Available

289 space appears as a key resource in the establishment of fouling communities (Osman 1977,

290 Sutherland 1981). *C. intestinalis* thus may play an important structuring role in these environments.

291 Consequently, its dominance at the entrance of our marina may explain the observed differences of

292 biodiversity and community structure among locations. This heterogeneous distribution could be

293 caused by various environmental factors such as predation (Schmidt & Warner 1986, Dumont et al.

294 2011, Gauff et al. 2022) and copper pollution which is known to negatively affect reproduction and

295 larval development of *C. intestinalis* (Bellas et al. 2001, 2004) and has been observed at the inner

296 part of the marina (Tab. 1; Kenworthy et al. 2018b, Gauff et al. 2022). The systematically different

297 community structures at the entrance and the inner of the marina could therefore be also explained

298 by these parameters influencing the presence of *C. intestinalis.*

299 The work presented in this article has highlighted that spatial structure of the communities and

300 some of its characteristics appear temporally preserved over all the studied periods. Fouling

301 communities appear always significantly different between the entrance and the inner location of

302 the marina, NIS cover is constantly higher toward the inner location of the marina compared to the

303 entrance and *C. intestinalis* seems to frequently play a major role in the communities' contrasts

304 among locations. This might be linked to spatial variability of environmental conditions, most

305 probably variable pollution levels. However, the temporal scale of our work has also revealed a large

306 variability in our results, particularly about the community structure of the middle of the marina

307 and the presence of NIS. While the distribution of these taxa is often more prevalent in the inner of

308 the marina, it is much more contrasted and visible at certain periods than at others. In June 2019,

309 NIS did not exceed an average of 30% cover, but this cover sometimes appears much higher at other

310 periods with more than 75% average cover in August 2019 in the middle and at the inner of the

311 marina. Such results illustrate how important it is to quantify this temporal variability to conclude

312 on the actual presence and distribution of these organisms throughout the year. Currently, most

313 community-based studies conducted in marinas are attempting to increase the spatial scope of their

314 results by integrating more and more different study sites (Webb & Keough 2000, Ashton et al. 2006,

315 Ferrario et al. 2017, Spagnolo et al. 2019). In contrast, the number of studies integrating a temporal

316 dimension is comparatively much smaller (Covazzi Harriague et al. 2007, Canning-Clode et al. 2013).

317 Our results thus illustrate that the development of temporal monitoring of marina communities

318 appears essential today in a context of management and assessment of the NIS populations of these

319 ecosystems. To do so, the photographic analysis method appears as a tool of interest since it

320 requires a lower logistical investment which could facilitate the implementation of future annual

321 and interannual monitoring of several marina communities. Photographic analysis could thus be

322 combined with rapid assessment studies, which have higher taxonomic resolution, to form a

323 complementary approach.

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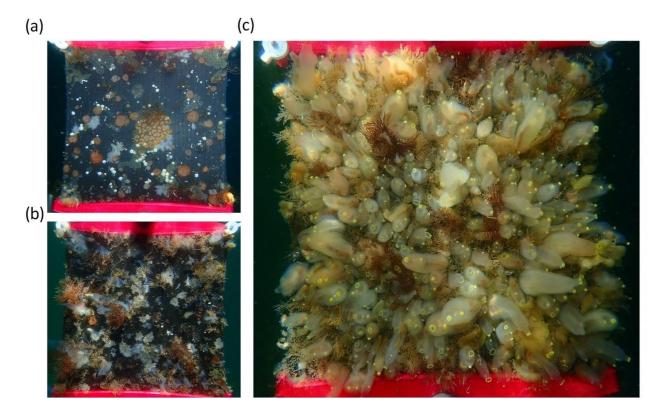434   in south-west Spain. Estuar Coast Shelf Sci 136:102–111.

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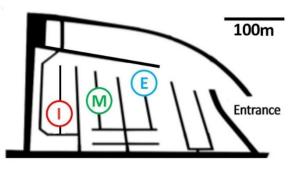514   16:345–360.

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Fig. 1: Examples of photographic data of macrobenthic communities recruited on PE panels in June 2019; (a) inner, (b) middle and (c) entrance location of the marina.

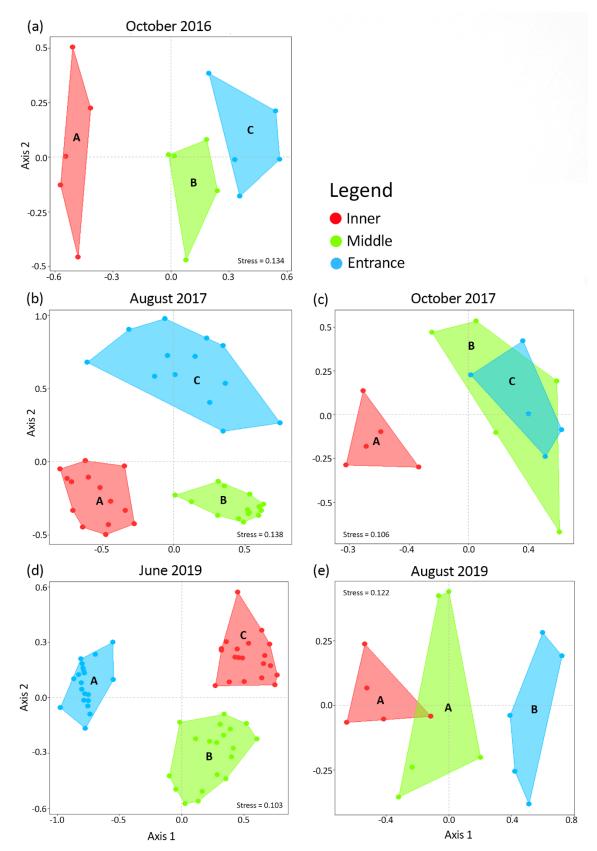| Pollutant		Site mean			
		Inner		Entrance	
		2016	**2019**	**2016**	**2019**
Metallic Trace Elements (MTE) mg.kg^{-1}	Cu63(MR)	93.3	84	44.2	33
	Pb208(LR)		85		46
	Zn66(MR)	446	236	175	111
Total hydrocarbons mg.kg^{-1}	(C10-C40)	1320		156	
Polycyclic Aromatic Hydrocarbon (PAH) µg.kg^{-1}	Benzo(*g,h,i*)perylene		407		283
	Fluorene		725		642
Polychlorinated Biphenyl (PCB) µg.kg^{-1}	tPCB		603		544
Pesticides µg.kg^{-1}	diazinon	< 50	0,6	< 50	1,5
	dieldrin		1,1		2,2
	pp'-DDD	< 10	10,7	< 10	2,5
	pp'-DDE	< 10	17,6	< 10	15,6
	pp'-DDT	< 10	0,6	< 10	0,1
	endosulfan-1	< 50	3,1	< 50	2,3
	methoxychlor		7,4		2,5

Tab. 1: Pollution levels (in mg.kg^{-1} or µg.kg^{-1}) at the inner and entrance location of the studied marina. Data from two publications using the same study locations as here was compiled. Pollutants that significantly varied between locations in Gauff et al. (2022) for 2019 and all detected pollutants in Kenworthy et al. (2018) for 2016 are indicated. Values below the detection limits are indicated for 2016 (with their detection limit).

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538 **Fig. 2:** Schematic diagram of the marina du Château in Brest and the different studied locations: (I) inner, (M) middle
539 and (E) entrance.

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542 **Tab. 2:** Different taxa and species identified via macro-morphological criteria as well as their ecological status within the
543 marina (sources CABI and WoRMS). The abbreviation "Obs" corresponds to an identification completed by the work
544 carried out in the laboratory (identification by molecular barcode and microscopy). NIS are indicated with an asterisk
545 and in bold.

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Species	Status	Criteria
Annelida		
Spirobranchus triqueter	Native	
Arthropoda		
Austrominius modestus	**Non-Indigenous**	
Bryozoa		
Bugula neritina	**Non-Indigenous**	
Bugulina flabellata	Native	Obs
Bugulina fulva	Cryptogenic	Obs
Cryptosula pallasiana	**Non-Indigenous**	
Electra pilosa	Native	
Tricellaria inopinata	**Non-Indigenous**	**Obs**
Watersipora subatra	**Non-Indigenous**	**Obs**
Tunicata		
Ascidiella aspersa & *scabra*	Native	
Asterocarpa humilis	**Non-Indigenous**	
Bortyllus schlosseri	Cryptogenic	
Ciona intestinalis	Native	Obs
Clavelina lepadiformis	Native	
Diplosoma listeranum	Cryptogenic	Obs
Phallusia mammilata	Native	
Styela clava	**Non-Indigenous**	
Mollusca		
Anomia ephippium	Native	

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Fig. 3: nMDS of global community data obtained from three different Château Marina locations in October 2016 (a), August 2017 (b), October 2017 (c), June 2019 (d) and August 2019 (e). Significant differences between the groups (Pairwise PERMANOVA, p < 0.05) are represented by the letters A-B-C; details of these results are available in Tab. 3.
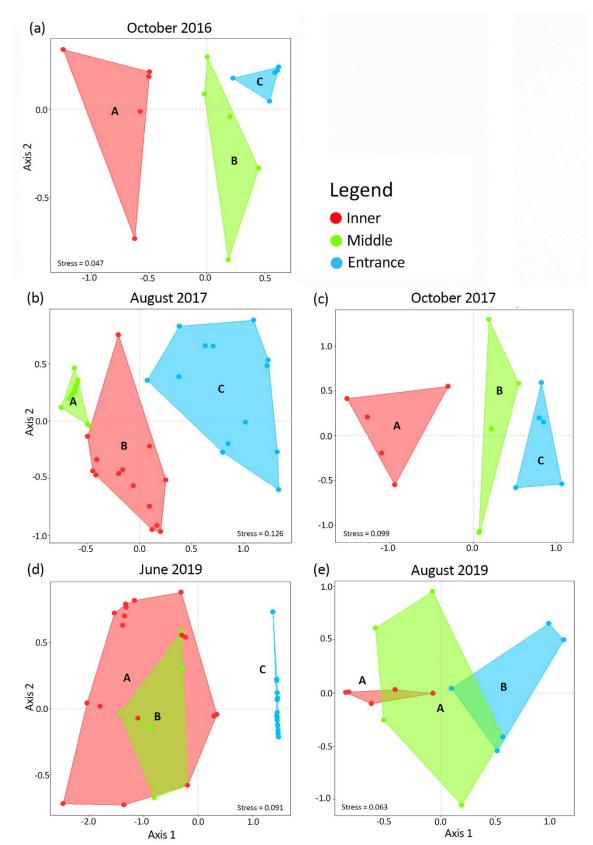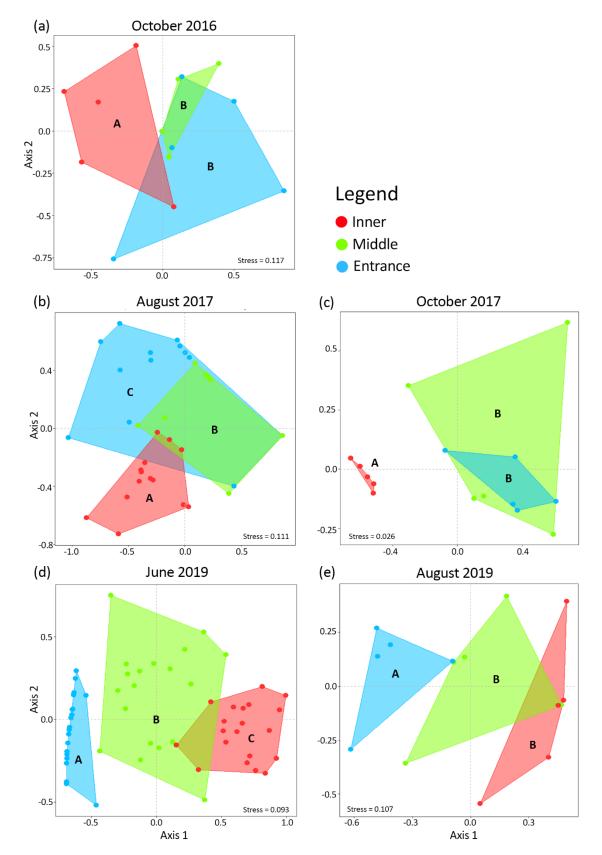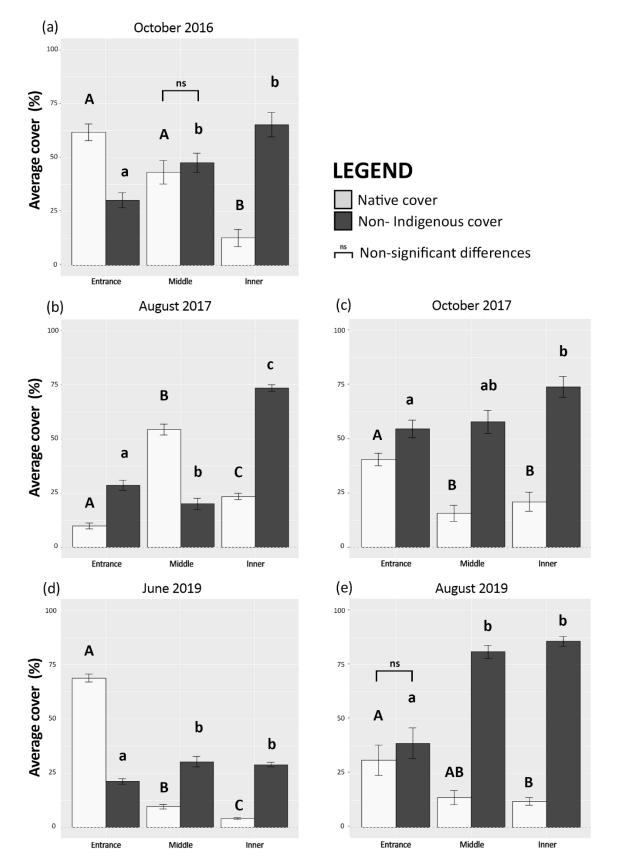
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Fig. 4: nMDS of native community data obtained from three different Château Marina locations in October 2016 (a), August 2017 (b), October 2017 (c), June 2019 (d) and August 2019 (e). Significant differences between the groups (Pairwise PERMANOVA, $p < 0.05$) are represented by the letters A-B-C; details of these results are available in Tab. 3.

Fig. 5: nMDS of the non-indigenous community data obtained from three different Château Marina locations in October 2016 (a), August 2017 (b), October 2017 (c), June 2019 (d) and August 2019 (e). Significant differences between the groups (Pairwise PERMANOVA, p < 0.05) are represented by the letters A-B-C; details of these results are available in Tab. 3.

579 **Tab. 3:** Results of the pairwise PERMANOVAs (Benjamini & Hochberg correction) testing for significant
580 differences among the data groups of different locations for October 2016, August 2017, October 2017,
581 June 2019 and August 2019. Significant results are indicated as follows: *: p < 0.05, **: p < 0.01, ***: p <
582 0.001 and ns: p > 0.05

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Period	Community	Comparisons	df	R2	p.value	p.adjusted	
October 2016	Complete	Inner vs Middle	1	0.322	0.008	0.013	*
		Inner vs Entrance	1	0.819	0.006	0.013	*
		Middle vs Entrance	1	0.178	0.015	0.015	*
	Native	Inner vs Middle	1	0.432	0.007	0.008	**
		Inner vs Entrance	1	0.562	0.008	0.008	**
		Middle vs Entrance	1	0.475	0.008	0.008	**
	Non-Indigenous	Inner vs Middle	1	0.352	0.023	0.034	*
		Inner vs Entrance	1	0.59	0.008	0.025	*
		Middle vs Entrance	1	0.291	0.057	0.057	ns
August 2017	Complete	Inner vs Middle	1	0.801	<0.001	<0.001	***
		Inner vs Entrance	1	0.686	<0.001	<0.001	***
		Middle vs Entrance	1	0.653	<0.001	<0.001	***
	Native	Inner vs Middle	1	0.613	<0.001	<0.001	***
		Inner vs Entrance	1	0.538	<0.001	<0.001	***
		Middle vs Entrance	1	0.688	<0.001	<0.001	***
	Non-Indigenous	Inner vs Middle	1	0.352	<0.001	<0.001	***
		Inner vs Entrance	1	0.59	<0.001	<0.001	***
		Middle vs Entrance	1	0.291	0.0058	0.0058	**
October 2017	Complete	Inner vs Middle	1	0.589	0.007	0.0086	**
		Inner vs Entrance	1	0.823	0.008	0.0086	**
		Middle vs Entrance	1	0.446	0.008	0.0086	**
	Native	Inner vs Middle	1	0.576	0.008	0.009	**
		Inner vs Entrance	1	0.815	0.009	0.009	**
		Middle vs Entrance	1	0.534	0.008	0.009	**
	Non-Indigenous	Inner vs Middle	1	0.568	0.007	0.011	*
		Inner vs Entrance	1	0.726	0.007	0.011	*
		Middle vs Entrance	1	0.057	0.749	0.749	ns
June 2019	Complete	Inner vs Middle	1	0.539	<0.001	<0.001	***
		Inner vs Entrance	1	0.858	<0.001	<0.001	***
		Middle vs Entrance	1	0.778	<0.001	<0.001	***
	Native	Inner vs Middle	1	0.281	<0.001	<0.001	***
		Inner vs Entrance	1	0.646	<0.001	<0.001	***
		Middle vs Entrance	1	0.862	<0.001	<0.001	***
	Non-Indigenous	Inner vs Middle	1	0.477	<0.001	<0.001	***
		Inner vs Entrance	1	0.730	<0.001	<0.001	***
		Middle vs Entrance	1	0.272	<0.001	<0.001	***
August 2019	Complete	Inner vs Middle	1	0.184	0.186	0.182	ns
		Inner vs Entrance	1	0.612	0.008	0.013	*
		Middle vs Entrance	1	0.496	0.007	0.013	*
	Native	Inner vs Middle	1	0.257	0.096	0.096	ns
		Inner vs Entrance	1	0.456	0.006	0.020	*
		Middle vs Entrance	1	0.315	0.024	0.036	*
	Non-Indigenous	Inner vs Middle	1	0.173	0.253	0.253	ns
		Inner vs Entrance	1	0.598	0.008	0.012	*
		Middle vs Entrance	1	0.521	0.008	0.012	*

(a) October 2016

(b) August 2017

(c) October 2017

(d) June 2019

(e) August 2019

LEGEND
- Native cover
- Non- Indigenous cover
- ns Non-significant differences

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585 **Fig. 6:** Comparisons of average covers of native species and non-indigenous species at 3 locations within the marina du
586 Château (Brest) in (a) October 2016, (b) August 2017, (c) October 2017, (d) June 2019 and (e) August 2019. Significant
587 differences within each category are indicated with letters, Significant differences between categories within the same
588 location are indicated using brackets for non-significant groups. An absence of a bracket indicates significant differences
589 between categories intra-location. Error Bars represent the error type of each average cover category.

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591 **Tab. 4:** Results of the pairwise Wilcoxon tests (Benjamini & Hochberg correction) testing for significant differences in
592 cover of Non-Indigenous and Native species between locations for October 2016, August 2017, October 2017, June 2019
593 and August 2019 and for significant differences *vice versa*. Significant results are indicated as follows: *: $p < 0.05$, **: p
594 < 0.01, ***: $p <$ ... 0.001 and
595 ns: $p > 0.05$.

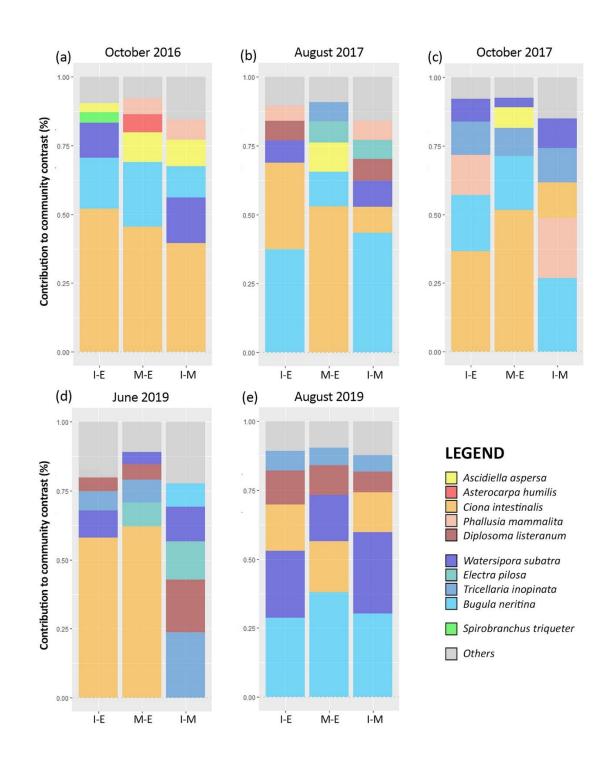| Period	Comparisons		W	p.adjusted	
October 2016	Native & NIS	Entrance NIS - Entrance Native	25	0.03	*
		Middle NIS - Middle Native	9	0.587	ns
		Inner NIS - Inner Native	0	0.03	*
	Native	Entrance vs Middle	22	0.076	ns
		Entrance vs Inner	25	0.03	*
		Middle vs Inner	0	0.03	*
	NIS	Entrance vs Middle	1.5	0.06	ns
		Entrance vs Inner	0	0.03	*
		Middle vs Inner	22.5	0.069	ns
August 2017	Native & NIS	Entrance NIS - Entrance Native	2	< 0.001	***
		Middle NIS - Middle Native	225	< 0.001	***
		Inner NIS - Inner Native	0	< 0.001	***
	Native	Entrance vs Middle	0	< 0.001	***
		Entrance vs Inner	7	< 0.001	***
		Middle vs Inner	0	< 0.001	***
	NIS	Entrance vs Middle	142	0.049	*
		Entrance vs Inner	0	< 0.001	***
		Middle vs Inner	225	< 0.001	***
October 2017	Native & NIS	Entrance NIS - Entrance Native	2	0.043	*
		Middle NIS - Middle Native	0	0.022	*
		Inner NIS - Inner Native	0	0.022	*
	Native	Entrance vs Middle	25	0.022	*
		Entrance vs Inner	24	0.032	*
		Middle vs Inner	17	0.431	ns
	NIS	Entrance vs Middle	9	0.548	ns
		Entrance vs Inner	2	0.045	*
		Middle vs Inner	22	0.068	ns
June 2019	Native & NIS	Entrance NIS - Entrance Native	400	< 0.001	***
		Middle NIS - Middle Native	9.5	< 0.001	***
		Inner NIS - Inner Native	0	< 0.001	***
	Native	Entrance vs Middle	380	< 0.001	***
		Entrance vs Inner	400	< 0.001	***
		Middle vs Inner	46.5	< 0.001	***
	NIS	Entrance vs Middle	81	0.0024	**
		Entrance vs Inner	54.5	< 0.001	***
		Middle vs Inner	175.5	0.694	ns
August 2019	Native & NIS	Entrance NIS - Entrance Native	8	0.451	ns
		Middle NIS - Middle Native	0	0.02	*
		Inner NIS - Inner Native	0	0.02	*
	Native	Entrance vs Middle	21	0.119	ns
		Entrance vs Inner	25	0.02	*
		Middle vs Inner	13	1.000	ns
	NIS	Entrance vs Middle	0	0.02	*
		Entrance vs Inner	0	0.02	*
		Middle vs Inner	18.5	0.287	ns

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626 **Fig. 7:** SIMPER analysis revealing the species most contributing to the contrast between communities of the different
627 locations in October 2016 (a), August 2017 (b), October 2017 (c), June 2019 (d) and August 2019 (e); I-E comparison of
628 the Inner-Entrance, M-E comparison of the Middle-Entrance and I-M comparison of the Inner-Middle.

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Tab. 5: Indicator species at the three Château Marina locations identified by the Multipattern analysis for October 2016, August 2017, October 2017, June 2019 and August 2019. NIS are indicated with an asterisk and in bold. Significant results are indicated as follows: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ and ns: $p > 0.05$.

Period	Locations	Indicator species	stat	p.value	
October 2016	Entrance	*Ciona intestinalis*	0.791	<0.001	***
	Inner	*Spirobranchus triqueter*	0.901	0.009	**
		Cryptosula pallasiana	0.894	0.017	*
		Bugula neritina	0.639	0.024	*
August 2017	Entrance	*Electra pilosa*	1.000	<0.001	***
		Tricellaria inopinata	0.734	<0.001	***
		Bugulina flabellata	0.568	0.002	**
		Bortyllus schlosseri	0.480	0.024	*
	Middle	*Ciona intestinalis*	0.888	<0.001	***
		Ascidiella aspersa	0.761	0.002	**
		Diplosoma listeranum	0.723	<0.001	***
	Inner	***Watersipora subatra***	0.941	<0.001	***
		Phallusia mammilata	0.917	<0.001	***
		Asterocarpa humilis	0.820	<0.001	***
		Bugula neritina	0.772	<0.001	***
		Spirobranchus triqueter	0.628	0.010	**
October 2017	Entrance	*Ciona intestinalis*	0.89	0.002	**
	Inner	*Phallusia mammilata*	0.966	0.002	**
		Watersipora subatra	0.869	0.002	**
		Asterocarpa humilis	0.839	0.010	**
		Spirobranchus triqueter	0.833	0.018	*
		Bugula neritina	0.645	0.014	*
June 2019	Entrance	*Ciona intestinalis*	0.998	<0.001	***
		Bugulina flabellata	0.949	<0.001	***
		Bugulina fulva	0.758	<0.001	***
	Middle	*Electra pilosa*	0.901	<0.001	***
		Tricellaria inopinata	0.783	<0.001	***
	Inner	***Cryptosula pallasiana***	0.890	<0.001	***
		Austrominius modestus	0.869	<0.001	***
		Watersipora subatra	0.838	<0.001	***
		Bortyllus schlosseri	0.837	<0.001	***
		Diplosoma listeranum	0.735	<0.001	***
		Ascidiella aspersa	0.658	<0.001	***
August 2019	Entrance	*Electra pilosa*	0.926	0.004	**
		Tricellaria inopinata	0.849	0.006	**
		Diplosoma listeranum	0.848	0.002	**
	Inner	*Spirobranchus triqueter*	0.895	0.002	**
		Cryptosula pallasiana	0.817	0.035	*
		Watersipora subatra	0.702	0.010	**