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1 **Assessment of an alternative *Pinctada margaritifera* spat collector in French Polynesia**

2

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13

14 **Abstract**

15 Plastic waste in the oceans is a growing concern due to its size diversity, its ubiquitous nature
16 and its impact on both marine organisms and ecosystems. While threatened by this marine plastic
17 litter, the aquaculture industries also represent one of its major sources. In French Polynesia, black-lip
18 pearl oyster (*Pinctada margaritifera*) farming is no exception. Wild spat collection has been described
19 as a source of pollution because of the numerous particularly fragile and brittle shade-mesh plastic
20 collectors used locally and often mismanaged when no longer usable. Thus, with the aim of helping to
21 reduce this pollution, the present study is focused on the assessment of reusable plate collector as a
22 potential alternative. We tested, using an *in situ* approach, the influence of the collecting surface
23 position (*i.e.* horizontal *vs* vertical), plate color (*i.e.* black *vs* orange) and density (*i.e.* 25 plates *vs* 50
24 plates) on *Pinctada margaritifera* and *Pinctada maculata* spat settlement. Our results showed that 50-
25 plates collectors, whatever their color and position, were more efficient to collect both kinds of spat
26 than shade-mesh collectors ($P < 0.0001$). They also induced a higher number of *Pinctada*
27 *margaritifera* spat than on the 25-plates collection device although there was no significant difference

28 in numbers of *Pinctada maculata* spat ($P < 0.0001$). However, every type of plate collector presented a
29 decreased mean length of the spat collected ($P < 0.0001$) compared to the shade-mesh collector.
30 Furthermore, horizontal positioning of the collecting surface greatly improved the spat numbers on
31 plate collectors ($P < 0.0001$) although there was no effect on shade-mesh collectors ($P > 0.05$).
32 Finally, our results indicated that, among all the devices tested, the black horizontal 50-plates collector
33 was the most efficient to collect spat and particularly *Pinctada margaritifera*. These first findings thus
34 tend to suggest that these black re-usable plate collectors could be an efficient alternative to the
35 currently used shade-mesh collectors.

36

37 **Keywords**

38 Pearl-farming, *Pinctada margaritifera*, *Pinctada maculata*, spat collectors

39

40 **1. Introduction**

41

42 With worldwide plastic production of 359 million tons in 2018 (PlasticsEurope, 2019), the
43 management of end-of-life plastics is one of the biggest environmental concerns of our time. In the
44 best-case scenario, after end of use, plastic waste is collected by local services to be recycled or
45 incinerated for energy recovery purposes (PlasticsEurope, 2019), and in the worst case, is littered in
46 the environment whether on land or in the ocean (Andrady, 2004; Geyer et al., 2017). Plastics
47 represent 60-80% of marine debris (Derraik, 2002; Lebreton et al., 2018), originating either in land
48 sources (70-80%) or sea-based sources (20-30%) (Andrady, 2011). Ocean contamination is therefore a
49 major issue, with about 4.8 to 12.7 Mt of plastic waste entering the oceans in 2010 (Jambeck et al.,
50 2015), especially since it cannot degrade completely due to its chemical composition (Gregory and
51 Andrady, 2004). Instead, several factors such as solar radiation, thermal oxidation or mechanical
52 actions break the chemical bonds between plastic components (Gregory and Andrady, 2004), resulting
53 in plastic fragments of all sizes, notably microplastics (<5 mm). Hard to remove, microplastics are
54 abundant and present everywhere in the oceans (Barnes et al., 2009; Eriksen et al., 2014; Jambeck et
55 al., 2015).

56 Size diversity and the ubiquitous nature of plastic marine debris make it available to a wide
57 range of marine organisms, harming them chemically (Teuten et al., 2009; Le Bihanic et al., 2020)
58 and/or physically (Gregory and Andrady, 2004). Moreover, marine ecosystems can also be affected
59 indirectly, as demonstrated by Katsanevakis et al. (2007) showing that an addition of plastic litter on
60 the sea-bed can induce a deviation in the structural community of benthos megafauna and its
61 composition. Barboza et al., (2019) also indicated in their review that plastics could impact predator-
62 prey relationships, smother the sea-floor (*i.e.* benthos community), disturb carbon cycling and
63 transport invasive species, pollutants and pathogens. Furthermore, for aquaculture industries another
64 concern is the transfer of these microplastics between trophic levels toward human (*i.e.* final
65 consumer) as underlined by Van Cauwenberghe and Janssen (2014). Yet, while being threatened by
66 marine plastic litter, aquaculture remains one of its sea-based sources as aquaculture materials are
67 mainly made from plastic. In their study, Lebreton et al. (2018) showed that 1.3% of the plastic litter
68 found in the Great Pacific Garbage Patch, located in the North Pacific Subtropical Gyre, derived from
69 aquaculture. Another study also showed that a large proportion of the floating marine debris they
70 studied in Chilean coastal zones came from sea-based aquaculture (Hinojosa and Thiel, 2009).
71 Likewise, Feng et al. (2020) in China demonstrated that the macro-algae farming industry released
72 1037 tons of plastics in the ocean and that it can be a source of microplastics.

73 In French Polynesia (FP), local communities, scientists and authorities have been concerned
74 for several years by waste production, recorded as around 147 000 tons in 2012 (Murzilli et al., 2012).
75 On islands, waste management is a sensitive issue because of the small land area available, the lack of
76 capital and financing options, the significant operational costs of waste management (transportation,
77 labour; etc.) and the vulnerability of the environment to extreme climatic events (Eckelman et al.,
78 2014). This is particularly the case for atolls and remote islands in FP, where those problems are
79 exacerbated. Thus, it is essential for islanders to reduce, or at least better control, their waste
80 production and to optimize waste management systems. This issue is of particular concern for the
81 pearl-farming industry operating in 23 remote islands mainly located in the Tuamotu-Gambier
82 archipelago. In these islands, the waste management system is basic, limited to a collection system for
83 household and green waste. No industrial waste treatment system exists. Yet, like most aquaculture

84 industries, pearl-farming uses plastic materials to rear the oysters, such as plastic ropes, nylon, shade-
85 mesh, buoys and plastic grating. However, there have been very few studies on waste production in
86 the pearl industry in Polynesian lagoons (Andréfouët et al., 2014; Gaertner-Mazouni et al., 2018). A
87 first estimate of the annual waste production of a farm of 15 ha using 15 collecting lines in Gambier
88 islands evidenced that around 2 tons of waste can be produced annually (Gaertner-Mazouni and
89 Rodriguez, 2016). These authors highlighted that some stages during pearl production, such as spat
90 collection, contribute significantly to waste production.

91 Spat collection constitutes the first stage of production in the pearl industry in French
92 Polynesia, representing 9464 collecting lines officially listed and deployed in lagoons (personal
93 communication, DRM, 2019). A collecting line consists in a 200 m plastic rope (diameter > 16 mm)
94 with 600 settlement supports (so-called 'spat collectors') on which larvae will attach. Spat collectors
95 used in French Polynesia are made of black plastic shade-mesh strip sewn accordion-style on a plastic
96 rope (Haws and Ellis, 2000), referred to as 'shade-mesh'. However, according to Gaertner-Mazouni et
97 al. (2018), they are particularly brittle and fragile with a three-year life span. Moreover, inefficient
98 collecting lines are sometimes abandoned due to the cost of retrieval compared to what they yield.
99 Either way, in-use, broken or abandoned collectors will stay in the lagoon for years and eventually
100 start to fragment or leach like every plastic, as suggested by Andréfouët et al. (2014), their brittleness
101 probably reinforcing this phenomenon.

102 In order to investigate this emerging threat, Gardon et al. (2018) exposed *Pinctada*
103 *margaritifera* to laboratory microbeads of polystyrene, in a preliminary study, and showed that its
104 reproduction activity was affected. Furthermore, these authors recently assessed the toxicity of
105 leachates of new and aged plastic pearl-farming materials (synthetic ropes and shade-mesh spat
106 collectors) used in French Polynesia on the embryo-larval development of *Pinctada margaritifera*.
107 Chemicals such as phthalates were found to leach from those plastic gears and toxicity was proven on
108 embryos after a 48 hour exposure at the smallest dose tested of 0.01 g.L⁻¹ (Gardon et al., 2020).
109 Therefore, to ensure pearl-farming sustainability, there is an urgent need to replace these shade-mesh
110 collectors by materials that are more resistant and environmentally-friendly.

111 In the literature, several settlement supports have been studied on bivalve species, either
112 natural (e.g. coconut husks, bundles of various plants) or artificial such as tiles and plastic supports.
113 However, none of the supports tested for *P. margaritifera* spat collection seemed to be more efficient
114 than the shade-mesh collector (Haws and Ellis, 2000; Arini and Jaya, 2011; Ishengoma et al., 2011;
115 Libini et al., 2013). According to the authors, numerous parameters have to be taken into account
116 when choosing a collector for targeted species to stimulate its spat settlement. They showed that the
117 shape, texture, color and position of the collector and its collecting surfaces could have an incidence
118 on settlement efficiency, depending on the oyster species (Galtsoff, 1964; Taylor et al., 1998; Saucedo
119 et al., 2005; Su et al., 2007; Arini and Jaya, 2011; Wang et al., 2017). It was reported that *Pinctada*
120 *margaritifera* spat settle preferentially on rigid rough dark materials due to a negative phototaxy of
121 larvae (Gervis and Sims, 1992; Friedman et al., 1998; Ehteshami et al., 2011; Ishengoma et al., 2011;
122 Libini et al., 2013).

123 Among currently available collecting supports, grooved plastic plate collectors, referred to as
124 'plates', which are commonly used for *Crassostrea gigas* spat collection in France, seemed to be an
125 interesting alternative to shade-mesh for various reasons. Firstly, they are made of rough semi-rigid
126 plastic, reusable, that can be positioned either horizontally or vertically. Secondly, they are available in
127 different colors, notably black, and are successfully used for the collection of bivalve spat (Ferra,
128 2008; Lescroart, 2017). Thirdly, these collectors are already available in French Polynesia for an
129 average cost of 18.96 € (tax incl.) for a 50-plate collector.

130 Another point to highlight is that, even if it is still plastic, the plate collector could be a more
131 environmental friendly alternative than shade-mesh collectors which have low longevity and release
132 toxic chemicals and MP in the environment in the very short term (Gardon et al., 2020). With the
133 shape of a round plate 1.7 mm thick and 150 mm wide, compared to the thin filament of 3mm width of
134 the shade-mesh, the plate collector is more resistant, notably to mechanical erosion and photo-
135 degradation. These properties confer on plate collectors a life-span from 3 to 6 years (up to 10 years
136 for the most durable ones) when they are used for collection of *Crassostrea gigas* spat i.e. subjected to
137 the tide cycle and exposed to direct UV radiation (Ferra, 2008; Lescroart, 2017). Thus, they are
138 reusable and their longevity could be even greater in French Polynesia as collection of *P.*

139 *margaritifera* spat consists in continuous immersion of the collector at 5-6 m depth during 1 to 2
140 years. Moreover, no chemical toxicity of this material has been reported to our knowledge.

141 Therefore, the aim of this study was to determine whether plates could replace shade-mesh
142 collectors and what would be the best conditions to do so. For this purpose, we designed a
143 comparative study of the collecting efficiency of plate and shade-mesh collectors on *Pinctada*
144 *margaritifera* spat settlement, by performing an *in situ* experiment. We assessed in particular the
145 influence of the type of collector, collecting surface position, color and density of plates on spat
146 settlement. To complete our study, a section discussing the cost and environmental impact of the best
147 alternative is provided at the end of the study.

148

149 **2. Material & Methods**

150

151 **2.1 Experimental site**

152

153 This study took place in the lagoon of Takapoto (145°20'W, 14°70'S) in French Polynesia
154 between November 2018 and March 2019. This lagoon is defined as a closed environment
155 characterized by poor water renewal due to the lack of shipping channels except narrow ones
156 (Andréfouët et al., 2001). Water temperatures range from 26 to 30 °C (Ricard et al., 1979) and salinity
157 from 38 to 41 PSU (Rougerie, 1979; Pagès and Prasil, 2002).

158

159 **2.2 Spat collectors**

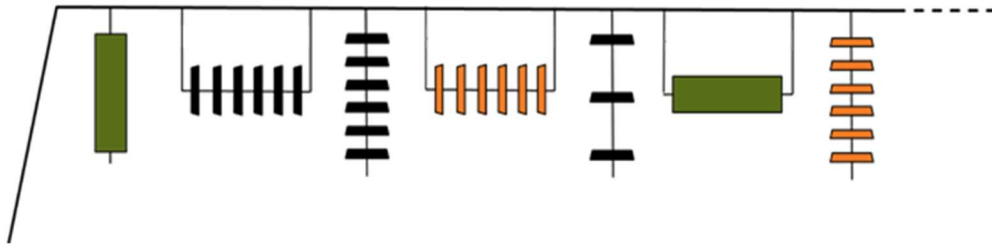
160

161 This experiment compared shade-mesh collectors and plate collectors. The shade-mesh
162 collectors were composed of black plastic shade-mesh strip 8 cm in width, corresponding to a
163 collecting surface of 1.28 m² (60g of plastic/m²), sewn accordion-style on a one meter plastic rope
164 with a diameter of 6 mm, currently used by Polynesian pearl farmers. Plate collectors were made of
165 grooved plastic plates 15 cm in width on a 1.2 m rigid PVC tube separated by a space of 2.5 or 5 cm
166 with a collecting surface of 0.025 m²/plate. Along with this comparison, the influence on settlement of

167 the position of the collecting surface (*i.e.* horizontal vs vertical), plate color (*i.e.* black vs orange) and
168 plate density for one tube was studied. Two densities were tested: 25 plates (half density plates, HDP)
169 vs 50 plates (normal density plates, NDP), corresponding respectively to an average collecting surface
170 area of 0.625 m² and 1.25 m².

171 In total, seven collecting devices were tested with 30 replicates each and attached alternately,
172 every 40 cm, on a main rope with buoys to avoid a possible collecting zone effect (Figure 1). The
173 vertical orange plates device had only 12 replicates as it had been tested previously and did not yield
174 consistent results (unpublished), only 42 orange plate collectors being available. They were placed at
175 the beginning, middle and end of the line to avoid any zone effect.

176



177

178 *Figure 1. Scheme of the first alternation of the seven devices on the main rope, from left to right : 1-*
179 *vertical shade-mesh, 2- vertical black plates, 3- horizontal black plates, 4- vertical orange plates, 5-*
180 *half density black plates, 6- horizontal shade-mesh, 7- horizontal orange plates. This pattern was*
181 *repeated 30 times along the 200 m rope.*

182

183 **2.3 Collector management and data collection**

184

185 The main rope with the collectors was immersed in the lagoon, next to the collecting lines of
186 pearl-farmers, at 6 m depth, which is the usual practice in French Polynesia. It was maintained at this
187 depth and location by means of buoys and mooring posts. To follow the progression of the collectors
188 during the experiment, the collecting line was checked every two to four weeks and depth was
189 adjusted (adding buoys) if necessary. After four months (123 days), the collectors were retrieved.
190 Monitoring was performed from photo and visual observations of each device. For each collector,
191 *Pinctada margaritifera* and *Pinctada maculata* spat were removed and counted. Afterwards, the total

192 weight of *P. margaritifera* and *P. maculata* spat.collector⁻¹ was measured. For each species a mean
193 weight per collector was calculated. Moreover, some *P. margaritifera* spat were randomly selected to
194 be measured. Our sampling effort was adapted to spat number as a maximum of 349
195 individuals.collector⁻¹ could be found: for collectors with less than 100 spat: all individuals were
196 measured, between 100 and 200 spat: half of them, more than 200: a quarter of them. To do so, they
197 were put on a millimeter paper, photographed and then length was measured by image analysis with
198 Image J ® (Schindelin et al., 2012).

199

200 **2.4 Statistical analysis**

201

202 In this study, the efficiency of one collecting device was represented by the average density of
203 spat of *P. margaritifera* and *P. maculata* settled on the structure, after four months of immersion. It
204 was calculated by the following equation:

$$205 \bar{D}_y \text{ (unit. cm}^{-2}\text{)} = \frac{1}{n_y \times S_y} \sum_{i=1}^{n_y} Ns_i$$

206 Where \bar{D} = Average density of spat (unit.cm⁻²); Ns = Number of spat, S_y = Surface area of
207 collection (cm²), y = 1, 2, 3, ... yth device, i = 1, 2, 3, ... nth replicate collector and n = number of
208 collectors.

209 Spat density, average biometry traits and individual length were first analyzed using a one-
210 way Anova testing the difference between devices. Prior to this analysis, normality of the model
211 residuals was verified using Shapiro test and the homoscedasticity of variances was tested using
212 Levene test. These analyses confirmed that the difference between the devices used in our experiment
213 was significant (one-way Anova, P < 0.0001). Thus, a three-way Anova was performed testing
214 incidence of the type, color and position and interactions between those factors. When interactions
215 (Anova, P> 0.05) were found between different explanatory variables, those effects were tested
216 independently with Student t-test; otherwise, for multiple comparisons, Tukey's Honestly Significant
217 Differences test was used. Moreover, to assess plates density effect on those variables (i.e. Spat
218 density, average biometry traits and individual length) a comparison was made between HDP (black

219 and horizontal) and black horizontal NDP with t-test. HDP was compared to shade-mesh collector as
220 well to see if it could be a potential alternative using t-test. Finally, Kendall's correlation was
221 determined between mean length and weight of spat per device and was 0.7229 ($P < 0.0001$). Thus, we
222 will present in this study only the results of mean spat length, as it was individual measures, *i.e.*
223 statistics took into account individual variability, and the differences between devices were similar for
224 the two variables. The results were considered as significant when the P-Value was lower than 0.05.

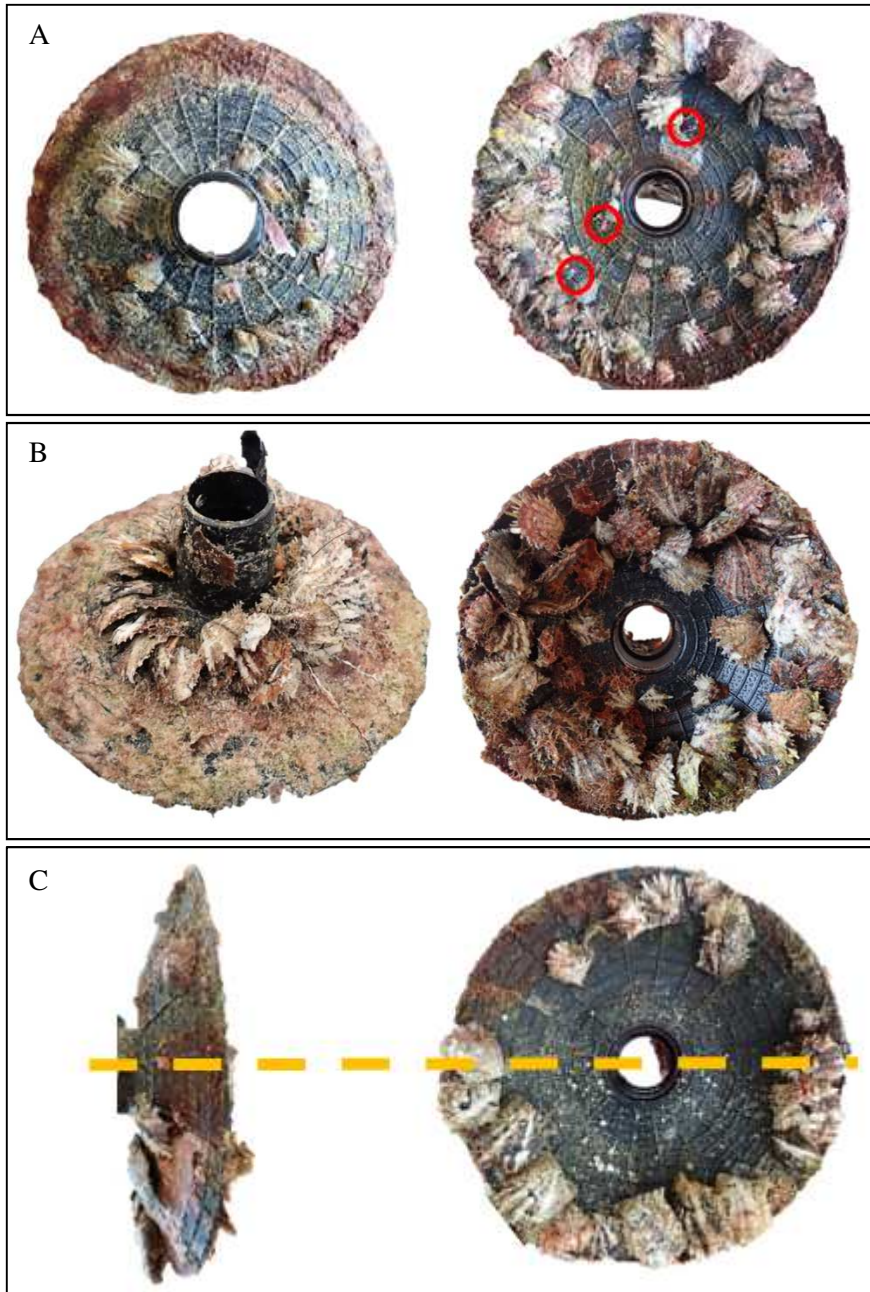
225

226 **3. Results**

227

228 A strong homogeneity was recorded in our observations, for each tested device. All spat (*P.*
229 *margaritifera* and *P. maculata*) on shade-mesh were mainly located in the center of the mesh whatever
230 its position. Conversely, for plate collectors, spat did not settle in the same place for each device. On
231 normal density plates (NDP) collectors, *Pinctada maculata* spat were mainly located on the edges and
232 *P. margaritifera* in the middle (Figure 2.A.). Spat were mainly on the concave side. On half density
233 plate (HDP) collectors, all spat on the convex side were located in the center, close to the PVC tube,
234 and all over the concave side (Figure 2.B.). Figure 2. C. represents a picture of a vertical plate as it was
235 set in the sea. It shows that all spat were concentrated on the side less exposed to the sunlight
236 compared to the horizontal plates (Figure 2.A.).

237



238

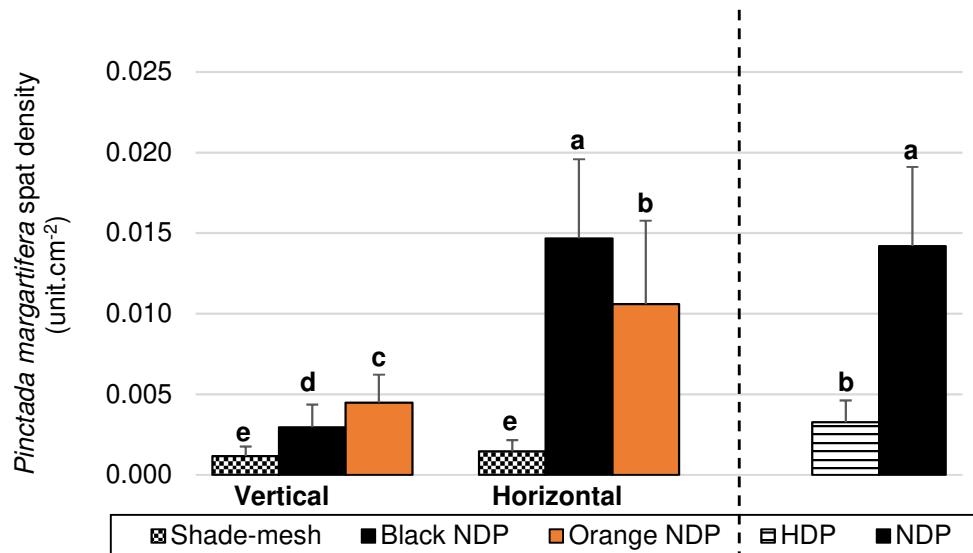
239 *Figure 2. From left to right pictures of (A) Convex (left) and concave horizontal black NDP, (B)*

240 *Convex and concave horizontal black HDP and (C) Convex (left) and concave vertical black plates*

241 *before retrieving spat. Spat circled in red are *Pinctada margaritifera* oysters, others are *P. maculata*.*

242 *The part of the plate above the orange dashed line in C is the one closest to the surface.*

243

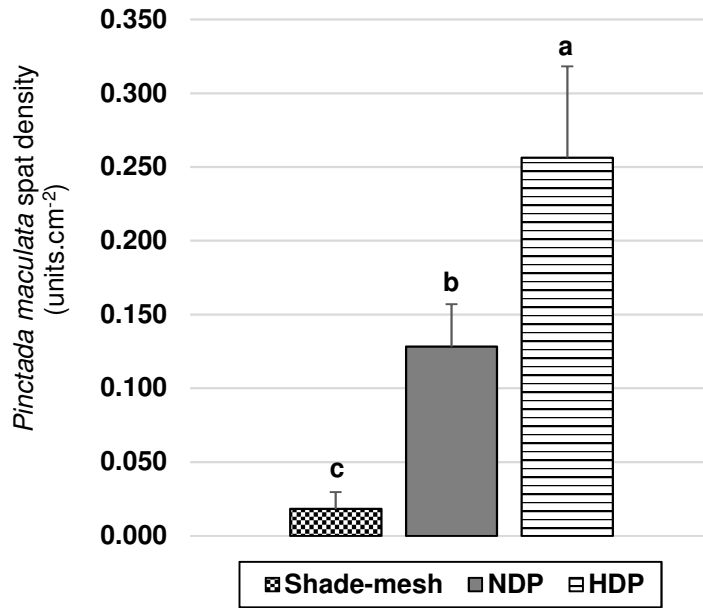


244
245

246 *Figure 3. Mean Pinctada margaritifera spat density (number of spats per cm²) for shade-mesh, orange*
 247 *NDP and black NDP device in function of the position of the collecting surface (left of the dashed line)*
 248 *and for black NDP and HDP (right of the dashed line). Error bars denote standard deviations. Within*
 249 *one graphic, devices with different letters show significant differences (P < 0.05).*

250

251 Mean density of spat of *Pinctada margaritifera* is shown in Figure 3. Shade-mesh collectors
 252 collected less than the others regardless of collecting surface position and plate color with a mean of
 253 0.0012 (\pm 0.0006) to 0.0015 (\pm 0.0007) spat.cm⁻² (T-test, P < 0.001). Black NDP also significantly
 254 recruited less in vertical position compared to orange NDP with 0.0030 (\pm 0.0014) and 0.0045 (\pm
 255 0.0017) spat.cm⁻² respectively (T-test, P= 0.0049). However, in horizontal position both NDP
 256 collectors recruited significantly more spat than in vertical position (Tukey, P < 0.0001), black NDP
 257 presenting the significantly highest mean of 0.0147 (\pm 0.0049) spat.cm⁻² (T-test, P= 0.0031) (Figure
 258 3). Yet position did not significantly affect shade-mesh recruitment (T-test, P= 0.0896). For HDP, the
 259 recruitment was significantly lower than NDP with only 0.0033 (\pm 0.0014) spat.cm⁻² (Kruskal Wallis,
 260 P < 0.0001) (Figure 3, right). Nonetheless, HDP still collected significantly more spat than shade-mesh
 261 collectors (T-test, P < 0.0001).



262

263 *Figure 4. Mean density of Pinctada maculata spat in function of type and density of collector. Error*
 264 *bars denote standard deviations. Devices with different letters show significant differences (P<0.05).*

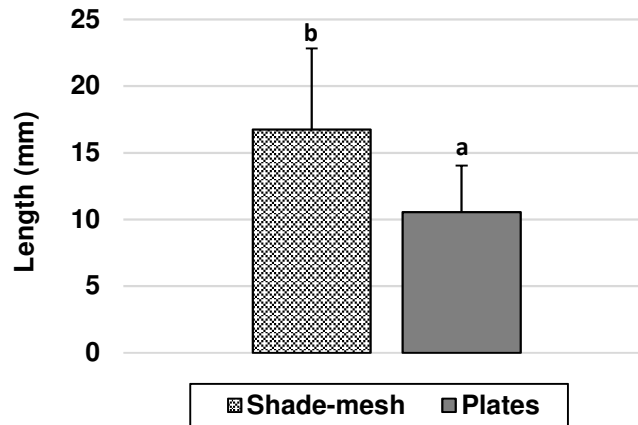
265

266 No differences of density of *Pinctada maculata* were found when color or position were
 267 compared (Anova, P> 0.05). However, it was significantly lower for shade-mesh type (Anova, P<
 268 0.0001), with 0.0182 (± 0.0115) spat.cm⁻² than for plate collectors. Likewise, it was significantly lower
 269 for NDP than for HDP with 0.1283 (± 0.0288) and 0.2563 (± 0.062) spat.cm⁻² respectively (Kruskal
 270 Wallis, P<0.0001) (Figure 4).

271

272 Furthermore, mean length of *Pinctada margaritifera* spat was significantly higher for shade-
 273 mesh collectors with 16.74 (± 6.08) mm than the plates type with 10.54 (± 3.49) mm (Anova, P <
 274 0.0001) (Figure 5). No other significant length differences were shown between the other devices
 275 (Anova, P > 0.05).

276



277

278 *Figure 5. Mean Pinctada margaritifera length in millimeters per type of collector at the end of the experiment.*

279 *Error bars denote standard deviations. Devices with different letters show significant differences ($P < 0.05$,*

280 *Anova).*

281

282 4. Discussion

283

284 In this study, despite the variability of *Pinctada margaritifera* spat density between collectors

285 of the same device, our results showed that spats were collected more efficiently when using black

286 horizontal plates. They also highlighted the influence of the type of collector, its position and its color.

287 Many studies have demonstrated that spat of various oyster species such as *Ostrea edulis*, *Pinctada*

288 *martensii* and *Pinctada maxima* preferentially settle on rigid support with a rough and textured surface

289 (Galtsoff, 1964; Pearce and Bourget, 1996; Taylor et al., 1998; Filgueira et al., 2007; Su et al., 2007;

290 Wang et al., 2017). Our results are also consistent with previous studies performed on *Pinctada*

291 *margaritifera* spat settlement (Gervis and Sims, 1992; Ehteshami et al., 2011; Ishengoma et al., 2011;

292 Libini et al., 2013). Even if both collectors have a rough or filamentous texture, plates are much more

293 rigid than shade-mesh. It may have helped spat to get a firmer grip on the collectors as was suggested

294 by Ehteshami et al. (2011), hypothesising that it would allow spat to place its byssal threads in a radial

295 pattern providing a stronger attachment compared to filament. Furthermore, the improvement of

296 collecting efficiency with the plate collector compared to shade-mesh could also be due to better

297 protection against predation due to its rigidity and conic shape. Moreover, the incidence of a specific

298 water flow on settlement has been underlined by various studies (Arakawa, 1990; Pearce and Bourget,

299 1996; Taylor et al., 1998; De La Roche et al., 2005). These two characteristics may have induced a
300 water flow that facilitated the access of spat to settlement support, compared to filamentous shape,
301 leading to better settlement efficiency as well.

302 In addition, our study supported the hypothesis of negative photo-taxis of *Pinctada*
303 *margaritifera* spat previously described by different authors for various oyster species (Gervis and
304 Sims, 1992; Holliday, 1996; Taylor et al., 1998; Saucedo et al., 2005; Su et al., 2007; Ehteshami et al.,
305 2011; Wang et al., 2017). These authors pointed out that spat preferred to settle on dark color
306 collectors and on substrate less exposed to light. In our experiment, we observed a higher settlement
307 rate with the horizontal position of plates which provides more shade than the vertical position. We
308 also observed that, in this position, spat mainly colonized the convex underside of the plates which is
309 less exposed to light than concave side. Similarly, spat on vertical plates were also mainly located on
310 the part less exposed to light as shown in the photos of collectors. Moreover, spat were principally
311 settled next to the PVC tube on the concave side. Thus, our results seem consistent with a high photo-
312 sensitivity of *P. margaritifera* spat. On the other hand, as described by Galtsoff (1964) and Taylor et
313 al. (1998), the two positions of plates induced a water flow dissimilarity that could also be responsible
314 for the difference of spat recruitment between them. Conversely, position did not significantly affect
315 collection efficiency of the shade-mesh collector. One assumption might be that space between plastic
316 filaments of the shade-mesh does not significantly change water flow between positions and lets the
317 light pass through so no part of it is more shaded than another.

318 Furthermore, different studies indicated that the color of collecting substrate has an influence
319 on settlement of larvae of *Pinctada* spp. including *Pinctada margaritifera* (Taylor et al., 1998;
320 Saucedo et al., 2005; Su et al., 2007; Arini and Jaya, 2011; Libini et al., 2013; Wang et al., 2017).
321 They suggested that black or dark colored substrates seem to be preferred by larvae for settlement. In
322 our case, we cannot draw any firm conclusions on color effect as it interacted directly with position
323 presenting opposite results when comparing the number of spat on orange and black plates (*i.e.* light or
324 dark color) for the two positions. In vertical position, orange plates had significantly higher density of
325 settled *Pinctada margaritifera* spat than black ones and vice-versa when horizontal. Libini et al.
326 (2013) also showed that black collectors collected more spat than others. However, yellow ones also

327 collected more than green or brown collectors, which are darker. Moreover, Ompi et al. (2018) also
328 studied settlement of *Pinctada maxima* spat on different substrates and did not find any effect of color.
329 This demonstrates how important interactions between factors are, notably, depending on the type of
330 collector, its position and color that should be considered with regard to the shade they will provide. In
331 our study, for the plate collector, position seemed to be the most important factor to attract *P.*
332 *margaritifera* spat compared to color, as it led to higher number of settled spat per collector when
333 plates were horizontal, regardless of color.

334 Our experiment also demonstrated that increasing space between plates, by removing one out
335 of two, significantly reduced the density of *Pinctada margaritifera* spat collected. The observed
336 difference could be explained by a rise in predation activity due to the increased space. In these atolls,
337 predators are various species of gastropods, crabs, worms and fishes (Coeroli et al., 1984; Gervis and
338 Sims, 1992; Friedman et al., 1998; Humphrey, 2008; Kishore et al., 2018). However, in Takapoto
339 lagoon, Coeroli et al. (1984) pointed out that fishes (Balistidae and Tetraodontidae) constitute the main
340 pearl-oyster predators. We may assume that a 2.5 cm space was too small to leave them access to *P.*
341 *margaritifera* spat, particularly with *Pinctada maculata* on the edges of plates, as shown in the photos,
342 but that a 5 cm space was sufficient. Moreover, increasing space may have also led to a stronger water
343 flow that could have made it harder for *P. margaritifera* larvae to settle (Galtsoff, 1964; Arakawa,
344 1990; Taylor et al., 1998; De La Roche et al., 2005). However, our results showed that increasing
345 space between plates also increased two-fold the density of *Pinctada maculata* spat collected
346 compared to NDP. Thus, the difference of density of *P. margaritifera* could also be due to more
347 intensive competition for space and food between species, *P. maculata* settling on nearly the whole
348 surface area of HDP and not leaving any space for *P. margaritifera* to settle.

349 Furthermore, NDP also seemed to be more efficient for the collection of *Pinctada maculata* spat
350 than the shade-mesh collector. There was 6 times more spat on them than on the shade-mesh collector,
351 which means that this shape seems more suitable for their settlement. Friedman and Bell (1996) also
352 studied the effect of different substrata on settlement of *P. margaritifera* and *P. maculata* in Solomon
353 Islands. One of the collectors tested was the black shade-mesh collector and the other was black plastic
354 sheeting (0.5 mm thick) threaded on a rope, and both were either 'bagged' or 'open'. As in our study,

355 their results showed that spat of both species were collected together with a positive Pearson
356 correlation of 0.62 between the number of spat of *P. margaritifera* and *P. maculata*. However, *P.*
357 *margaritifera* preferred to settle on shade-mesh collectors without distinction of whether settlement
358 supports were bagged or open whereas *P. maculata* spat preferred to settle on open collectors
359 regardless of the collector material. Their hypothesis was that these spat seemed to search for
360 maximum water exchange. They explained that the looser texture of the open plastic sheeting collector
361 seemed to allow *P. maculata* spat to maintain a strong water exchange. Similarly, they indicated that
362 those that settled on shade-mesh were usually found on the edges which also enabled them to have
363 better water exchange. Likewise, in our case, plate shape may have offered *P. maculata* spat better
364 water exchange than the shade-mesh filamentous shape with a different water flow. Moreover,
365 increasing space between plates must have provided a better water exchange than NDP too.
366 Interestingly, *P. maculata* were also usually found on the edges of plates which tends to confirm the
367 hypothesis of Friedman and Bell (1996). In addition, unlike *P. margaritifera*, there was no incidence
368 of position or color of plates. This could mean that *P. maculata* larvae are less photosensitive than
369 black-lip pearl-oyster spat, less preyed on or too numerous in the lagoon for it to be shown. *P.*
370 *maculata* oysters have long been considered as biofouling and were mainly thrown into the lagoons
371 (Murzilli et al., 2012). This probably led to the development of *P. maculata* oyster beds and to the
372 extensive spread of the population in the lagoons. The number of *P. maculata* collected on plate
373 collectors raises the question of the suitability of this type of collector. The majority of pearl farmers
374 usually consider *P. maculata* as a pest because this species is in direct competition for space and food
375 with *P. margaritifera* (Addessi, 1999). However, if we compare the abundance of *P. margaritifera* and
376 *P. maculata* measured during our study on shade mesh collectors to those obtained for the black
377 horizontal plate collector, the conclusion is the opposite. On this plate collector, we found 9.2 times
378 more *P. maculata* spat than *P. margaritifera* spat whereas on shade-mesh collector this ratio is 14.
379 Likewise, when we compare the two devices, there are 9 times more *P. margaritifera* on plates than on
380 the shade-mesh collector whereas there are only 6.1 times more *P. maculata*. All these findings clearly
381 show that the shade-mesh collector tended to collect more *P. maculata* proportionally to *P.*
382 *margaritifera* than black horizontal NDP.

383 Lastly, our results showed that mean length of *P. margaritifera* spat was lower on plates after
384 four months of collection than on shade-mesh without showing any effect of color, density of plates or
385 position. Thus, only the collector shape seemed to have an influence on this biometric trait. It is
386 important to point out that the variable of mean length presented in this paper cannot be assimilated to
387 the measurement of the growth of the collected spat. *Pinctada margaritifera* is known to spawn all
388 year long (Gervis and Sims, 1992). This means that, in our study, there might have been several
389 recruitment 'waves' (e.g cohort) leading to a high variability of age and size of oysters on one
390 collector. It seems that plate shape was more suitable for successive settlements. In contrast, the
391 settlement of oysters appears limited when biofouling covered the shade-mesh filamentous shape.
392 However, this length difference is also likely due to the higher number of spat collected by the plate
393 collectors. A negative relationship between growth and number of individuals per area has been
394 described by Filgueira et al. (2007) on Galician mussel, which has been highlighted by other authors
395 as well (Fréchette et al., 1996; De La Roche et al., 2005; Guíñez, 2005). They explained that it induces
396 space competition and food limitation which could affect growth. In our case, space and food
397 competition with *P. maculata* could also explain the difference recorded (Addessi, 1999). Biofouling
398 development could also influence *Pinctada margaritifera* growth by reducing water flow and oxygen
399 availability (Taylor et al., 1997; Pit and Southgate, 2003). Thus, further studies are needed to better
400 understand the factors that control the spat recruitment and growth on the collectors. Moreover, longer
401 tests over the current collection period (12 to 24 months) are needed to conclude on the eventual
402 impact of spat length collected with plates on pearl-farming production and farmers' practices.

403 In conclusion, our study showed that black horizontal NDP could be a good alternative to
404 shade-mesh collectors when focusing on short-term collection performance. However, many factors
405 need to be taken in account before validation of this potential alternative. They concern environmental
406 impact, practicality for pearl-farmers, and cost-benefit analysis. Plate collectors are made of plastic, so
407 the quantity of plastic waste produced after several years of use is of importance. However, from our
408 results on spat collection, initial calculations can be performed. Here, if we consider the longevity of a
409 plate collector as around 6 years because they will be immersed practically continuously (Table 1),
410 annual plastic waste production by shade-mesh collector expressed in relation to its efficiency (*P.*

411 *margaritifera* spat collection) is 1.715 g.spat⁻¹ while black horizontal NDP will produce only 1 g.spat⁻¹.
 412 But if we estimate the cost per spat collected, then black horizontal NDP appears more expensive, at
 413 0.0172 €, compared to the shade-mesh collector (0.0123 €). These values represent only rough
 414 estimates, as other costs should be taken into account such as material costs (buoys to maintain the line
 415 afloat, the plate collector being heavier) and operating costs (transportation constraints of plate
 416 collectors, which are more voluminous, fuel, etc.). For these additional costs, the efficiency of the
 417 collectors in spat collection is also to be considered (e.g. twelve times fewer plate collectors needed to
 418 obtain the same amount of *P. margaritifera* spat), as it could counterbalance the final production cost
 419 per spat. So additional studies, involving economists, are needed to conclude on the
 420 affordability/feasibility of a change in practices for pearl farmers. This will be the next step of our
 421 approach.

422

423 *Table 1: Comparison elements of black horizontal NDP collector and shade-mesh collector after 4 months of*
 424 *immersion*
 425

	Black horizontal NDP	Shade-mesh
Collection surface (cm ²)	12488	12833
Cost tax included (€)	18.96	0.59
Plastic weight (g)	1100	77
Collector longevity (year)	6	3
Plastic waste produced per year (g)	183.3	25.67
<i>P. margaritifera</i> spat numbers per collector	183	16
<i>P. margaritifera</i> spat density (unit.cm ⁻²)	0.0147	0.0012
Plastic waste per <i>P. margaritifera</i> spat collected per year (g)	1	1.715

426

427 **Author contribution**

428 **M. Crusot:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – Original
 429 draft, Writing – review & editing, Visualization, Project management.

430

431 **C. Lo:** Conceptualization, Methodology, Writing – review & editing, Visualization, Funding acquisition.

432

433 **N. Gaertner-Mazouni:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing
434 – Original draft, Writing – review & editing, Visualization, Supervision, Project administration,
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442

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