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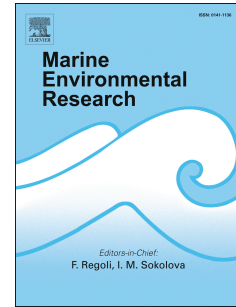
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1 Meiofauna distribution in a mangrove forest exposed to shrimp farm effluents (New Caledonia).

2

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4

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12 **Abstract**

13 Meiofauna abundance, biomass and individual size were studied in mangrove sediments subjected to  
14 shrimp farm effluents in New Caledonia. Two strategies were developed: i) meiofauna examination  
15 during the active (AP) and the non-active (NAP) periods of the farm in five mangrove stands  
16 characteristics of the mangrove zonation along this coastline, ii) meiofauna examination every two  
17 months during one year in the stand the closest to the pond (i.e. *Avicennia marina*). Thirteen  
18 taxonomic groups of meiofauna were identified, with nematodes and copepods being the most  
19 abundant ones. Meiofauna abundance and biomass increased from the land side to the sea side of the  
20 mangrove probably as a result of the increased length of tidal immersion. Abundance of total  
21 meiofauna was not significantly different before and after the rearing period. However, the effluent-  
22 receiving mangrove presented twice the meiofauna abundance and biomass than the control one.  
23 Among rare taxa, mites appeared extremely sensitive to this perturbation.

24

25 Key words: mangrove, meiofauna, shrimp farming, environmental status, New Caledonia

26

## 27 **1 Introduction**

28           In New Caledonia, shrimp ponds cover 680 ha, producing ~ 2,000 metric tons of shrimps per  
29 year (Della Patrona and Brun, 2009). In contrast to other parts of the world, farms are built on salt  
30 flats, developing upstream the mangrove forests, and there were no direct losses of mangroves due to  
31 pond construction. However, pond effluents are discharged into the adjacent mangroves, considered to  
32 be a “natural biofilter” that can reduce or eliminate impacts on the surrounding World Heritage listed  
33 lagoon and coral reef (Thomas et al., 2010; Molnar et al., 2013). The impact and fate of shrimp farm  
34 effluents in mangrove ecosystems can be studied using physico-chemical parameters, nutrients  
35 concentration, quantity and quality of organic matter. (McKinnon et al., 2002; Costanzo et al., 2004;  
36 Lacerda et al., 2006; Mirto et al., 2007; Pusceddu et al., 2008). Benthic organisms, which are sensitive  
37 to physical, chemical and biological disturbances, can also act as relevant ecological indicators of the  
38 status of the receiving ecosystem (Lamparadariou et al., 2005). Actually, benthic trophic status based  
39 on organic matter variables is not sufficient to provide a sound assessment of the environmental  
40 quality of the ecosystem, which can be obtained combined with a study on meiofaunal variables  
41 (Bianchelli et al., 2016). Meiofauna has been used as ecological descriptors in numerous studies  
42 dealing with the impact of fish farms (Vezzulli et al., 2008; Grego et al., 2009; Mirto et al, 2010; 2012,  
43 2014; Bianchelli et al., 2016) and to a lesser extent of mussel farms (Mirto et al ., 2000; Danavoro et  
44 al., 2004), oyster farms (Castel et al., 1989; Dinet et al., 1990), and algae farms (Olafsson et al., 1995).  
45 The general outcome from the literature is that aquaculture farms biodeposition typically alter  
46 meiofaunal abundance, diversity, biomass and species composition. The disappearance of the rare  
47 taxa, representing <1% of the total meiofauna abundance, were usually also described under fish farm  
48 influence (Mirto et al., 2010).

49 To understand the putative impact on effluents on meiofauna variables in mangrove, one has also to  
50 understand the natural distribution of meiofauna in this specific ecosystem. However, few references  
51 were interested in meiofauna distribution along a tidal gradient under semi-arid climate (Debenay et  
52 al., 2015). Environmental cues such as temperature, salinity, length of tidal immersion, redox  
53 conditions and sediment grain size are the most important factors regulating the zonation patterns of  
54 meiofauna in mangrove estuaries (Alongi, 1987a,b; Anzari et al., 1993; Thilagavathi et al., 2011).

55 These parameters can vary according to the mangrove stand (Vanhove et al., 1992; Marchand et al.,  
56 2004; Chinnadurai and Fernando, 2007) and its position in the tidal zone that induces difference in  
57 waterlogging, leading notably to different pore water salinity (Marchand et al., 2011).

58 In the mangrove studied herein, the influence of the effluents on C, N, and P dynamic as well as on the  
59 physico-chemical characteristics of the sediment were already demonstrated (Molnar et al., 2013;  
60 2015; Aschenbroich et al., 2015). Organic matter exported from shrimp farm stimulated oxygen  
61 demand and nutrient regeneration rates. However, the major role of mangrove sediments was to  
62 process the effluent PON loads and to export them directly in dissolved forms to the surrounding  
63 lagoon waters, or indirectly by stimulating bacterial and phytoplankton biomass production. No sign  
64 of saturation, eutrophication or anoxia of the effluent receiving mangrove was observed. Thus, we  
65 suggested that the mangrove was only a partial filter for the shrimp farm effluent. In the specific  
66 context, our first hypothesis is that the abundance, biomass and composition of meiofauna collected in  
67 the effluents receiving mangrove were not severely affected by shrimp farm effluents. Our second  
68 hypothesis is that the distribution, abundance, individual weight and biomass of meiofauna taxa will  
69 differ between mangrove stands as a result of their specific physico-chemical properties.

70 Our objectives were thus: i) to assess the influence of shrimp farming effluents on meiofauna  
71 distribution, ii) to determine the influence of the mangrove stand on this distribution. To reach our  
72 goals, we developed two sampling strategies: i) collection of surface sediments during the active (AP)  
73 and non-active periods (NAP) of the farm in the different mangrove stands characteristic of zonation  
74 under semi-arid climate, ii) a one-year survey of the meiobenthos distribution in a stand where the  
75 effluents are released (i.e. *Avicennia marina*) compared to a control one. Abundance and biomass of  
76 meiobenthos were measured, as well as the Chl-a content of surface sediments. To our knowledge, this  
77 study is the first one using meiofauna as ecological indicators in mangrove sediments receiving shrimp  
78 farm effluents.

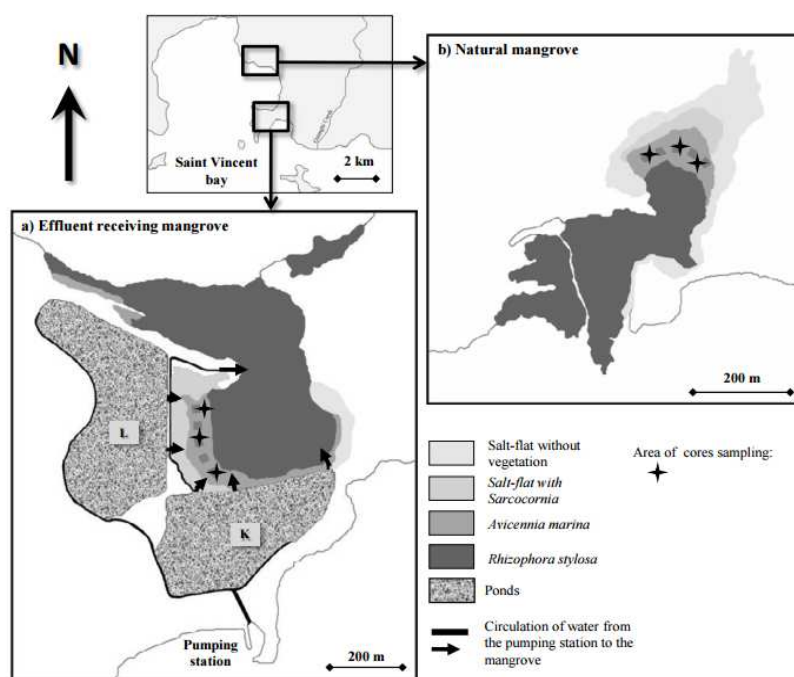
79

## 80 2 Material and methods

### 81 2.1 Study site and sampling strategy

82 The work was carried out in two mangroves of similar size located in Saint Vincent Bay  
 83 (Boulouparis, New Caledonia) that display the same mangrove zonation: i) at the back edge of the  
 84 mangrove swamp, the area is characterized by salt flats, a highly saline zone submerged only at high  
 85 spring tides and covered sparsely in the most downstream stretches with *Sarcocornia quinqueflora* and  
 86 *Suaeda australis* bushes; ii) a second stand of vegetation, downstream, is characterized by the  
 87 presence of *Avicennia marina*; iii) finally, the seaward edge is characterized by *Rhizophora*  
 88 *stylosatrees*, which are always submerged at high tide.

89 The control mangrove area (21°54'S, 166°04'E) covered 22 ha (Figure 1) is free from any aquaculture  
 90 or agriculture influences. The effluent-receiving mangrove (21°56'S, 166°04'E; of total area 28 ha,  
 91 located 2 km from the control mangrove) receives effluent discharges from the 2 ponds (K and L) of  
 92 the “Ferme Aquacole de la Ouenghi” shrimp farm (FAO).



93  
 94 **Figure 1** Map showing: i) the location of the effluent receiving (a) and control mangrove (b) in  
 95 Saint Vincent Bay (New Caledonia); ii) the effluent outlets: at the west and east side of the K and  
 96 L numbered Ponds (exposed site); iii) the locations of sampling sites b are symbolized by crosses.

97

98 Like the majority of shrimp farms in New Caledonia, FAO operates a semi-intensive rearing system.

99 Ponds were stocked with blue shrimp, *Litopenaeus stylirostris*, at an abundance of  $\sim 17 \text{ ind.m}^{-2}$  in

100 December 2008, and reared for  $\sim 8$  months. The shrimp were fed with locally produced feed pellets

101 (35-40% protein), which were added daily throughout the rearing period, with inputs increasing from

102  $\sim 0.25$  to  $\sim 3.5 \text{ kg.ha}^{-1}.\text{d}^{-1}$  over the rearing cycle as the shrimps grew. The volume of water discharged

103 into the mangrove corresponded to the volume of the daily water renewed, and increased progressively

104 with the growth of postlarvae and adult organisms from 0 to about 20% of the volume of the pond per

105 day. The ponds were drained in July 2009 after the last shrimp harvest and allowed to dry for a period

106 of about three to four months prior to the start of the next breeding cycle.

107 The effect of shrimp effluents on mangrove meiofauna was investigated by means of two

108 complementary approaches: dual-season spatial studies in the whole effluent-receiving mangrove and

109 one-year monitoring in the *Avicennia* stand both in control and effluent receiving mangroves.

110 The spatial studies were carried out in the mangrove areas adjacent to FAO during two distinct periods

111 of farm activity: the non-active period (NAP, November 2009) one month before the beginning of

112 rearing, and the active period (AP, June 2010) characteristic of breeding running at full load.

113 Forty-five geo-referenced samples were collected throughout the whole mangrove area, subdivided in

114 accordance with the objective of the study into five vegetation zones=stands in relation to their

115 different immersion time, roots systems and suspected effluent plume effect: n°1 salt flat "S", n°2 *A.*

116 *marina* "A", n°3 mixed zone harboring *A. marina* and *Rhizophora stylosa* "MAR", n°4 central zone

117 with *R. stylosa* "CR", and n°5 seaward edge with *R. stylosa* "ER".

118 Sediment samples were collected in triplicate for meiofaunal analysis by means of Plexiglas cores

119 (inner diameter 3.6 cm, corresponding to  $\sim 10.7 \text{ cm}^2$  surface area) to a depth of 2 cm. Sediment

120 samples were immediately fixed with buffered 4% formaldehyde solution until laboratory analyses

121 and stained with a few drops of Rose Bengal ( $0.5 \text{ g.l}^{-1}$ ).

122 In both *Avicennia* stands (control and effluent-receiving), eight sampling campaigns were conducted

123 from February 2009 to February 2010. Sampling campaigns were conducted to cover the entire

124 production cycle of the farm, with four campaigns during the rearing period, and four during the



125 “drying” period. Five sub-areas were defined for each *Avicennia* stand (effluent-receiving and  
126 control), and five replicates were collected in each sub-area. One replicate was obtained by pooling 5  
127 sub-samples.

128

## 129 **2.2 Analytical methods**

### 130 **2.2.1 Meiofauna analysis**

131 In the laboratory each sample was rinsed and filtered on 1000 and 45  $\mu\text{m}$  mesh sieves. The  
132 45 $\mu\text{m}$  mesh residue sieve was centrifuged three times in the Ludox HS40 ( $d = 1.15$ ). The animals were  
133 counted on a 200-wells glass plate and identified to major groups through an adequate detailed  
134 observation (microscopic ampliation or with a 80x binocular magnifier) according reference manuals  
135 (Higgins and Thiel, 1988; Giere, 1993). Meiofaunal biomass was estimated from size measurements of  
136 different animals. The length and width of up to 30 organisms per major taxon were measured using a  
137 dissecting microscope fitted with a micrometer scale. These measurements were used for further  
138 conversion into biomass, using the specific conversion factors for each taxonomic group following  
139 Wieser (1960) and Warwick and Price (1979) for nematodes, Warwick and Gee (1984) and Riemann  
140 et al. (1990) for copepods, Gradinger et al. (1999) for crustacean nauplii, Ruttner-Kolisko (1977) and  
141 Bottrell et al. (1976) for rotifers, and Guo et al. (2005) and Nozais et al. (2005) for the other groups.

142

### 143 **2.2.2. Chlorophyll a analysis**

144 Chl-a was extracted from freeze-dried sediments using a 93% methanol solution and their  
145 concentrations were determined fluorometrically (Yentsch and Menzel, 1963). The fluorometer used  
146 was a Turner Designs TD700 equipped with an optical kit n°7000-961 including an excitation filter of  
147 340-500 nm wavelength, and an emission filter up to 665 nm wavelength. Pigments in methanol were  
148 then excited in the fluorometer with a 450 nm wavelength beam of light and fluorescence emitted at  
149 664 nm. MPB is the microphytobenthic biomass ( $\text{mg Chl-a.m}^{-2}$ ), converted to autotrophic carbon ( $\text{mg}$   
150  $\text{C.m}^{-2}$ ) assuming a C:Chl-a ratio of 40:1 (de Jonge, 1980 in Nozais et al., 2005).

151

### 152 2.3 Statistical analysis

153 Principal component analysis (PCA) was used to analyze: i) the dual season spatial study data,  
154 in which observations (meiofauna abundance and biomass) are described by several inter-correlated  
155 quantitative dependent variables (i.e. spatial study, vegetation, period), ii) the one-year monitoring in  
156 effluent-receiving and controlled *A. marina* stand data (environmental status effect vs. control,  
157 campaign date).

158 PRIMER 6 software was used for multivariate analysis. Data matrices were used to create triangular  
159 similarity matrices, based on the Bray–Curtis similarity coefficient. Differences in meiofauna  
160 composition among factors were tested using one-way or two-ways analysis (as appropriate) of  
161 similarity (ANOSIM) and the statistical test was computed after 5,000 permutations. No  
162 transformation was applied to the data and factors used for analysis. Where differences in meiofauna  
163 composition were detected between factors (Status, date), similarity of percentage tests (SIMPER)  
164 were used to determine which meiofauna taxa drove the observed differences between the two sets of  
165 data. Differences in abundance of meiofauna between sampling times and vegetation stands were  
166 tested using analysis of variance. Prior to ANOVA, Chl-a data were  $\log(x+1)$  transformed and all data  
167 were tested for homoscedasticity (Bartlett test) and normal distribution (Shapiro–Wilk). Tukey's HSD  
168 post-hoc tests were then used to determine differences between groups. Chl-a data were, first,  
169 analyzed by a non-parametric Kruskal-Wallis test, and then by a Wilcoxon test to compare mean  
170 values for pairs (control mangrove vs. effluent-receiving mangrove, between campaigns). For  
171 kinorhynchs and mites data homoscedasticity and normal distribution of residuals condition were not  
172 fulfilled. So kinorhynch and mites data were tested using a non-parametric test (Kruskal–Wallis test).  
173 Van Der Waerden test was used to convert the ranks from Kruskal-Wallis one-way analysis of  
174 variance to quantiles of the standard normal distribution called normal scores and the test was  
175 computed from these normal scores. Regression analysis were used to identify relationship between  
176 MPB (Microphytobenthos) and total meiofauna biomass. All these tests were performed using the R  
177 version 2.9.0 2009 software and for all tests the probability  $\alpha$  was set at 0.05. The initial hypothesis  $H_0$   
178 (means of the groups are equal to one another) is rejected if the p-values  $< \alpha$  i.e. at least one group is  
179 different from the other one.

180

181 **3. Results**182 **3.1. Dual-season spatial study in the different stands of the effluent-receiving mangrove**183 **3.1.1. General characteristics of meiofauna distribution**

184 Within the surface sediment of the mangrove receiving shrimp farm effluents, a total of 13  
 185 taxonomic groups of meiofauna was identified during the two spatial studies carried out in November  
 186 2009 and June 2010 (Table 1).

187 **Table 1 Mean abundances (Nb x 10 cm<sup>2</sup> ± Standard Deviation) of meiobenthic taxa recorded**  
 188 **during Non-Active and Active Periods in all (global) and different mangrove stands affected by**  
 189 **shrimp farm effluents for twenty-five years. “S” salt-marsh, “A” *A.marina*, ”MAR” mixed zone**  
 190 **harboring *A. marina* and *Rhizophora stylosa*, “CR” central zone with *R. stylosa* and “ER”**  
 191 **seaward edge with *R. stylosa*.**

Taxa	Non Active Period (NAP)									
	S		A		MAR		CR		ER	
	Abund.	S.D	Abund.	S.D	Abund.	S.D	Abund.	S.D	Abund.	S.D
Amphipoda	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.2 ± 0.8	0.2	2.3 ± 5.4	2.3
Bivalvia	0.0 ± 0.0	0.0	0.4 ± 1.0	1.0	0.4 ± 1.0	1.0	0.0 ± 0.0	0.0	2.3 ± 3.1	3.1
Copepoda	17.4 ± 32.6	32.6	45.3 ± 33.4	33.4	54.6 ± 66.8	66.8	52.4 ± 48.2	48.2	72.3 ± 98.3	98.3
Gastropoda	2.5 ± 5.5	5.5	0.1 ± 0.3	0.3	0.0 ± 0.0	0.0	0.3 ± 0.5	0.5	2.1 ± 2.1	2.1
Halacaroida	0.9 ± 1.5	1.5	0.3 ± 0.7	0.7	0.6 ± 1.1	1.1	0.8 ± 1.1	1.1	1.3 ± 2.0	2.0
Kinorhyncha	0.2 ± 0.5	0.5	0.6 ± 1.2	1.2	0.1 ± 0.3	0.3	0.5 ± 1.9	1.9	5.1 ± 11.6	11.6
Crustacean nauplii	23.6 ± 37.4	37.4	4.1 ± 4.7	4.7	16.9 ± 28.4	28.4	4.0 ± 9.8	9.8	19.0 ± 27.5	27.5
Nematoda	584.7 ± 588.7	588.7	761.7 ± 411.3	411.3	881.8 ± 697.7	697.7	932.1 ± 454.8	454.8	1255.1 ± 414.2	414.2
Oligochaeta	1.3 ± 1.7	1.7	7.8 ± 18.8	18.8	1.1 ± 1.7	1.7	2.3 ± 3.4	3.4	7.3 ± 5.9	5.9
Ostracoda	1.8 ± 3.9	3.9	1.1 ± 1.5	1.5	0.3 ± 0.4	0.4	0.4 ± 1.5	1.5	2.1 ± 2.9	2.9
Polychaeta	0.5 ± 0.7	0.7	5.4 ± 12.4	12.4	5.9 ± 15.6	15.6	12.0 ± 17.9	17.9	51.1 ± 44.8	44.8
Rotifera	1.5 ± 3.3	3.3	19.1 ± 33.7	33.7	6.0 ± 13.3	13.3	0.6 ± 1.4	1.4	0.3 ± 0.4	0.4
Tardigrada	53.2 ± 116.9	116.9	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0
Turbellarians	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0

192

193

Taxa	Active Period (AP)									
	S		A		MAR		CR		ER	
	Abund.	S.D	Abund.	S.D	Abund.	S.D	Abund.	S.D	Abund.	S.D
Amphipoda	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.3 ± 0.9	0.9	0.0 ± 0.0	0.0	0.8 ± 1.7	1.7
Bivalvia	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	2.6 ± 4.4	4.4
Copepoda	24.3 ± 32.2	32.2	58.1 ± 36.6	36.6	44.4 ± 77.3	77.3	37.7 ± 37.4	37.4	79.7 ± 41.3	41.3
Gastropoda	0.4 ± 0.6	0.6	0.4 ± 0.5	0.5	0.2 ± 0.5	0.5	0.2 ± 0.5	0.5	2.7 ± 4.4	4.4
Halacaroidea	1.0 ± 1.7	1.7	0.8 ± 0.9	0.9	0.2 ± 0.5	0.5	0.5 ± 1.2	1.2	2.9 ± 3.5	3.5
Kinorhyncha	0.0 ± 0.0	0.0	0.1 ± 0.4	0.4	0.5 ± 1.3	1.3	0.1 ± 0.6	0.6	15.0 ± 23.7	23.7
Crustacean nauplii	101.1 ± 114.0	114.0	18.6 ± 30.3	30.3	13.6 ± 25.9	25.9	0.5 ± 1.0	1.0	6.3 ± 8.9	8.9
Nematoda	235.0 ± 268.2	268.2	656.1 ± 565.9	565.9	535.6 ± 55.8	55.8	727.7 ± 389.6	389.6	1798.8 ± 1143.0	1143.0
Oligochaeta	0.1 ± 0.3	0.3	0.7 ± 1.4	1.4	0.6 ± 1.0	1.0	1.5 ± 2.4	2.4	4.5 ± 5.7	5.7
Ostracoda	62.4 ± 130.0	130.0	10.8 ± 20.6	20.6	2.9 ± 8.5	8.5	0.0 ± 0.0	0.0	6.3 ± 8.3	8.3
Polychaeta	2.0 ± 4.5	4.5	8.9 ± 12.9	12.9	1.4 ± 2.5	2.5	8.1 ± 14.7	14.7	49.9 ± 36.7	36.7
Rotifera	0.0 ± 0.0	0.0	2.8 ± 6.8	6.8	10.4 ± 24.7	24.7	1.3 ± 4.4	4.4	8.12 ± 15.6	15.6
Tardigrada	0.7 ± 1.1	1.1	0.3 ± 0.5	0.5	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0
Turbellarians	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.1 ± 0.3	0.3	0.1 ± 0.3	0.3

194

195

196 With regard to meiofauna abundance, PCA “inter” inertia was explained by spatial study (0.8%),

197 period (0.8%) and vegetation (15.2%). Both vegetation and period factors represent 22.8% of total

198 inertia. In terms of biomass, PCA “inter” inertia was explained by spatial study (2.3%), period (2.3%)

199 and vegetation (14.4%). Both vegetation and period represent 24.3% of total inertia (Figure 2).

200 Meiofauna abundance (ANOVA,  $p < 0.05$ ) and biomass (ANOVA,  $p < 0.05$ ) were significantly different

201 in the five mangrove stands. As expected, nematodes (70-94%) and copepods (3-8%) were the most

202 abundant taxa, with 500-1,500 ind.10 cm<sup>-2</sup> and 50-100 ind.10 cm<sup>-2</sup>, respectively. Bianchelli et al.

203 (2010) and Pusceddu et al. (2011) have used the term “rare meiofauna taxa” for taxa representing

204 &lt;1% of the total meiofauna abundance. Thus, seven groups belong to this category “rare taxa” in the

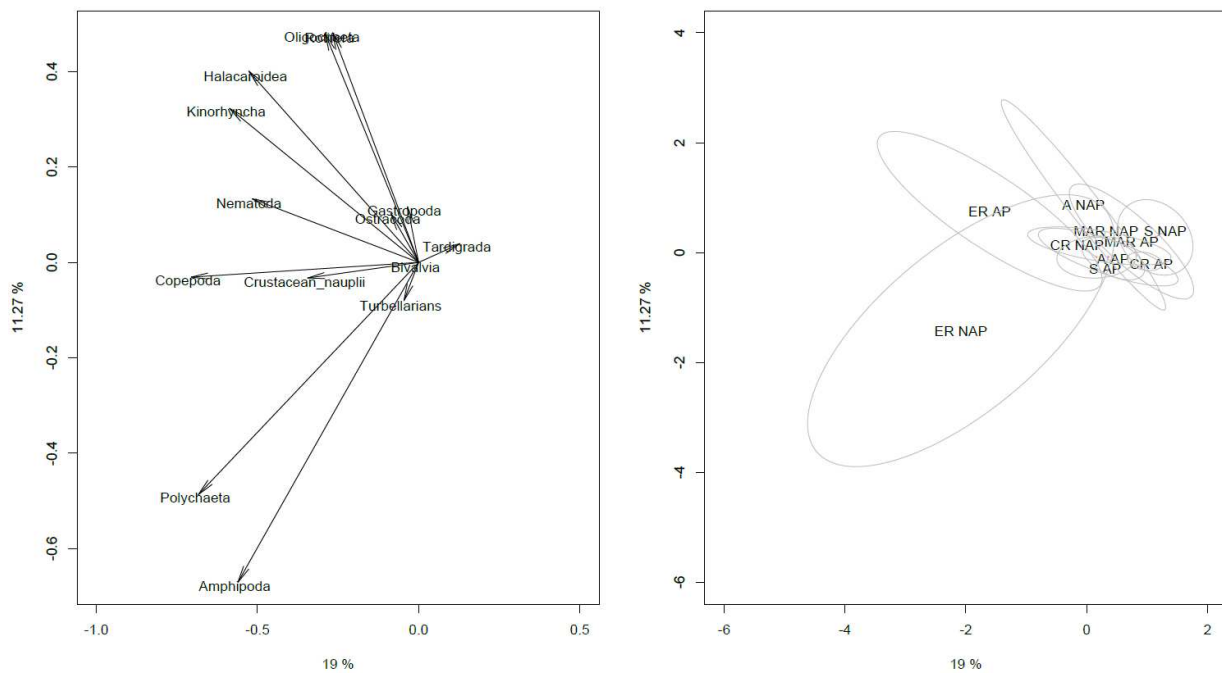
205 effluent-receiving mangrove: turbellarians, tardigrada, kinorhyncha, halacaroidea, gastropoda, bivalvia

206 and amphipoda whereas pygngonida has been found twice in one sample out of five.

207 Meiofauna abundance decreased from the land side to the sea side of the mangrove, the minimum

208 being in the “S” salt flat with 427 ind.10 cm<sup>-2</sup>, and the maximum in the “ER” seaward *Rhizophora*209 stand, with more than 1,420 ind.10 cm<sup>-2</sup>. The grey mangroves *A. marina*, “A”, the mixed grey and stilt210 mangrove, “MAR”, and the inner *R.stylosa*, “CR”, stands showed an abundance around 750 ind.10211 cm<sup>-2</sup>. Total meiofauna biomass exhibited similar spatial patterns as abundance.

212



213

214 **Figure 2 Principal Components Analysis (PCA) of the dual-season spatial study in effluent-**  
 215 **receiving mangrove stands using meiofauna biomass. Left panel: loadings representing the**  
 216 **extent to which the variables are correlated to principal components. Right panel: component**  
 217 **scores.**

218

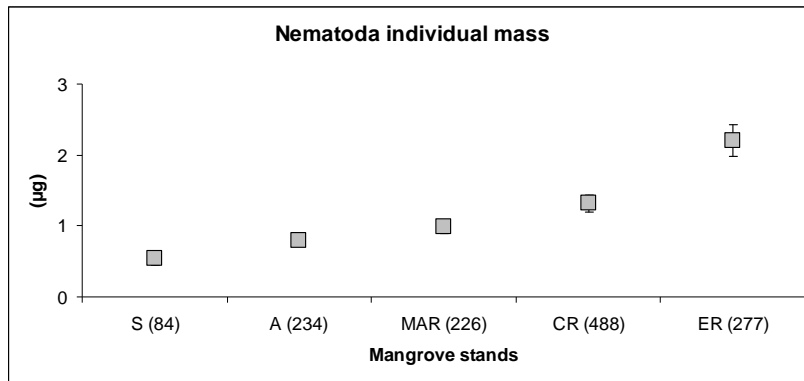
### 219 *3.1.1.1 Distribution of the most abundant taxa: nematodes and copepods*

220 Nematode and copepod abundance (ANOVA,  $p_{\text{Nem}} < 0.05$ ;  $p_{\text{Cop}} = 0.05$ ) and biomass  
 221 (ANOVA,  $p_{\text{Nem}} < 0.05$ ;  $p_{\text{Cop}} = 0.05$ ) were significantly different in the five mangrove stands  
 222 ( $p < 0.05$ ). Their abundance slightly increased towards the sea, i.e. from “S” to “ER”.

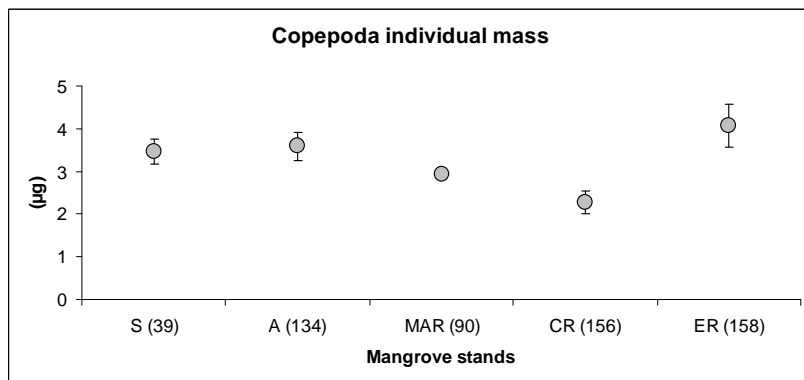
223 Nematoda represented the largest biomass (37-74%) of meiofauna present in all the mangrove stands.  
 224 With exception of 47% in salt flat “S” during AP, the proportion of copepods in terms of biomass was  
 225 about 30% in all the mangrove stands. Relative biomass contribution of polychaeta (third biomass  
 226 contributor) increased towards the sea, and was very significant in the outer stilt mangrove “ER” (17-  
 227 23%). Individual mass of nematodes also showed a remarkably progressive increase towards the sea  
 228 side, with individual mass increasing fourfold, from 0.5 to 2  $\mu\text{g}$  (Figure 3). Copepod individual mass  
 229 followed a different pattern. Individual mass around 3  $\mu\text{g}$  was observed in 4/5 vegetation types: salt

230 flat, grey mangrove, mixed grey and stilt mangroves, and seaward stilt mangrove, whereas smaller  
 231 specimens were observed in the inner stilt mangrove (Figure 3).

232



233



234

235 **Figure 3 Individual mass (µg) of nematodes and copepods in the different stands of the effluent-**  
 236 **receiving mangrove (mean ±S.D). “S” salt-marsh, “A” *A.marina*, ”MAR” mixed zone**  
 237 **harboring *A. marina* and *Rhizophora stylosa*, “CR” central zone with *R. stylosa* and “ER”**  
 238 **seaward edge with *R. stylosa*. (average ± SD); N (numbers of weighted specimens)**

239

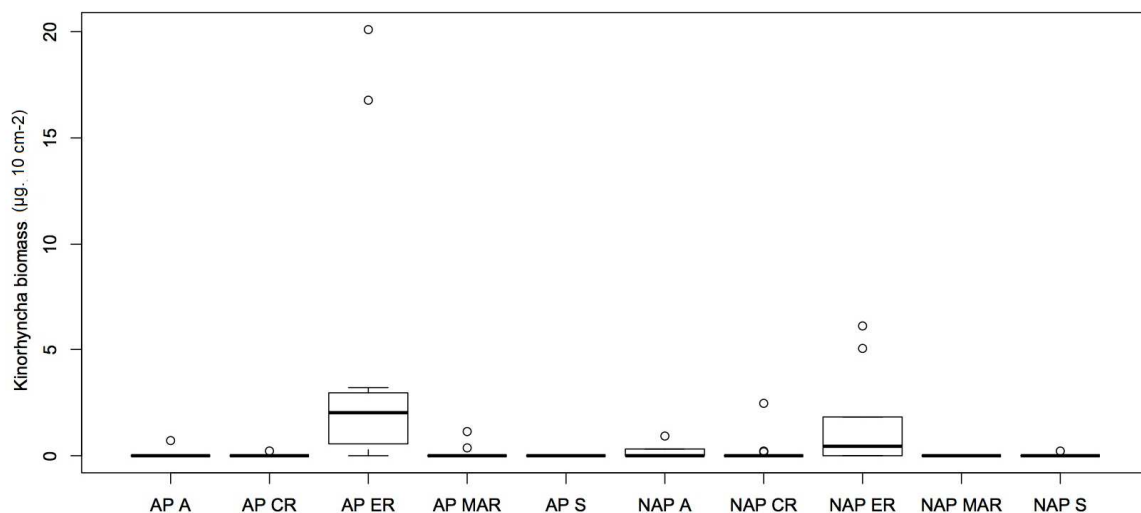
240

### 3.1.1.2 Distribution of rare taxa

241 Turbellarians have been found in ER in three samples out of five during AP. Tardigrada  
 242 (relative abundance =  $0.79 \pm SD 7.13\%$ ) seemed to be restricted to less flooded sediments i.e S and to a  
 243 lesser extent A sediments. They reached 53.2 and 0.7 ind.10 cm<sup>-2</sup> in S during non active period and  
 244 active period, respectively, and 0.3 ind.10 cm<sup>-2</sup> in A during the active period.

245 Amphipoda ( $0.00 \pm SD 0.03\%$ ) and bivalvia ( $0.06 \pm SD 0.21\%$ ) rarely exceeded 1.0 ind.10 cm<sup>-2</sup> and  
 246 have been found more abundant in sediments that are most often flooded (CR and ER). Halacaroidea

247 (mites) ( $0.06 \pm \text{SD } 0.13\%$ ) and gastropoda ( $0.04 \pm \text{SD } 0.40\%$ ) were ubiquitously collected in five  
 248 stands in very low abundance  $<3 \text{ ind } .10 \text{ cm}^{-2}$  whatever the period. In addition, anecdotal finding of  
 249 one pyngonida has been done once in ER in one sample out of five during NAP. Kinorhynchs  
 250 represented only  $0.16 \pm \text{SD } 0.52\%$  of the total meiofauna abundance. Kinorhynch abundance and  
 251 biomass were significantly different in the five mangrove stands (Kruskal-Wallis,  $p < 0.05$ ) with lowest  
 252 abundances in S, A, MAR, CR and highest in ER. They displayed their highest biomass in “ER” in  
 253 both spatial studies (van der Waerden test;  $\text{Chisq} = 41.83$ ;  $p.\text{chisq} = 3.52e-06$ ) (Figure 4). Same results  
 254 were obtained with their abundance (not shown). Mites abundance and biomass were significantly  
 255 different in the five mangrove stands ( $p < 0.05$ ) with lowest abundances in S, A, MAR, CR and highest  
 256 in ER (van der Waerden test;  $\text{Chisq} = 17.56$ ;  $p.\text{chisq} = 0.0015$ ).



257

258 **Figure 4 Importance of kinorhynchs biomass ( $\mu\text{g } 10 \text{ cm}^{-2}$ ) in different mangrove stands in both**  
 259 **spatial studies according van der Waerden test. vdW test identified 3 groups. AP-ER belongs to**  
 260 **1st group; NAP-ER belongs to 2nd group while other pairwise form “activity-stand” belong to**  
 261 **3<sup>rd</sup> and/or both two different groups. “S” salt-marsh, “A” *A.marina*, ”MAR” mixed zone**  
 262 **harboring *A. marina* and *Rhizophora stylosa*, “CR” central zone with *R. stylosa* and “ER”**  
 263 **seaward edge with *R. stylosa*. “AP” Active Period. “NAP” Non active period.**

264

265

266 **3.1.2. Meiofauna response to crop effluent pressure over an 8-month period in**  
267 **the effluent-receiving mangrove (NAP vs. AP)**

268 **3.1.2.1 Total meiofauna abundance**

269 Abundance of total meiofauna was not significantly different ( $p > 0.05$ ) before ( $1033 \pm \text{SD } 86$   
270  $\text{ind. } 10 \text{ cm}^{-2}$ ) and after ( $921 \pm \text{SD } 129 \text{ ind. } 10 \text{ cm}^{-2}$ ) farm activity (NAP vs. AP) in the whole  
271 mangrove ( $p > 0.05$ ) or in each stand separately ( $p > 0.05$ ). Among thirteen meiofauna groups  
272 determined during the two sampling seasons, ten, including the two major groups nematodes and  
273 copepods, showed similar abundance and similar distribution in the different mangrove stands over the  
274 two spatial studies. In addition, during the AP, the abundance of crustacean nauplii and ostracods was  
275 up to 8 times higher compared to the NAP in the stand the closest to the ponds: the salt-flat “S” and  
276 the grey mangrove “A”.

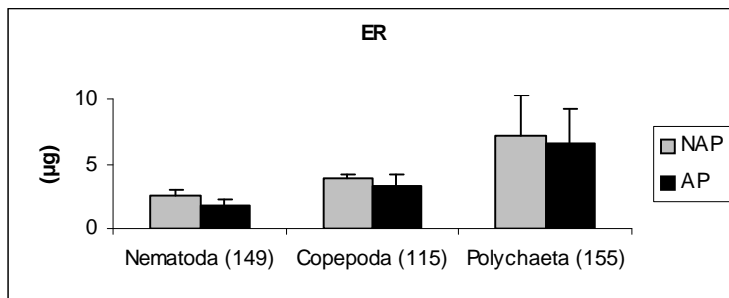
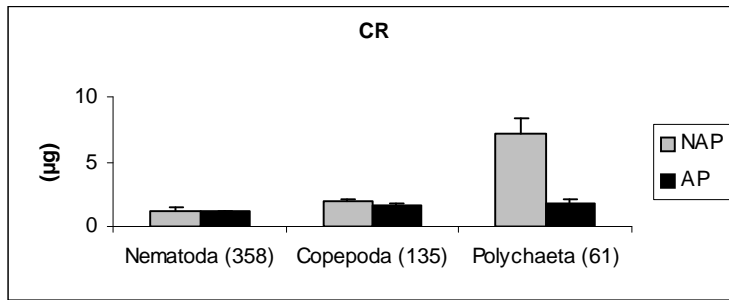
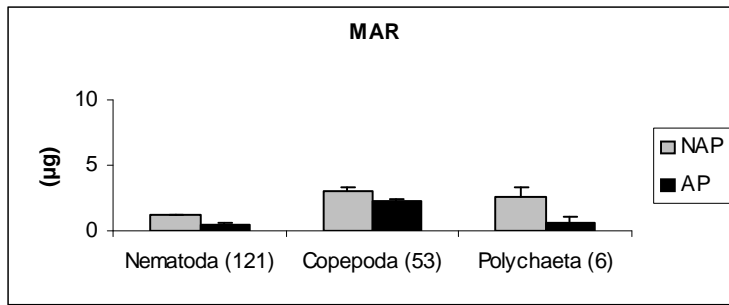
277

278 **3.1.2.2 Total meiofauna biomass**

279 Biomass of total meiofauna was significantly different before ( $635 \pm \text{SD } \mu\text{g } 10\text{cm}^{-2}$ ) and after  
280 ( $383 \pm \text{SD } 40 \mu\text{g } 10 \text{ cm}^{-2}$ ) farm activity (norma.residu.p.value= 0.84; bartlett.p.value= 0.19) in  
281 effluent-receiving mangrove (Stand: F.value =12.04; Pr..F.= 9.46e-08; Spatial study: F.value = 15.96;  
282 Pr..F.= 1.40e-04). During the NAP, meiobenthic biomass was up to 2 times higher compared to the AP  
283 in the CR (Tukey.p.value= 3.06e-02) and MAR ( $p = 8.49\text{e-}02$ ; n.s). Significant larger specimens of  
284 nematodes, copepods and polychaetes ( $p < 0.05$ ) were observed in “MAR”, “CR” and “ER” during non-  
285 active period NAP of shrimp farm waste release, partially explaining total meiofauna biomass  
286 difference (Figure 5).

287





291 Figure 5 Individual mass ( $\mu\text{g}$ ) of nematodes, copepods and polychetes in “MAR” mixed zone  
 292 harboring *A. marina* and *Rhizophora stylosa*, “CR” central zone with *R. stylosa* and “ER” seaward  
 293 edge with *R. stylosa* recorded during active period AP and non-active period NAP of shrimp farm  
 294 waste release. (average  $\pm$  SD); N (numbers of weighted specimens).

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### 3.1.2.3 Rare taxa

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There were three times more kinorhynchs in “ER” during the AP (Kruskal-Wallis for Spatial study/Vegetation,  $p < 0.05$ ). The abundance of waterbears (tardigrada) in “S” was 50 times higher during the NAP ( $p < 0.05$ ). Turbellarians, halacaroidea, gastropoda, bivalvia and amphipoda did not showed significant differences in their abundance during AP and NAP. Pygogonida were found in “ER” only during the NAP.

### 3.2. One-year monitoring in effluent-receiving and controlled *A. marina* stand

#### 3.2.1 Meiofauna

##### 3.2.1.1 Total meiofauna abundance in both control and effluent-receiving

##### *A. marina* stands

On average, mean total meiofauna abundance in the effluent-receiving mangrove stand ( $305.3 \pm \text{S.D } 38.3 \text{ ind.10 cm}^{-2}$ ) was twice the control mangrove stand ( $165.2 \pm \text{S.E } 29.1 \text{ ind.10 cm}^{-2}$ ), ( $p < 0.05$ ). With regard to meiofauna abundance, PCA "inter" inertia was explained by environmental status (3.7%) and campaign dates (15.2%). Both status and dates represent 30.2% of total inertia. In the sediment of the control *A. marina* stand, total meiofauna abundance values were fairly stable from February to June 2009, with an average value around  $50 \text{ ind.10 cm}^{-2}$ , without any significant differences during the 4 sampling campaigns (Wilcoxon Test,  $p > 0.05$ ). Then, abundance increased sharply until September, reaching a maximum of  $439.2 \pm \text{SD } 219.2 \text{ ind.10 cm}^{-2}$ . From September 2009 to November 2009, it decreased quickly and stabilized at values around  $125 \text{ ind.10 cm}^{-2}$  (24 November 2009 to 8 February 2010). In the sediment of the effluent-receiving mangrove, when the farm was active, total meiofauna abundance increased significantly from February ( $100.1 \pm \text{SD } 0.3 \text{ ind. } 10 \text{ cm}^{-2}$ ) to June 2009 ( $347.4 \pm \text{SD } 266.1 \text{ ind.10 cm}^{-2}$ ) and stabilized at around  $325 \text{ ind.10 cm}^{-2}$  from June to August ( $312.9 \pm \text{SD } 106.5 \text{ ind.10 cm}^{-2}$ ). After the final drain (August), i.e during the non-active period, abundance increased again and reached a maximum in September ( $538.9 \pm \text{SD } 285.8 \text{ ind.10 cm}^{-2}$ ). It then decreased sharply to stabilize at around  $300 \text{ ind.10cm}^{-2}$  (24 November 2009 to 8 February 2010).

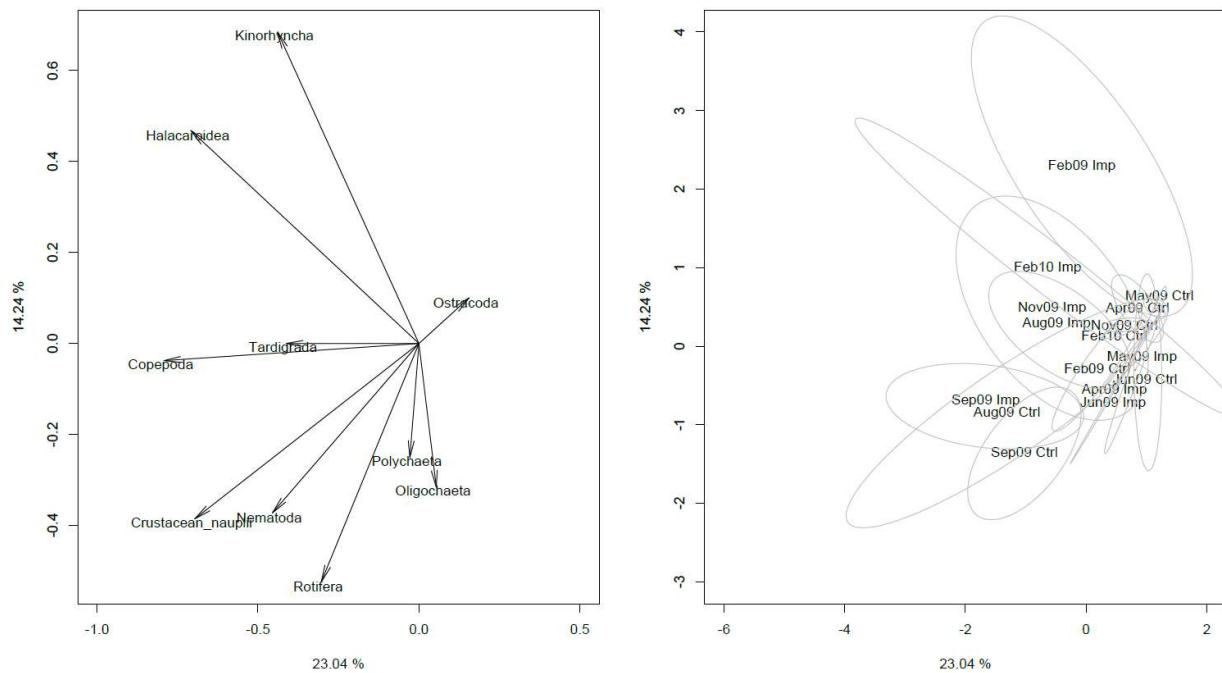
##### 3.2.1.2 Total meiofauna biomass in both control and effluent-receiving *A.*

##### *marina* stands

The difference was also significant with regard to biomass ( $p < 0.05$ ). On average, effluents-receiving *A.marina* sediments had a meiofaunal biomass twice as large as the control sediments with  $211.2 \pm \text{SD } 34.3$  and  $118.5 \pm \text{SD } 19.2 \mu\text{g.10cm}^{-2}$ , respectively. In terms of biomass, PCA "inter" inertia was explained by environmental status (3.0%) and campaign dates (17.6%). Both status and dates represent 30.7% of total inertia (Figure 6). Total meiofauna biomass differed significantly in

331 terms of Environmental status (F.value=15.75; Pr..F. 2.04e-04) and campaign date (F.value= 16.22;  
332 Pr.F= 1.54e-11) in both control and effluent-receiving *A.marina* stands. In the control mangrove, the  
333 total biomass was low and stable from February to June 2009 without any significant differences  
334 during the 4 sampling campaigns (Wilcoxon Test,  $p>0.05$ ), with values around  $35 \mu\text{g}.10\text{cm}^2$ . It then  
335 increased, peaking at  $302.7 \pm \text{SD } 91.0 \mu\text{g}.10\text{cm}^2$  in September, and eventually decreased to  $109.5 \pm$   
336  $\text{SD } 33.2 \mu\text{g} .10\text{cm}^2$  in February 2010. In the sediment of the effluent-receiving mangrove, when the  
337 farm was active, total meiobenthic biomass values were fairly stable from February to June, with an  
338 average value around  $90 \mu\text{g}.10 \text{ cm}^2$ , without any significant differences during the 4 sampling  
339 campaigns (Wilcoxon Test,  $p>0.05$ ). In August after the final drain, the biomass increased, reaching  
340  $282.4 \pm \text{SD } 124.1 \mu\text{g}.10\text{cm}^2$ . During the non-active period of the farm, from August to February, total  
341 meiobenthic biomass increased, with a mean value of  $300.6 \pm \text{SD } 219.9 \mu\text{g}.10\text{cm}^2$  (Figure 9).  
342 ANOSIM showed significant differences ( $R=0.4199$ ,  $p<0.05$ ) between meiofauna biomass  
343 compositions in both control and exposed *A. marina* stands (factor “status”) during the 8 sampling  
344 campaigns from February 2009 to February 2010 (factor “Date”). Similarity of percentage tests  
345 (SIMPER) of cumulative contributions of most influential species showed that composition is mainly  
346 driven by nematodes and copepods. Actually, copepods (62 and 64% of total biomass in effluent-  
347 receiving and control mangroves) and nematodes (36% and 34%, respectively) were the most  
348 influential groups in terms of biomass (SIMPER analysis) and contributed at least 75% to the  
349 difference between groups (cumulative dissimilarity contribution) i.e. 0.786 and 0.774, respectively  
350 for nematodes and copepods in effluents-receiving sediments, 0.768 and 0.772 for nematodes and  
351 copepods in control sediments, and 0.797 and 0.787 for nematodes and copepods in both sediments  
352 (Effluents-receiving vs. control same date).

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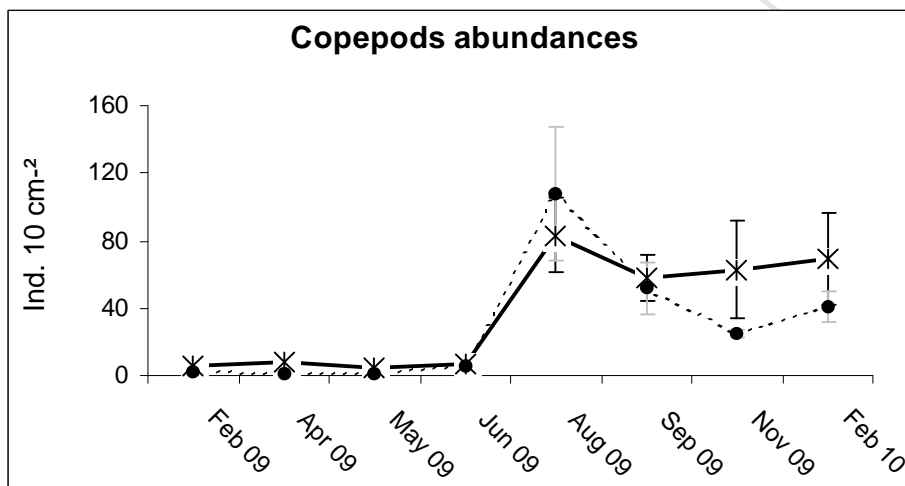
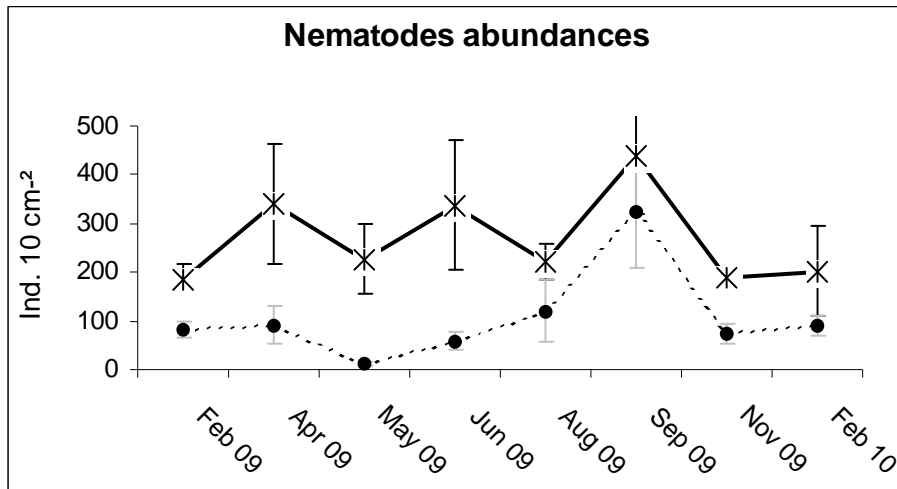
355 **Figure 6 Principal Components Analysis (PCA) of the one-year monitoring in exposed and**  
 356 **controlled *A. marina* stands using meiofauna biomass. Left panel loadings representing the**  
 357 **extent to which the variables are correlated to principal components. Right panel: component**  
 358 **scores.**

359

360 **3.2.1.3 Temporal changes of nematodes and copepods abundances and**  
 361 **biomass in both control and effluent-receiving *A. marina* stands**

362 The temporal variations of abundance of nematodes and copepods differed between the  
 363 control and the effluent-receiving *A. marina* vegetation. During the year, nematode abundance varied  
 364 between 200 and 350 ind.10cm<sup>-2</sup>, except for a peak at 450 ind.10cm<sup>-2</sup> in September in the effluents-  
 365 receiving sediments. In the control mangrove, nematodes abundance remained low from February to  
 366 June (< 100 ind.10cm<sup>-2</sup>), and then increased to the same values as those measured in the effluent-  
 367 receiving mangrove. From February to June, the abundance of copepods was low and stable with no  
 368 more than 10 ind.10 cm<sup>-2</sup> in both sites. From July, a dramatic 900% increase occurred synchronously  
 369 in both sites, with abundances reaching 100 ind.10cm<sup>-2</sup> in August. However, after this increase, the

370 abundance of copepods slightly decreased but remained high in the effluent-receiving mangrove (60 to  
 371 80 ind.10 cm<sup>-2</sup>), whereas it gradually decreased to 25 ind.10cm<sup>-2</sup> in the control site (Figure 7).



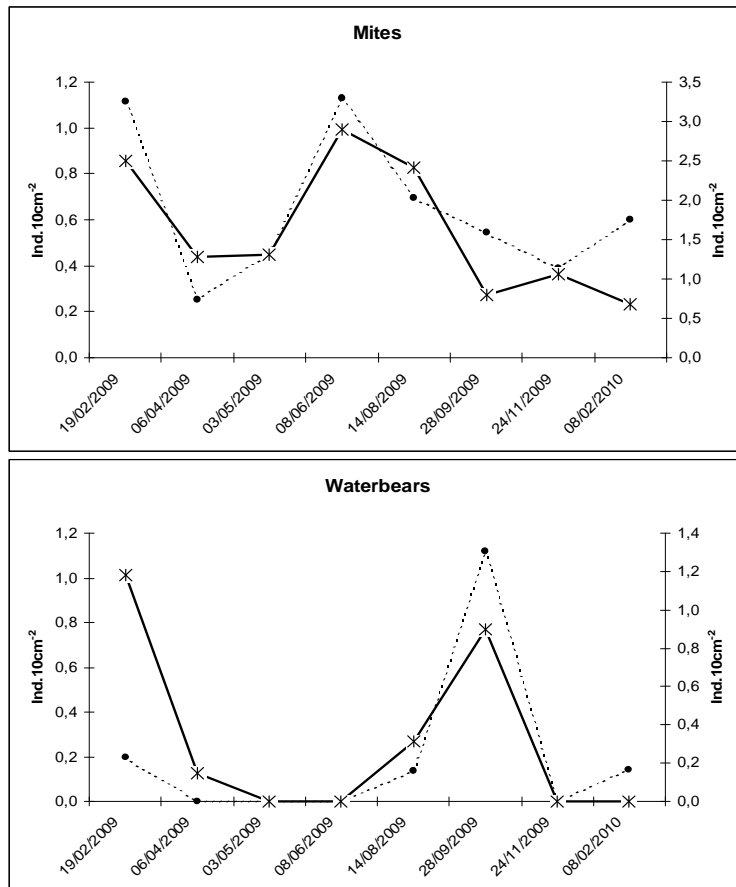
374 **Figure 7 Nematoda abundance (above) and copepoda abundance (below) (ind. 10 cm<sup>-2</sup>)**  
 375 **measured within sediment in the effluent-receiving mangrove and in the control mangrove**  
 376 **during 8 campaigns between February 2009 and February 2010. The impact of effluents**  
 377 **(February to June) barely registers on copepods while it is very noticeable on nematodes**  
 378 **(Control sediment: dotted line with black circle; Effluents receiving sediment: solid line with**  
 379 **stars; (average ± SD)**

380

381

382 **3.2.1.4 Temporal changes of rare taxa abundances in both control and**  
383 **effluent-receiving *A. marina* stands**

384 Three rare taxa were found in very low quantities in the sediments of effluent-receiving and control  
385 vegetations. Turbellarians have been observed in February 2010 in the effluent-receiving *A.marina*  
386 stand ( $0.6 \pm \text{SD } 1.4 \text{ ind.}10 \text{ cm}^{-2}$ ) and in September 2009 in the control *A.marina* stand ( $0.4 \pm \text{SD } 0.4$   
387  $\text{ind.}10 \text{ cm}^{-2}$ ). Gastropoda have been found in February 2010 in the effluent-receiving *A.marina* stand  
388 ( $0.4 \pm \text{SD } 0.5 \text{ ind.}10 \text{ cm}^{-2}$ ) and in November 2009 in the control *A.marina* stand ( $0.1 \pm \text{SD } 0.3 \text{ ind.}10$   
389  $\text{cm}^{-2}$ ). Pygogonida have been observed only in February 2010 in the effluent-receiving *A.marina*  
390 stand ( $4.3 \pm \text{SD } 9.5 \text{ ind.}10\text{cm}^{-2}$ ). Neither bivalves nor amphipods have been observed. Kinorhynchs  
391 have been found only in the effluent receiving *A.marina* sediments during 2009 and 2010 hot seasons  
392 i.e  $0.3 \pm \text{SD } 0.6 \text{ ind.}10 \text{ cm}^{-2}$  (Feb.2009);  $0.1 \pm \text{SD } 0.3 \text{ ind.}10 \text{ cm}^{-2}$  (Nov.2009) and  $0.1 \pm \text{SD } 0.3 \text{ ind.}10$   
393  $\text{cm}^{-2}$  (Feb.2010). Mites (halacoidea) and waterbears (tardigrada) have been found almost all year round  
394 in both control and effluent-receiving *A. marina* mangroves (Figure 8). Seasonal patterns of their  
395 abundance in receiving-effluents sediments mirrored those observed in the control mangrove. On  
396 average over the year, waterbears abundances did not differ significantly in effluents receiving and  
397 control *A.marina* sediments ( $p>0.05$ ), whereas mites were significantly more abundant in control  
398 sediments ( $p<0.05$ ).



399

400

401 **Figure 8** Rare taxa abundances (halacaroidea: mites, tardigrada: waterbears) (ind. 10 cm<sup>-2</sup>)  
 402 measured within sediments in the effluent-receiving and in the control *A.marina* vegetations  
 403 during 8 campaigns between February 2009 and February 2010 (Control sediments: dotted line  
 404 with black circle; effluent-receiving sediments: solid line with stars).

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### 3.2.2 Microphytobenthos

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#### 3.2.2.1 Microphytobenthos temporal evolution

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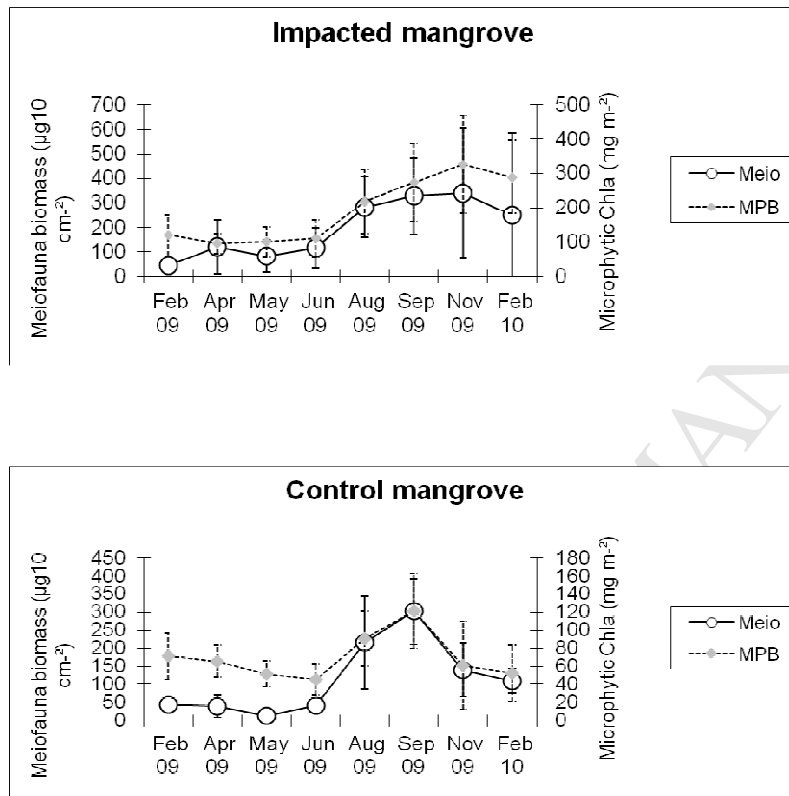
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414

Microphytobenthic Chl-a concentrations were significantly different between the two sites ( $p < 0.05$ ). The surface sediment of the effluent-receiving *Avicennia* stand presented three times higher Chl-a concentration than the control sediment, with on average  $198.0 \pm \text{SD } 14.9 \text{ mgChl-a.m}^{-2}$  and  $73.5 \pm \text{SD } 4.2 \text{ mgChl-a.m}^{-2}$ , respectively. From February to June, microphytobenthic Chl-a concentrations were relatively stable in the control mangrove (Wilcoxon Test,  $p > 0.05$ ), while they increased and peaked in September and decreased to February. The seasonal change of microphytobenthic Chl-a concentrations in the effluent-receiving mangrove was different to that in the control mangrove. From

415 February to June, when the farm was active, concentrations ranged between  $119.3 \pm \text{SD } 60.3$  and  
 416  $110.2 \pm \text{SD } 54.4 \text{ mgChl-a.m}^{-2}$ , without any significant differences during the 4 sampling campaigns  
 417 (Wilcoxon Test,  $p > 0.05$ ). In August after the final drain, the concentrations increased, reaching  $217.2$   
 418  $\pm \text{SD } 92.7 \text{ mgChl-a.m}^{-2}$ . During the non-active period of the farm, from August to February,  
 419 microphytobenthic Chl-a concentrations increased, with a mean value of  $269.0 \pm \text{SD } 113.5 \text{ mgChl-}$   
 420  $\text{a.m}^{-2}$  (Figure 9).



421

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423

424 **Figure 9** Temporal variations in total meiofauna biomass ( $\mu\text{g } 10 \text{ cm}^{-2}$ ) and microphytobenthic  
 425 **Chl-a** ( $\text{mg m}^{-2}$ ) (average  $\pm$  SD) in control mangrove and effluent receiving mangrove. Seasonal  
 426 patterns in meiofaunal biomass mirror the patterns of microphytobenthic Chl-a.

427

### 428 3.2.2.2 Parallel microphytobenthos and meiofauna temporal evolution

429 Pearson product-moment correlation coefficient indicated that meiofauna and microphytobenthos  
 430 biomass were positively correlated and followed very similar patterns at both sites (control  $t = 4.2159$ ,  
 431  $df = 6$ ,  $p\text{-value} = 0.005586$ ,  $r = 0.8646534$ ; impacted  $t = 5.9269$ ,  $df = 6$ ,  $p\text{-value} = 0.001028$ ,  $r =$   
 432  $0.9241838$ ) during the same period.



433

## 434 **4 Discussion**

### 435 **4.1. General characteristics of meiofauna distribution in the whole mangrove area** 436 **receiving shrimp farm effluents**

437 In the whole mangrove area that has received shrimp farm effluents over a period of 25 years,  
438 meiofauna abundance ranged between 70 and 5,137 ind.10 cm<sup>-2</sup>, which is similar to natural mangrove  
439 sediments worldwide (Coull, 1999). The top three contributors to biomass identified in the effluent-  
440 receiving mangrove were nematodes (57.3%), copepods (31.3%) and annelids (6.3%) confirming that  
441 such taxa are the most ubiquitous taxa in mangroves, as observed in Brazil (Netto and Galluci, 2003),  
442 in Vietnam (Xuan et al., 2007; Mokievsky et al. 2011), and in India (Chinnadurai and Fernando, 2006;  
443 Chinnadurai and Fernando, 2007; Thilagavathi et al., 2011). Consequently, we suggest that 25 years of  
444 release of aquaculture effluents into the mangrove has not caused any severe changes in benthic  
445 meiofauna in terms of total abundance or biomass. This conclusion is in agreement with that of  
446 Molnar et al. (2014), who did not find any signs of saturation, eutrophication or anoxia of the sediment  
447 of the same effluents receiving mangrove.

448

### 449 **4.2. Meiofauna distribution in the whole mangrove in relation to mangrove stand and** 450 **farm activity**

#### 451 **4.2.1. Meiofaunal distribution in the whole mangrove during the non-active** 452 **period: the influence of mangrove zonation.**

453 Meiofauna abundance and biomass increased from the land side to the sea side of the  
454 mangrove, i.e. from the closest to the furthest point of the effluent discharge. At first glance, this result  
455 may suggest a situation of hyper-eutrophication (Environment Canada, 2010). Nevertheless, individual  
456 mass and consequently total biomass did not show any specific changes in relation to the distance  
457 from the discharge point. In New Caledonia, from the salt flat to the *Rhizophora* stand, the physico-  
458 chemical parameters of the sediment present different gradients, notable salinity, water content, and  
459 organic matter (Deborde et al., 2015). We thus suggest the abundance and biomass increases towards  
460 the sea was rather related to the decreasing salinity of pore waters from the land side to the sea side of

461 the mangrove (Molnar et al., 2014), salinity being recognized as a key parameter of meiofauna  
462 distribution. In mangrove ecosystems, salinity is mainly driven by the length of tidal immersion and  
463 thus by the elevation of the soil, and thus increases towards the land. The salinity gradient is also  
464 responsible for the mangrove species distribution along the tidal zone, the ability of mangrove trees to  
465 cope with high salinity differs among species (Marchand et al., 2011). Actually, in New Caledonia,  
466 pore-water salinity in salt flats can reach more than 80 ‰ (Marchand et al., 2011), and sediment  
467 temperature can be as high as 43°C or more, inducing high evaporation (Leopold et al., 2015).  
468 Meiofauna biomass and abundance differed between vegetation, but some differences were also  
469 observed within sediments of the same mangrove species. The fringing *R. stylosa* presented higher  
470 abundance and biomass than the inner *Rhizophora* stand. In fact, *Rhizophora* trees, growing at the  
471 edge of the sea, present higher abundance and more developed root system than inland, and this can  
472 create a favorable environment for the development of numerous taxa. Furthermore, this particular  
473 sediment consists of a coarser grain size linked to the high energy of the sea side zone, as well as a  
474 lower organic content of the sediment linked to tidal flushing (Marchand et al., 2004), which may  
475 induced better sediment oxygenation than in the inner *Rhizophora* zone, which is known to be strongly  
476 anoxic (Deborde et al., 2015). With regard to the *Avicennia* stand, which is situated between the salt  
477 flat and the *Rhizophora* stand, its sediments have high biomass and the highest abundance of  
478 meiofauna. In addition to sediment grain size and food availability, *Avicennia* pneumatophores  
479 probably act as a more effective barrier than the *Rhizophora* stilts for meiofauna (Chinnadurai and  
480 Fernando (2007). Furthermore, *Avicennia*'s specific root system, by diffusing oxygen into the  
481 sediment (Marchand et al., 2004), may create more favorable conditions for meiofauna development.  
482 Eventually, *Avicennia sp.* leaves, which have high nitrogen content and low C/N ratios, decompose  
483 faster (Robertson, 1988), and may be more easily accessible to meiofauna than *Rhizophora* leaves that  
484 are rich in tannins, which by acidity and/or toxicity adversely affect meiofauna (Alongi, 1987c).  
485

486 **4.2.2. Evolution of meiofauna distribution in the whole mangrove between the**  
487 **active and the non active periods.**

488 Over the course of the 8 month rearing cycle, the total N and P loads to the mangrove were  
489 approximately 2.3 and 0.5 tons of N and P, respectively, which are equivalent to loads of 79 kg N ha<sup>-1</sup>  
490 and 19 kg P ha<sup>-1</sup> (Molnar et al., 2013). Short-term effects of effluent release on total meiofaunal was  
491 expected, and thus samples were collected during the farm's active and non-active periods. At the  
492 whole mangrove scale, we did not observe any significant differences in terms of abundance but in  
493 terms of biomass between AP and NAP periods, which may seem paradoxical. It is known that  
494 intraspecific variation of animal size may be correlated with organic enrichment (Weston, 1990 ; Grall  
495 and Chauvaud, 2002). In fact, significant smaller specimens of nematodes, copepods and polychaetes  
496 were found during the active period. In the present study, releasing effluents into the mangrove led to  
497 a decrease in the length of sediment air exposure, a reduced availability of dissolved oxygen in pore  
498 waters, and thus to more hypoxic conditions (Molnar et al., 2014). One explanation would be that the  
499 transient combination of moderate organic enrichment and reduced availability of dissolved oxygen in  
500 pore waters during the AP may selectively promote the smaller species. Additionally, effluent release  
501 occurred during the cold season, when the metabolism of benthic organisms is at its minimum (Santos  
502 et al., 1996), and the final drain occurred just before the seasonal temperature increase, a period during  
503 which the microphytobenthos biomass increased in the control mangrove. The seasonal variations may  
504 be responsible for this difference in biomass. A reversal of the NAP (June) vs. AP (November)  
505 situation from the one studied in the present study would have been extremely informative to  
506 distinguish the respective influence of farm and seasons. However, owing to reduced profits, New  
507 Caledonian shrimp farmers no longer stock their ponds in the cold season (May-June) with a view to  
508 harvest in the hot season (November-December). Consequently, this sampling strategy cannot be  
509 developed in New Caledonia. Hence, we have carried out a parallel one-year monitoring of meiofauna  
510 distribution in the *Avicennia* stand, the closest vegetated stand to the ponds, both in the effluent-  
511 receiving mangrove and in a control stand.

512

513 **4.3. Respective influence of season and farm activity on meiofauna in the *A. marina* stand**  
514 **receiving the effluents.**

515 **4.3.1 Response of specific taxa**

516 **4.3.1.1 Most abundant taxa: nematodes and copepods**

517 At the crop scale, the impact of effluents was not identifiable on copepods but was noticeable  
518 on nematodes in the *Avicennia* stand (Figure 7). Copepods abundance remained low throughout the  
519 farm's active period. Consequently, the massive arrival of water enriched in potential food sources did  
520 not stimulate copepods development. We suggest that main changes displayed by copepods  
521 populations are mainly related to their natural cycle (reproduction), as their blooms occurred  
522 simultaneously in the effluent-receiving and in the control mangroves characterized by significant  
523 different microphytobenthos biomass. Nematode populations displayed an opposite trend than that of  
524 copepods. Shrimp farm effluents seemed to highly stimulate their development, while they remained  
525 low in the control site over the period February to June 2009. Shrimp farm wastes contain highly  
526 diversified phytoplankton cells up to 20 millions cel.ml<sup>-1</sup> (Della Patrona and Brun, 2009) that  
527 constitute a very important food source for epistrate feeders, that are known to directly assimilate it  
528 (Olafsson and Elmgren, 1997). We thus suggest that these phytoplankton-rich effluents directly and  
529 specifically enhance epistrate-feeder populations that are the dominant trophic nematofauna group in  
530 the *A.marina* stand (Chinnadurai and Fernando, 2007). Under the influence of anoxic conditions, the  
531 general pattern consists of an increase in "less sensitive" nematodes in conjunction with a decrease in  
532 "very sensitive" copepods (Vezzulli et al. 2003; Moreno et al., 2008). However, the semi-intensive  
533 rearing system of New Caledonia did not lead to such severe conditions and did not disrupt copepods  
534 life cycle as reported in sediments subjected to mussel farm biodeposition (Danovaro et al., 2004) or  
535 to some peculiar well managed fish farms (Holmer et al., 2008; Mirto et al., 2010).

536

537 **4.3.1.2 Rare taxa**

538 The large dominance of nematodes, copepods and polychaetes can mask the presence or the  
539 evolution of other taxa (Bianchelli et al., 2010; Pusceddu et al., 2011). Some rare taxa are recognized  
540 as providing more reliable and clear results on the degree of eutrophication than ubiquitous groups

541 (Mirto et al., 2010; Gambi et al., 2010. In this regard, kinorhynchs, is the most frequently rare taxon  
542 examined in aquaculture studies (Mazzola et al., 1999; Mazzola et al., 2000; Nadjek et al., 2007;  
543 Holmer et al., 2008; Grego et al., 2009). In the present study, kinorhynchs populations displayed an  
544 opposite trend to what was expected. They were present in impacted sediments and absent in control  
545 ones. Actually, we assume that owing to its characteristics (low water and OM contents, high pore  
546 water salinity and elevated insolation), the sediment of the control site had probably insufficient  
547 trophic capacity for kinorhynchs development. Furthermore, this taxon was only observed during the  
548 hot season (November to February), confirming their elevated requirements. Mites, which are usually  
549 observed in intertidal environments (Marshall et al., 2001), may be very abundant in tropical estuaries  
550 (Nozais et al., 2005). In this study, mites abundance peaked during the cold season (June to August)  
551 while usually maximum abundance of meiofauna peaks in the warm months (Giere, 1993). However,  
552 individual taxa or species may reach maximum abundance at different periods (Higgins and Thiel,  
553 1988) *a fortiori* in tropical conditions where differences in temperatures are less pronounced. As stated  
554 for tardigrades, seasonal shrimp farm activity (AP) did not disturb the natural cycle of mites in  
555 *A.marina* sediments. However, mites were found three times less abundant in the effluents-receiving  
556 *A.marina* stand than in the control one. Unlike kinorhynchs, mites were ubiquitously found in the  
557 different mangrove stands and almost all year round. Consequently, we suggest that this rare taxon  
558 may be a useful indicator of long term shrimp farm biodeposition in mangrove.

559

#### 560 **4.3.2. Total meiofauna and microphytobenthos parallel changes**

##### 561 **4.3.2.1 Response of microphytobenthos to shrimp farm effluents**

562 In addition to the meiofauna distribution, we were also interested in the Chl-a content of the  
563 surface sediment in the *A.marina* stand. Actually, along the mangrove zonation in New Caledonia,  
564 Leopold et al. (2013) showed that the *A.marina* stand, with its intermediate position in the tidal zone,  
565 was the preferential zone for the development of MPB, because i) the canopy cover was not dense and  
566 enabled solar radiation to reach the soil and ii) ideal soil water content, not immersed all the time and  
567 never dry. In the present study, the surface sediment of the effluent-receiving *A.marina* stand had a  
568 Chl-a concentration three times higher and a meiofaunal biomass, as well as a total abundance, twice

569 as large as the control sediment, demonstrating the influence of shrimp farming on this mangrove.  
570 However, Chl-a concentrations never exceeded a threshold above which, it is possible to consider an  
571 eutrophication of the ecosystem, which is consistent with previous results showing the light evolution  
572 of the effluent-receiving mangrove (Molnar et al., 2013; 2014; Debenay et al., 2015). Thus,  
573 microphytobenthos (MPB) biomass can be a good descriptor of shrimp farm effluents disturbance in  
574 mangrove.

575

#### 576 **4.3.2.2 Complex interaction between meiofauna and microphytobenthos**

577 Seasonal patterns in meiofaunal biomass mirrored the patterns of microphytobenthic Chl-a  
578 highlighting a possible causal trophic relationship. Actually, most of meiofauna taxa are important  
579 consumers of microphytobenthos (Nozais et al., 2005). In open areas, contradictory results have been  
580 observed (Mirto et al., 2007). La Rosa et al. (2001) reported that meiofaunal and microphytobenthic  
581 biomass increased synchronously in response to organic enrichment under fish cages. At the opposite,  
582 Vezzulli et al. (2003) reported that meiofauna abundance was not correlated to the microphytobenthos  
583 or that of bacteria. In our study site, Aschenbroich et al. (2015) have shown that mangrove benthic  
584 organic matter is qualitatively and quantitatively affected by shrimp farm effluent release and that  
585 responses to environmental condition changed depend on mangrove stand characteristics.  
586 Additionally, it was demonstrated that i) the OM exported from the ponds stimulated oxygen demand  
587 and nutrient regeneration rates in sediments of the closest mangrove stand, resulting in large effluxes  
588 of dissolved organic and inorganic nutrients into the overlying water, ii) benthic primary productivity  
589 at sediment surface was enhanced, even after the cessation of the release, iii) microalgal communities  
590 shifted (Aschenbroich et al., 2015; Molnar et al., 2013; 2014; Debenay et al., 2015). We thus suggest  
591 that qualitative and quantitative changes in MPB can directly influence meiofauna development in  
592 *A.marina* stand, however it is difficult to determine the respective influence of natural seasonal  
593 evolution and effluents on MPB development. In fact, trophic interaction between different benthic  
594 components as organic matter quality (carbohydrates, proteins, lipids, Chl-a contents), meiofauna,  
595 bacteria and protozoa are very complex (Danovaro et al., 2004; Vezzulli et al., 2003).

596

## 597 **5. Conclusions**

598

599 This study suggests that abundance, biomass and individual mass of meiofauna taxa rather  
600 vary according mangrove zonation than under the influence of shrimp farm effluents. Meiofauna sea-  
601 land gradients cannot be attributed to an eventual flume effect of shrimp farm wastes but rather to  
602 different biogeochemical properties of sediments induced by different mangrove roots systems and/or  
603 length of tidal immersion. The *Rhizophora* stand was characterized by the highest meiofauna biomass,  
604 however the stand with the largest ecological interest was the *A.marina* stand, which harbored both  
605 higher meiofaunal abundance and higher microphytobenthic biomass. We suggest that the meiofauna  
606 development was linked to the quantity and quality of the MPB, which were driven both by the release  
607 of effluents and the climatic seasonal evolution. The fact that the final drain of the shrimp ponds  
608 occurred just before the seasonal temperature increase induced a boosted algal bloom and meiofauna  
609 development. It is thus difficult to conclude on the influence on releasing shrimp farm effluents on  
610 meiofauna distribution in mangrove on a short term. However, the long-term (25 years) effect of  
611 effluents led to a situation where mangrove sediments presented higher meiofaunal abundance and  
612 biomass than the control one, and more interestingly, were characterized by additional taxonomic  
613 groups compared to the control site. Consequently, our results suggest that semi-intensive farming in  
614 the investigated system (FAO) has a low impact on the environmental quality of the adjacent  
615 mangrove. However, it does not seem appropriate to extrapolate this result to all New Caledonian  
616 farms, because the amount of effluents released in the mangrove per area unit, and the way they are  
617 released may differ from one farm to another. Shrimp farming is one of the main cause of mangrove  
618 destruction worldwide, this study shows that other practices exist, and that mangrove and shrimp-  
619 farming can co-exist.

620

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- 13 taxonomic groups of meiofauna were identified in mangrove sediments
- Meiofauna abundance and biomass increased from the land side to the sea side
- Meiofauna distribution was controlled by the mangrove zonation
- Short term effect of aquaculture effluents on meiofauna distribution were not evidenced
- Long term effects were higher meiofaunal diversity, abundance and biomass

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