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## First simultaneous assessment of macro- and meiobenthic community response to juvenile shellfish culture in a Mediterranean coastal lagoon (Thau, France)

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1           **First simultaneous assessment of macro- and meiobenthic community response to**  
2           **juvenile shellfish culture in a Mediterranean coastal lagoon (Thau, France)**

3  
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16  
17  
18   **Abstract**

19   Shellfish aquaculture has the potential to alter benthic assemblage composition with  
20   subsequent modifications of ecosystem functioning. While the impacts of aquaculture on the  
21   taxonomic structure of macro- and — to a lesser extent — meiofauna, have been widely  
22   studied, the functional changes of these communities remain relatively unknown. The recent  
23   development of biological trait analysis (BTA) has made it possible to produce information  
24   about how ecosystem functioning could change across specific terrestrial or aquatic system.  
25   In the present study, we used a BTA in parallel with standard taxonomic analysis to evaluate  
26   how well the two approaches detected the potential influence of juvenile oyster culture on the  
27   benthic community in the French Mediterranean Thau Lagoon. Two sites were sampled under  
28   farm structures and compared with two reference sites beyond the influence of farming. This  
29   study is the first detailed parallel description of macro- and meiofauna (nematodes). A total of  
30   118 and 41 taxa were determined for macrofauna and nematodes respectively. Some taxa  
31   were more abundant or exclusively observed under farm structures such as *Chaetozone gibber*

32 and *Neanthes acuminata* for macrofauna and *Daptonema fallax* and *Anticoma eberthi* for  
33 nematodes.

34 Overall, our results indicate that biological traits and functional indices both detected the  
35 impact of shellfish culture on benthic assemblages, whereas macrofauna taxonomic indices  
36 revealed no difference. Our results thus suggest that trophic and ecological groups are  
37 particularly good indicators of the effects of shellfish culture. This study confirms the  
38 relevance of the functional approach, and more generally of multi-index approaches, to detect  
39 the influence of aquaculture on benthic communities. Further work is required to test multiple  
40 traits in different regions and under different systems, but this work paves the way for  
41 environmental impact assessment using a trait based approach.

42

43 *Key-words:* Environmental Impact Assessment; Aquaculture; Benthic indicators; Trait based  
44 Approach; Macrofauna; Meiofauna

45

## 46 **1. Introduction**

47 Coastal ecosystems are facing drastic environmental changes due to many human pressures  
48 that threaten the integrity of communities and may have major impacts on ecosystem  
49 functioning and its ability to sustain ecosystem services (Halpern et al. 2008, Cardinale et al.  
50 2012). In shallow coastal ecosystems, benthic communities play a central ecological role in  
51 benthic-pelagic coupling through their feeding and movements that promote nutrient and  
52 oxygen exchange throughout the sediment layer. Thus, any alteration of these communities  
53 may have implications for ecosystem structure and performance (Thrush et al. 2006,  
54 Kristensen et al. 2014) and understanding how they respond to disturbance is crucial to  
55 predict future changes and help maintain and improve the quality of marine ecosystems. To  
56 date, most studies have focused on the response of benthic macrofaunal communities to

57 disturbances (Pratt et al. 2015, Dimitriou et al. 2017, Ellis et al. 2017). Recently, interest has  
58 been growing in the study of meiofauna, since it has been shown that these organisms may  
59 modulate biological interactions in the sediment layer (Bonaglia et al. 2014, Lacoste et al.  
60 2018a) and play a significant role in benthic ecosystem processes and services (Schratzberger  
61 and Ingels 2017). Due to their widespread distribution, short life and reproductive cycles, high  
62 abundance and diversity, meiofauna are thus beginning to be used as biological indicators for  
63 the assessment and monitoring of aquatic ecosystems (Semprucci et al. 2015, Zeppilli et al.  
64 2015, Bianchelli et al. 2018).

65 Bivalve aquaculture has various environmental impacts (McKindsey et al. 2011, Brenner et  
66 al. 2014) including the accumulation of biodeposits on the bottom under culture sites that may  
67 results in local organic enrichment. Such disturbance typically affect benthic communities  
68 according to the Pearson and Rosenberg (1978) model of organic enrichment with a  
69 progressive increase in the abundance of enrichment-tolerant species in the vicinity of farms,  
70 whereas communities directly under the farming structures are dominated by a few  
71 opportunistic species (Callier et al. 2007, 2009, McKindsey et al. 2011). Several studies have  
72 reported lower richness and/or abundance of organisms, as well as differential community  
73 composition of macrofauna (Christensen et al. 2003, Harstein and Rowden 2004, Lacoste et  
74 al. 2018b), or meiofauna (Mirto et al. 2000, Mahmoudi et al. 2008, Netto and Valgas 2010)  
75 under farming structures compared with reference sites. One notable feature of these studies is  
76 the focus on structural attributes of the benthos (e.g. taxonomic composition, number of  
77 species) whereas functional changes are disregarded. Only a few studies have used a  
78 functional approach to investigate the impact of interactions between the environment and  
79 fish (Papageorgiou et al. 2009, Dimitriadis and Koutsoubas 2011), or mussel (Lacoste et al.  
80 2019) aquaculture.

81 Growing awareness that changes in biodiversity may modify ecosystem functioning (e.g.  
82 Loreau et al. 2001, Naeem et al. 2009) has led to the recognition that impacts on the  
83 functional diversity of benthic assemblages, as opposed to their structural features, should be  
84 better quantified (e.g. Strong et al. 2015, Cernansky 2017). Due to functional redundancies in  
85 communities, observed changes in species composition may be buffered (Walker 1992,  
86 Snelgrove 1998) such that structural impacts on benthic assemblages are not always matched  
87 by their functional counterparts (Bolam 2012). Otherwise, trophic pathways (indicator of  
88 ecosystem functioning) may be impacted without apparent changes of the overall benthic  
89 diversity (Dubois et al. 2007). Thus, functional-based approaches, including biological trait  
90 analysis and the computation of functional diversity (FD) indices, provide useful proxies to  
91 investigate the effect of human disturbances at the ecosystem-functioning level (Mouillot et  
92 al. 2006, 2013) and to anticipate the potential loss of ecosystem services associated with  
93 biodiversity erosion (Cardinale et al. 2012). Moreover, this approach can be used to compare  
94 communities that have no species in common but do share traits.

95 Thau Lagoon is a Mediterranean marine ecosystem of notable economic importance due to  
96 the many ecosystem services it supports, in particular, the significant oyster farming activity  
97 (about 7 000 tons per year, near 10% of the national production). A few authors have  
98 described changes in macrofaunal communities in relation to oyster culture (Mazouni et al.  
99 1996, Duport et al. 2007, Thouzeau et al. 2007), and one study showed spatial variation of  
100 meiofauna abundance (Guelorget et al. 1994). But to date, no list has been published of  
101 meiofauna assemblages in the lagoon, even though these communities may respond more  
102 rapidly to biodeposition than macrofauna do and could be good indicators of lagoon  
103 ecological status. Potential associated functional changes in macro- and meiofauna remain  
104 unknown. Thus, in a global context of increasing intensity and frequency of climatic and  
105 anthropogenic disturbances, there is a need to describe and predict changes in functional

106 community structure to anticipate potential losses of ecosystem services associated with  
107 biodiversity modifications in coastal areas such as Thau Lagoon.

108 The aims of this study were therefore 1/ to document macrobenthic and meiobenthic  
109 (nematode) communities in relation to oyster culture in Thau Lagoon and 2/ to compare the  
110 response of some selected taxonomic and functional attributes to disturbances caused by  
111 shellfish culture for macro- and meiofauna assemblages. To our knowledge, this is the first  
112 time the impact of bivalve aquaculture on macro- and meiobenthic communities has been  
113 studied simultaneously using a traditional taxonomic based approach coupled with a  
114 functional approach.

115

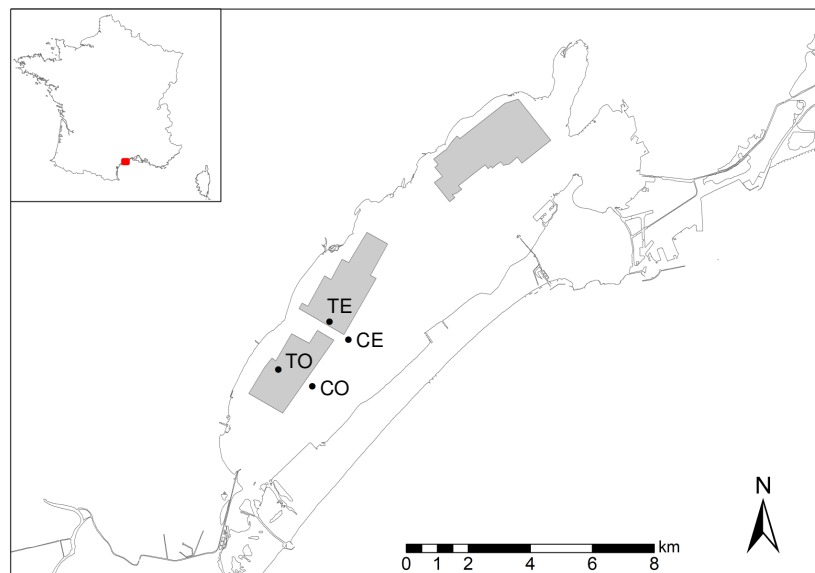
## 116 **2. Materials and Methods**

### 117 *2.1. Study area and sampling strategy*

118 Thau Lagoon is a French Mediterranean semi-enclosed shallow lagoon (mean depth 4 m)  
119 covering an area of 68 km<sup>2</sup> (Fig. 1). It is the most important French Mediterranean shellfish  
120 farming zone with an annual production of about 7 330 tons of oysters in 2016 (DDTM34  
121 2017) reared on spatially organized structures, called “tables”. Each table is 50 × 12 m long  
122 and supports between 800 and 1200 suspended nets or ropes holding juvenile or adult oysters.  
123 Sixteen tables form a ‘block’ and the blocks are separated from each other by a distance of  
124 150m. Annual variations in water temperature and salinity in the lagoon are large, varying  
125 from 3 °C to 30 °C and from 33 to 40 PSU respectively (Messiaen et al. 2019).

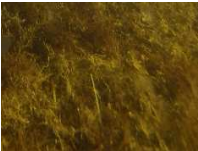
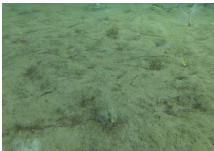
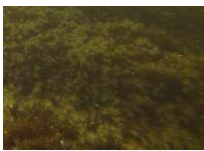
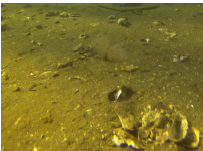
126 The study was conducted in a dedicated shellfish-farming zone in the western part of the  
127 lagoon (Fig. 1). As a part of a research program (see Richard et al. 2017, 2019), the study  
128 focused on juvenile production, whose impact on ecosystem has never previously been  
129 evaluated in Thau Lagoon. Samples were collected at two sites in the farmed zone directly  
130 below juvenile tables (TO and TE sites) and at two additional sites located at a distance of

131 200 m outside the farming zone (CO and CE sites) (Fig. 1). Some characteristics of the four  
 132 sites are given in Table 1, the main difference being algal cover (*Halopytis* sp.) which was  
 133 greater at TO and CO sites. Sampling was done during three surveys in 2015 (September 29–  
 134 October 2; October 28–November 4 and December 1–December 4, hereafter referred to as  
 135 October, November and December) covering the whole juvenile production period. Each date,  
 136 three replicate sediment cores (9 cm diameter, 10 cm depth) were randomly collected by  
 137 scuba divers at each site. The samples were preserved in 10% buffered formalin and later  
 138 transferred into 70% alcohol.



139  
 140 Figure 1. Location of the four sampling sites in Thau lagoon. The two T sites (TE & TO) were directly  
 141 under the tables and the two reference sites (CE & CO) were located at a distance of 200 m from the  
 142 shellfish farming area.  
 143

144  
 145 Table 1. Summary description of the 4 sampled sites

	Reference sites		Farm sites	
	CO	CE	TO	TE
Depth	3.7 m	4.2 m	3.6 m	4.2 m
Bottom observation				
Culture system	-	-	Juvenile oysters ca. 1.1 t per table*	Juvenile oysters ca. 1.4 t per table

146 \*Calculation from initial measurements on a sub-sample

147

## 148 *2.2. Abundance and species richness*

149 Back to the laboratory, the content of each core sample was passed through a 1mm mesh  
150 sieve. Macrofauna in the > 1mm fraction was sorted and identified to the lowest taxonomic  
151 resolution possible, up to species in most cases. The < 1 mm fraction was decanted over a 40-  
152 µm sieve. All nematodes were sorted under a stereo-microscope, transferred to glycerol  
153 through a series of ethanol-glycerine solutions, mounted on glycerine slides (Somerfield et al.  
154 1994) and identified to the lowest taxonomic level possible using the generic keys of Platt and  
155 Warwick (1983, 1988) and Warwick et al. (1998) and species descriptions in the literature.  
156 Abundance (N) and species richness (S) of macrofauna and nematodes were characterized.  
157 The Shannon-Wiener diversity index (H') and Pielou's evenness (J') were calculated for each  
158 site. Taxa that were observed only once were removed from analyses (Clarke and Warwick  
159 2001); all results are expressed per core (63 cm<sup>2</sup>).

160

## 161 *2.3. Functional analysis*

162 The common step in all functional ecology studies is to characterize the functional strategy of  
163 each species of interest by identifying a relevant combination of functional traits (Violle et al.  
164 2007). There is currently no consensus on the methodology for selecting the most appropriate  
165 traits for a given study (Marchini et al. 2008). As the aim of the present study was to use traits  
166 as a proxy for assemblage response to shellfish culture, we decided to focus on the biological  
167 traits and ecological groups of macrofauna that have already been shown to be good  
168 indicators of organic enrichment in this specific context (Borja et al. 2000, Lacoste et al.  
169 2019). To our knowledge, this is the first study to simultaneously evaluate the functional  
170 response of both macrofauna and meiofauna (nematodes) to bivalve aquaculture. We thus  
171 selected nematodes biological traits that have been shown to respond to organic enrichment



172 (Alves et al. 2013, Kandravicius et al. 2018), with the assumption that they are comparable  
173 with those of macrofauna. Thus, selected traits for both assemblages were related to trophic  
174 guilds, ecological strategy, and interaction with sediments (Table 2). Each selected trait was  
175 subdivided into several modalities (Table 2). Organisms identified up to species level were  
176 classified in functional groups based on existing literature (e.g. Wieser 1953, Thistle et al.  
177 1995, Queiros et al. 2013, WoRMS Editorial Board 2018). When reliable information was not  
178 available, data from the closest phylogenetic neighbor were used or the taxon was excluded  
179 from subsequent analysis. All nematodes and 44 macrofaunal (accounting for 91% of all  
180 individuals identified) taxa were thus retained for functional analysis. The subsequent ‘trait  
181 per taxon’ matrix was combined with the ‘taxa per sample’ matrix (abundance) to create a  
182 ‘trait per sample’ matrix. The relative abundance of the functional groups was then calculated  
183 by summing abundance across all taxa allocated to each functional group at a given site per  
184 sampling date. Finally, the two matrices were used to calculate functional indices (functional  
185 richness: FRic, functional evenness: FEve and functional dispersion: FDis) as outlined by  
186 Villéger et al. (2008) and Laliberté and Legendre (2015) for both macrofauna and nematodes.  
187 Using only categorical traits, FRic was defined as the number of unique trait combinations  
188 (equivalent to singular species).

189 Although its results are ambiguous (Semprucci et al. 2015), the Index of Trophic Diversity  
190 (ITD, Heip et al. 1985) is popular and widely used for nematodes. We thus calculated ITD for  
191 both nematodes and macrofauna, using their respective trophic guilds, with the aim of  
192 evaluating its consistency in the case of disturbances caused by shellfish culture. The index  
193 was calculated following Heip et al. (1985):  $ITD = \sum \theta^2$ , where  $\theta$  is the percentage contribution  
194 of each feeding type. Thus, a low ITD value corresponds to high trophic diversity (each group  
195 accounting for the same percentage).

196  
197

198 Table 2. List of biological traits and respective categories selected for macrofauna and nematodes.  
 199

	Category	Level	Code
<b>Macrofauna</b>	Feeding group	Filter/suspension-feeder	F
		Grazer/Detritivorous	Gr
		Deposit-feeder	D
		Subsurface deposit-feeder	SubD
		Carnivorous	C
	Ecological group	Very sensitive	I
		Tolerant	II
		Indifferent	III
		2 <sup>d</sup> -order opportunistic	IV
		1 <sup>st</sup> -order opportunistic	V
	Mobility	Fixed in a tube	I
		Limited movement	II
		Slow movement	III
		Free movement (burrow system)	IV
	Reworking type	Epifauna	E
Surficial modifier		S	
Biodiffusers		B	
Upward conveyors		UC	
Upward/downward conveyors		UDC	
<b>Nematodes</b>	Feeding groups	Selective deposit-feeder	1A
		Non selective deposit-feeder	1B
		Epigrowth feeder	2A
		Predators/omnivores	2B
	Cp-score	Opportunistics	2
			3
		to	4
		Persisters	5
	Adult shape	Long/thin	Lt
		Slender	Sl
	Amphid shape	Circular	Cr
		Indistinct	Id
		Pocket-like	Pk
		Rounded or elongate loop	REL
		Spiral	Sp

200

201 *2.4. Statistical analysis*

202 The effect of Date × Condition (Farm vs Reference) on univariate measurements of benthic  
 203 communities (taxonomic and functional indices) was tested using linear mixed effect models  
 204 (IMEM; modelling normally distributed responses) with site as a random factor nested in

205 Condition. The effect of factors was considered as significant when the null vs main effect  
206 models (using maximum likelihood estimation) differed significantly (ANOVA,  $p < 0.05$ ).  
207 Assumptions were assessed visually by examination of residual plots (Quinn and Keough  
208 2002, Bates et al. 2015). All mixed effect models were constructed in R using *lme4* (Bates et  
209 al. 2015) and Tukey's HSD tests as implemented in library *emmeans* (Lenth 2019) were used  
210 as post hoc tests to compare conditions at each date if IMEM showed a significant effect of  
211 the interaction. Variation in multivariate assemblage structure (abundance data) was  
212 visualized using model-based ordination (generalized linear latent variable model) with the R  
213 package *gllvm* (Niku et al. 2019) and evaluated using multivariate linear modelling with the R  
214 package *mvabund* (Wang et al. 2012), assuming a negative binomial distribution. Species that  
215 contributed significantly to differences among conditions were identified using the *p.uni*  
216 argument that allows univariate 'species-by-species' comparisons in *mvabund*. Variation in  
217 assemblage functional composition between dates and conditions ('traits by site' matrix) was  
218 also compared using the same multivariate approach.

219

## 220 **3. Results**

### 221 *3.1. Taxonomic indices*

222 A total of 2 679 organisms belonging to 118 taxa were identified in the macrofaunal  
223 community in this study. Annelida (36.7%) and Mollusca (35.5%) were the most widely  
224 represented group followed Arthropoda (25.8%). The mollusk *Musculus subpictus* alone  
225 represented 24.2% of the total sample, whereas we found a single individual of 42 taxa, which  
226 were subsequently removed from analyses.

227 A total of 778 nematodes belonging to 41 taxa were identified. Two taxa, *Paracomesma*  
228 *dubium* and *Spirina gerlachi*, represented 34% of the organisms recorded and 6 taxa were

229 represented by a single individual. As this is the first time the nematodes have been described  
 230 for Thau Lagoon, a complete list of taxa is provided in Table S1.

231 Table 3 lists the values of each index averaged per Condition (for all sampling dates).  
 232 Statistical results are those of the Condition effect (pairwise comparison), except in case of a  
 233 significant effect of Date  $\times$  Condition interaction. Although there was a trend of more taxa  
 234 and greater abundance of macrofauna at reference sites, the Condition effect was not  
 235 statistically significant (Table 3). The effect of Condition on the diversity indices was neither  
 236 significant (Table 3).

237 A similar pattern was observed for nematodes, with the greatest abundance and richness  
 238 values observed at reference sites (Table 3). IMEM revealed a significant effect of  
 239 Date  $\times$  Condition on S, H' and J', with contrasts indicating higher values of S and H' in  
 240 December at reference sites ( $S = 11 \pm 3$  and  $H = 2.1 \pm 0.1$ ) compared to farm sites ( $S = 4 \pm 1$   
 241 and  $H = 1.3 \pm 0.2$ ).

242

243 Table 3. Macrofauna and nematodes characteristics (mean  $\pm$  SE, n = 3) under juvenile oyster tables  
 244 (Farm sites) and beyond the farming zone (Reference sites). Abundance (number of individuals, N)  
 245 and richness (number of taxa, S), Shannon diversity (H') and Pielou's evenness (J'). Statistical effect of  
 246 factors (Fc) Condition (Cd: Farm vs Reference) or Date  $\times$  Condition interaction (D  $\times$  Cd). \**Significant*  
 247 *difference at the 0.05 level.*

		<b>Farm</b>	<b>Reference</b>	Fc	df	L-ratio	p
<b>Macrofauna</b>	<b>N</b>	40 $\pm$ 8	106 $\pm$ 16	Cd	3	5.592	0.133
	<b>S</b>	13 $\pm$ 1	21 $\pm$ 2	Cd	3	2.941	0.401
	<b>H'</b>	2.1 $\pm$ 0.1	2.3 $\pm$ 0.1	Cd	3	2.740	0.480
	<b>J'</b>	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1	Cd	3	5.791	0.122
<b>Meiofauna</b>	<b>N</b>	12 $\pm$ 1	31 $\pm$ 5	Cd	3	5.962	0.113
	<b>S</b>	6 $\pm$ 1	10 $\pm$ 1	D $\times$ Cd	5	12.356	0.030*
	<b>H'</b>	1.5 $\pm$ 0.1	1.9 $\pm$ 0.1	D $\times$ Cd	5	20.704	<0.001*
	<b>J'</b>	0.9 $\pm$ 0.1	0.8 $\pm$ 0.1	D $\times$ Cd	5	12.538	0.028*

248

### 249 3.2. Community structure

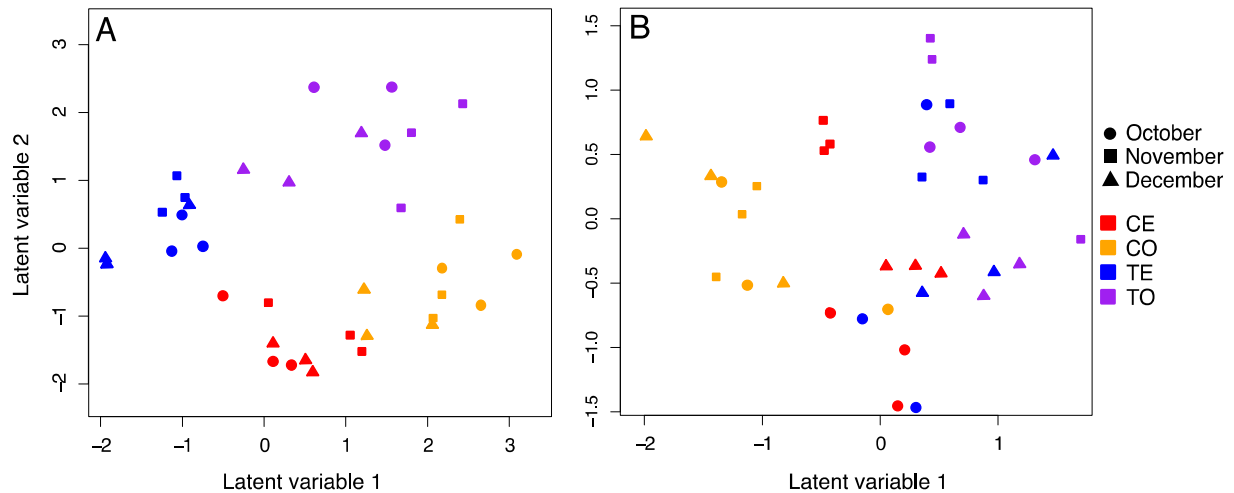
250 The model-based projection of macrofauna community structure (Fig. 2A) showed a clear  
 251 distinction between conditions. The multivariate test confirmed the significant difference  
 252 between communities at the farm vs at the reference sites (Table 4). Univariate comparisons  
 253 were significant for five taxa; three of which were more abundant at the farm sites  
 254 (*Chaetozone gibber*, *Notomastus sp.* and *Neanthes acuminata*) and two that were observed  
 255 only at reference sites (*Phascolion strombus* and *Pusillina lineolata*).

256 The ordination based on nematodes abundance (Fig. 2B) also showed a distinction between  
 257 communities from the two conditions (Farm vs Reference), confirmed by the significance of  
 258 the multivariate test (Table 4). Differences between sites within conditions were less clear  
 259 relative to macrofauna as there was a major overlap of symbols representing samples from  
 260 different sites (especially TO and TE) in the ordination graph (Fig. 2B). Nematode  
 261 assemblages also varied among dates (Table 4). Pairwise comparisons indicated that  
 262 assemblages differed significantly between October and November ( $p = 0.041$ ). Among  
 263 species that best explained variation among sites, *Paracomerosoma dubium*, *Anticomopsis*  
 264 *longicaudata* and *Spirina gerlachi*, were more abundant at the two reference sites.  
 265 Conversely, one taxon was more abundant under farming structures than at the reference sites  
 266 (*Daptonema fallax*) and three taxa were present only at the two farm sites (*Anticoma eberthi*,  
 267 *Eurystomina sp.* and *Metalinhomoeus sp.*).

268  
 269 Table 4. Results of the GLM testing the effect of Date, Condition and their interaction on macrofauna  
 270 and nematode assemblages composition (abundance). \*Significant difference at the 0.05 level.

		df	Dev.	<i>p</i>
<b>Macrofauna</b>	Condition	1	341.3	0.001*
	Date	2	214.7	0.109
	Condition × Date	2	121.2	0.089
<b>Meiofauna</b>	Condition	1	141.83	0.001*
	Date	2	125.58	0.019*
	Condition × Date	2	78.07	0.055

271  
 272  
 273



274

275 Figure 2. Model based biplot (unconstrained ordination) of abundance data for (A) macrofauna and  
 276 (B) nematodes. Samples (cores) are shown in colors indexed by Site and Date (symbols).

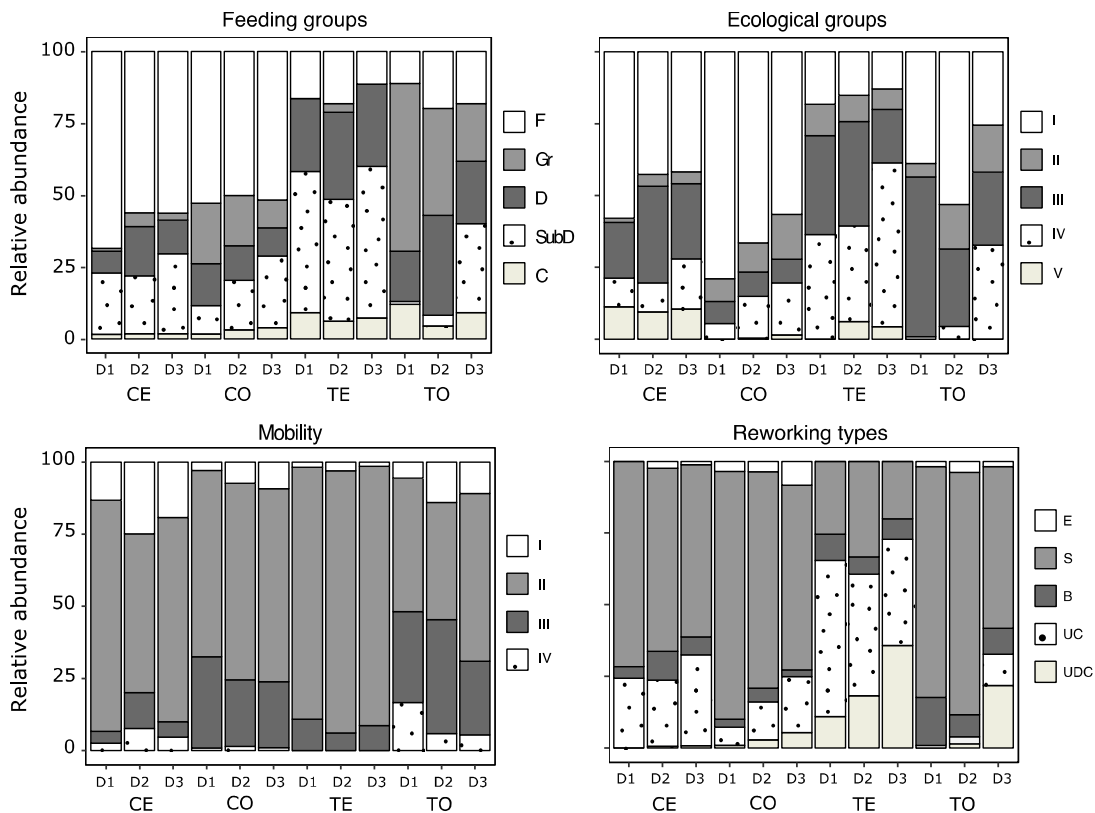
277

### 278 3.3. Functional analysis

#### 279 3.3.1 Macrofauna functional composition

280 Figure 3 shows the relative abundance of each biological trait. Although there was some  
 281 variability within conditions and between dates, macrofauna at the reference sites was mainly  
 282 characterized by filter-feeder organisms (ca 50%: Fig. 3), which are mostly sensitive to  
 283 organic enrichment (ecological group I, Fig. 3). The proportion of filter-feeders was lower in  
 284 the farming zone, whereas the proportion of deposit feeders and grazers increased (Fig. 3).  
 285 These communities were dominated by animals that are mostly tolerant to organic enrichment  
 286 or first-order opportunistic (ecological groups III and IV: Fig. 3). Mobility and reworking  
 287 types did not allow us to clearly distinguish farming and reference sites (Fig. 3), except that  
 288 upward-downward conveyors (UC and UDC: Fig. 3) appeared to be more abundant at the  
 289 farm sites. The high abundance of surficial reworkers (S) at site TO (Fig. 3) was mainly due  
 290 to the presence of many Arthropoda, also belonging to grazers (Gr) and ecological group I,  
 291 and the the bivalve *Musculus subpictus*. These taxa were mainly found in association with the  
 292 algae *Halopithys* sp. The GLM indicated a significant effect of Condition on the functional

293 composition of assemblage ( $df = 1$ ,  $Dev. = 225.45$ ,  $p < 0.001$ ), with no significant effect of  
 294 the interaction (Date  $\times$  Condition,  $df = 2$ ,  $Dev. = 48.39$ ,  $p = 0.393$ ).



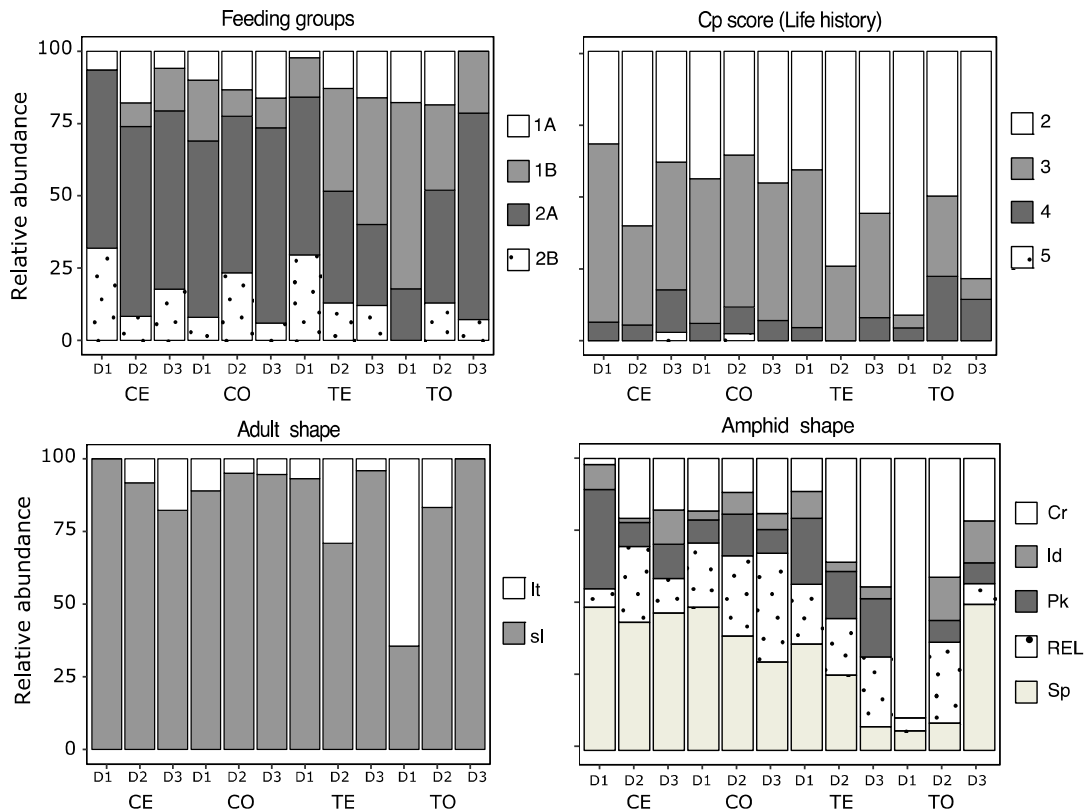
295  
 296 Figure 3. Relative abundance of the different macrofauna functional groups at the farm sites (TE, TO)  
 297 and at the reference sites (CE, CO) on the three sampling dates (D1: October, D2: November, D3:  
 298 December). Feeding groups: F: filter-feeder, Gr: grazer, D: deposit-feeder, SubD: subsurface deposit-  
 299 feeder, C: carnivorous; Ecological groups from very sensitive (I) to opportunistic (V) ; Mobility from  
 300 fixed (I) to burrower (IV) ; Reworking types: E: epifauna, S: surficial modifier, B: biodiffuser, UC:  
 301 upward conveyor, UDC: up-/downward conveyor.

302  
 303

### 304 3.3.2. Nematode functional composition

305 The most representative feeding group at reference sites was epigrowth feeders (2A) whereas  
 306 the percentage of detritivores (1B) was increased at the farm sites (Fig. 4). Overall, the  
 307 community at the two reference sites was dominated by taxa with a cp score of 3 (Fig. 4) and  
 308 amphids with a spiral shape (Sp: Fig. 4). At the two farm sites, the percentage of opportunistic  
 309 nematodes (cp score of 2: Fig. 4) with circular amphid (Cr) increased (Fig. 4). The adult  
 310 shape did not clearly differ between farming zone sites and those outside the farming zone  
 311 (Fig. 4). The high percentage of long/thin adults (lt) at site TO in September was mainly due

312 to the presence of *Metalinhomoeus longiseta*. The GLM indicated a significant effect of  
 313 Condition on assemblage functional composition (df = 1, Dev. = 154.49,  $p < 0.001$ ), with no  
 314 significant effect of the interaction (Date  $\times$  Condition, df = 2, Dev. = 48.22,  $p = 0.212$ ).  
 315



316  
 317 Figure 4. Relative abundance of the different nematode functional groups at the farm sites (TE, TO)  
 318 and at the reference sites (CE, CO) on the three sampling dates (D1: October, D2: November, D3:  
 319 December). Feeding groups: 1A: selective or 1B: non selective deposit-feeder, 2A: epigrowth feeder,  
 320 2B: predators; Life history: opportunistic (2) to persister (5); Adult shape: lt: long/thin, sl: slender ;  
 321 Amphid shape: Cr: circular, Id: indistinct, Pk: pocket-like, REL: rounded or elongate, Sp: spiral.  
 322

### 323 3.3.2. Functional indices

324 The lowest FRic values of both macrofauna and nematodes were recorded under the tables  
 325 (TE and TO) (Table 5). The Date  $\times$  Condition interaction had a significant effect for  
 326 nematodes, with contrasts indicating significantly higher FRic values at reference sites in  
 327 December ( $9.2 \pm 1.6$ ) compared with the farm sites ( $4 \pm 0.6$ ). Macrofauna FEve was higher  
 328 under the tables in November ( $0.72 \pm 0.06$ ) and December ( $0.72 \pm 0.04$ ) than at the reference  
 329 sites ( $0.57 \pm 0.04$  and  $0.56 \pm 0.04$  respectively in November and December) whereas no



330 difference was found for nematodes. The Date  $\times$  Condition interaction had a significant effect  
 331 on nematodes FDis but with no difference between conditions each date.

332 The ITD for macrofauna was significantly lower at the farm sites (Table 5) indicating that  
 333 these sites presented a higher trophic diversity than did reference sites. For nematodes, the  
 334 Date  $\times$  Condition interaction had a significant effect on ITD but no difference was found  
 335 between conditions at each date.

336

337 Table 5. Macrofauna and nematodes functional characteristics (mean  $\pm$  SE, n = 3) at sites under the juvenile  
 338 oyster tables (Farm sites) and outside the farming zone (Reference sites). (FRic: functional richness, FEve:  
 339 functional evenness, FDis: functional dispersion, ITD: Index of Trophic Diversity). Statistical effect of factors  
 340 (Fc) Condition (Cd: Farm vs Reference) or Date  $\times$  Condition interaction (D  $\times$  Cd) \*Significant difference at the  
 341 0.05 level.

		<b>Farm</b>	<b>Reference</b>	Fc	df	L-ratio	<i>p</i>
<b>Macrofauna</b>	<b>FRic</b>	8 $\pm$ 1	12 $\pm$ 1	D $\times$ Cd	5	10.657	0.05
	<b>FEve</b>	0.7 $\pm$ 0.1	0.5 $\pm$ 0.1	D $\times$ Cd	5	11.805	<0.05*
	<b>FDis</b>	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	Cd	3	1.520	0.677
	<b>ITD</b>	0.4 $\pm$ 0.1	0.7 $\pm$ 0.1	Cd	3	9.237	<0.05*
<b>Meiofauna</b>	<b>FRic</b>	6 $\pm$ 1	9 $\pm$ 1	D $\times$ Cd	5	12.326	<0.05*
	<b>FEve</b>	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	Cd	3	2.481	0.479
	<b>FDis</b>	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	D $\times$ Cd	5	13.69	0.017*
	<b>ITD</b>	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	D $\times$ Cd	5	13.043	<0.05*

342

343

#### 344 4. Discussion

##### 345 4.1. First description of nematodes in Thau Lagoon

346 With the exception of marine benthic ecosystems in Italy and Tunisia (Semprucci et al. 2008,  
 347 Boufahja et al. 2014), there is a serious gap in knowledge concerning the Mediterranean  
 348 meiofauna. This is particularly true for coastal lagoons, for which the spatial and temporal  
 349 distribution of meiofauna, including free-living nematodes, is not well documented or is  
 350 underestimated, despite they often greatly contribute to the biodiversity of coastal areas  
 351 (Balsamo et al. 2012). Nematodes can provide critical information on the functioning of  
 352 ecosystems and have already been proposed as potential indicators of anthropogenic

353 disturbance (Schratzberger et al. 2004, Danovaro et al. 2008, Moreno et al. 2011, Pusceddu et  
354 al. 2011). Our study is the first to provide a complete list of nematode taxa observed in the  
355 French Mediterranean Thau lagoon. Compared to the few studies that have documented other  
356 Mediterranean coastal areas, the number of taxa reported here from Thau Lagoon is similar  
357 but abundances were in the lower range (Mirto et al. 2000, Danovaro et al. 2004, Mahmoudi  
358 et al. 2008). The community structure showed many similarities with checklists of Italian and  
359 Tunisian lagoons, published respectively by Semprucci et al. (2008) and Boufahja et al.  
360 (2014). Ten of the common species found in Thau Lagoon have also been found in Italy (e.g.  
361 *Anticoma eberthi*, *Longicyatholaimus longicaudatus*) and 18 species were recorded in both  
362 Thau Lagoon and Tunisia (e.g. *Spirinia gerlachi*, *S. parasitifera*, *Thalassironus britannicus*,  
363 for details, see Table S1). These shared records could be linked to common environmental  
364 parameters among areas and to water exchanges between the two Mediterranean basins,  
365 which make large-scale dispersal between Tunisia, Italy and France possible. The checklist of  
366 nematodes resulting from this study could be the basis to further documenting meiobenthic  
367 diversity in Mediterranean coastal areas, with the aim to advance our understanding of its  
368 spatio-temporal variability.

369

#### 370 4.2. Response of macrofaunal and nematode assemblages to shellfish culture

371 Overall, community structure (taxonomic and functional) differed between conditions, with  
372 lower abundance and richness (some significant, some not) at farm sites, as expected from  
373 previous studies (Mirto et al. 2000, Christensen et al. 2003, Callier et al. 2008). The presence  
374 of opportunistic species under the farm tables (e.g. *Neanthes acuminata*, *Prionospio fallax*,  
375 *Daptonema* sp.) whose abundance is favored under organically enriched conditions, showed  
376 that biodeposition by juvenile oysters impacts the benthic environment below farm structures  
377 in the study area. However, the high and similar species evenness values (J) at the farm and

378 reference sites revealed the absence of strongly dominant species that would be expected in an  
379 organically enriched environment (Pearson and Rosenberg 1978). The organic enrichment  
380 due to the presence of juvenile oysters is likely low compared with that of adult oysters, but  
381 the continuous exploitation of the area for farming may have modified community  
382 characteristics compared with outside of the farming zone.

383 The results of the analysis of nematodes revealed differences in both univariate indices and  
384 community structure at the farm and reference sites. Our results for nematodes are in  
385 agreement with the study of Mirto et al. (2000) who reported decreased meiofauna richness  
386 and abundance in aquaculture sites relative to reference areas (Mirto et al. 2000). Inversely, a  
387 study in Bizerta Lagoon indicated increased nematode abundance at farm sites relative to  
388 control sites (Mahmoudi et al. 2008). The latter authors suggested that increased abundance  
389 was due to differential tolerance to organic matter, decreased competition, and a subsequent  
390 competitive release of more resistant species due to sediment organic enrichment. However,  
391 other factors (biotic and abiotic) could also explain the distribution of species since, for  
392 example, *Paracomesma dubium* was more abundant at farm sites in the study by Mahmoudi  
393 et al. (2008), whereas in our study, we observed a higher abundance at reference sites.  
394 Temperature variations may have contributed to explain temporal changes since when  
395 significant, the effect of Condition was mostly detected in December.

396 In this study, macrofaunal taxonomic indices failed to detect a significant effect of shellfish  
397 culture whereas the multivariate approach did, as already reported in other studies (Grant et  
398 al. 2012, Lacoste et al. 2018a). Previous studies in Thau Lagoon produced conflicting results  
399 on the impact of aquaculture on macrofauna, ranging from low (Mazouni et al. 1996, Duport  
400 et al. 2007) to severe with a completely defaunated site in a deep shellfish area (Thouzeau et  
401 al. 2007). These authors highlighted the concomitant role of depth, algal cover and  
402 aquaculture in community composition. Our study also suggests that algal cover played an

403 important role, at least for the macrofauna community. Some arthropods, (e.g. *Gammarella*  
404 sp., *Cymodoce truncata*) were found attached to the algae *Halopithys* sp., so the TO and CO  
405 sites had specific assemblages structure due to these taxa.

406 The general trend thus supports the hypothesis that juvenile oysters slightly influenced both  
407 macrobenthic and nematode communities, although the variability between sites within  
408 conditions may have masked statistical differences in some indicators. Although this may  
409 require a labor-intensive sampling effort, it is recommended to sample several farm and  
410 reference sites to disentangle the influence of aquaculture on benthic communities, if such an  
411 influence exists, from the natural variability. This is particularly important in transitional  
412 waters as coastal lagoons where high natural variability may exist due to rapid environmental  
413 changes.

414

#### 415 *4.3. Insights from the functional approach*

416 To date, the effect of aquaculture on benthic communities has usually been evaluated using  
417 traditional taxonomic approaches (Lacoste et al. 2020). Here, we demonstrate that some  
418 species traits can be used to predict the response of macro- and meiofauna to disturbances  
419 caused by shellfish culture since the distribution of traits and functional indices performed  
420 well in distinguishing between the farm and reference sites.

421 For both assemblages, trophic group and ecological group/life history were the traits that best  
422 responded to the presence of oyster tables. As reported in other studies, the presence of  
423 aquaculture facilities often decreases the abundance and diversity of filter-feeding organisms  
424 (Forrest and Creese 2006, Dubois et al. 2007, Fabi et al. 2009) whereas increased organic  
425 supply in farm zones favors the presence of non-selective deposit feeders. Following the  
426 classification of Grall and Glemarec (1997) and Borja et al. (2000), farming zones favored the  
427 presence of tolerant and opportunistic taxa whereas reference sites were dominated by

428 sensitive organisms. Among the meiofauna, epigrowth feeders were the most abundant  
429 organisms at reference sites and were replaced by non-selective deposit feeders in the farming  
430 zone. The reduction in the abundance of epigrowth feeders at the farm sites may be due to  
431 excluding competition with other groups but we can also hypothesize a lower abundance of  
432 diatoms under oyster tables due to a shading effect (McKindsey et al. 2011).

433 Amphids are complex sensorial organs of nematodes used in the search of food and partners,  
434 and are useful for taxonomical identification. To date, there is no information linking amphids  
435 to a particular type of marine environment, except Semprucci et al. (2018), who described a  
436 potential link between inconspicuous amphids and rich food sources areas. Accordingly, our  
437 results suggest that the more intense and regular food supply at farm sites may limit the  
438 advantage of large amphids (REL, Sp) and hence that nematodes with smaller amphids (Cr)  
439 are more abundant in farming zones. However, this preliminary result requires further  
440 investigation to highlight potential links between morphological features of nematodes and  
441 their marine habitat.

442 Functional diversity (FD) indices were computed for macrofauna and nematodes to evaluate  
443 the relevance of these indices in the case of aquaculture-induced disturbances. Nematode  
444 FRic was reduced at the farm sites and FD indices revealed the influence of aquaculture on  
445 macrofauna assemblages that was not detected using taxonomic indices. This confirms the  
446 usefulness of the functional approach, and more generally of multi-index approaches to detect  
447 the influence of farming (Borja et al. 2009, Lacoste et al. 2019). Interestingly, macrofauna FD  
448 indices indicated a higher trophic diversity and functional evenness at farm sites whereas no  
449 changes were detected at the richness level. The actual level of disturbance may therefore  
450 have shifted community composition that promoted a more even use of the trophic niche,  
451 which, in turn, may have increased the productivity of the system (Mason et al. 2005).  
452 Compared with the taxonomic approach, functional analysis not only offers the opportunity to

453 study the response of communities to the environment but also the subsequent impact of the  
454 changes on ecosystem properties (Lavorel and Garnier 2002, Lacoste et al. 2020), which is  
455 essential to evaluate the sustainable exploitation of aquaculture systems.

456

#### 457 *4.4 Limitation and future outlook of the functional approach*

458 This work provides insights into the functional response of benthic communities to  
459 aquaculture-induced disturbance. We proposed the use of four selected traits that may respond  
460 both for macrofauna and nematode assemblages, that we assumed referred to similar  
461 behavior. The choice of biological traits may strongly influence the calculation of functional  
462 indices (Petchey and Gaston 2006). It was not our objective to test the most appropriate traits  
463 to use from a wide selection of possible choices. Rather we wished to highlight the potential  
464 of functional analysis to evaluate the impact of aquaculture using various kinds of benthic  
465 organisms (macrofauna and nematodes) and traits that have already been shown to respond to  
466 organic enrichment (Borja et al. 2000, Alves et al. 2013, KandrataVICIUS et al. 2018, Lacoste  
467 et al. 2019). Future experiments that examine multiple traits in different regions and systems  
468 will be needed to evaluate if functional analysis is a reliable tool to assess the benthic impacts  
469 of aquaculture.

470 The number of taxa and the completeness and accuracy of the species trait data play a  
471 significant role in the estimation of functional trait metrics (Pakeman and Quested 2007,  
472 Pakeman 2014). In this exploratory study, we chose to use the maximum number of taxa for  
473 which traits were available. However, the inclusion of arthropods, for example, is  
474 questionable since it appears that these organisms were mostly associated with *Halopithys* sp.  
475 algae and are very mobile, so they could also have been classified as epifauna instead of  
476 infauna and disregarded. When we excluded them from the functional analysis (data not

477 shown), we obtained differences in the representation of relative abundances based on traits,  
478 but no difference with respect to FD indices.

479 Although further studies are needed to identify the traits that react most consistently in a  
480 given manner or the taxonomic groups to considered, this work provides interesting  
481 information on the potential of the trait based approach to detect disturbances caused by  
482 aquaculture. Along with theoretical work, we think that further studies encompassing  
483 different kinds of disturbance across ecosystems will enable the implementation of a  
484 framework intended to include the functional approach in benthic monitoring programs.

485

## 486 **Conclusion and Perspectives**

487 This study documented the macrobenthic and the nematode communities in relation with  
488 juvenile oyster culture in Thau Lagoon. The trait-based approach we propose in this work  
489 shows the feasibility of detecting the impact of shellfish culture on the functional structure of  
490 both macro- and meiofauna communities in coastal lagoons, although further studies are  
491 needed to identify general patterns, within and across biogeographical regions and  
492 aquaculture systems. Combining taxonomic and functional approaches may add relevant  
493 ecological information and help obtain a clearer understanding of the functioning of benthic  
494 communities that are impacted by anthropogenic activities. For aquaculture, as well as other  
495 human-induced disturbance, the functional approach will undoubtedly enhance our ability to  
496 monitor activities and aid compliance with conservation strategies such as the Water  
497 Framework Directive Strategy (EC 2000), which requires an understanding of both how  
498 function varies spatially and how it responds to human disturbances. Several gaps that  
499 represent many research opportunities should however be addressed to 'operationalize' such  
500 approach. As examples i) new tools such as eDNA would allow to get rapid identification of

501 organisms once taxonomic validation of database will be improved, ii) further description of  
502 benthic organisms biological and ecological traits should be completed to feed databases.

503

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515

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