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

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Abstract

Shellfish aquaculture has the potential to alter benthic assemblage composition with subsequent modifications of ecosystem functioning. While the impacts of aquaculture on the taxonomic structure of macro- and — to a lesser extent — meiofauna, have been widely studied, the functional changes of these communities remain relatively unknown. The recent development of biological trait analysis (BTA) has made it possible to produce information about how ecosystem functioning could change across specific terrestrial or aquatic system.

In the present study, we used a BTA in parallel with standard taxonomic analysis to evaluate how well the two approaches detected the potential influence of juvenile oyster culture on the benthic community in the French Mediterranean Thau Lagoon. Two sites were sampled under farm structures and compared with two reference sites beyond the influence of farming. This study is the first detailed parallel description of macro- and meiofauna (nematodes). A total of 118 and 41 taxa were determined for macrofauna and nematodes respectively. Some taxa were more abundant or exclusively observed under farm structures such as Chaetozone gibber.
and *Neanthes acuminata* for macrofauna and *Daptonema fallax* and *Anticoma eberthi* for nematodes.

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

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

### 1. Introduction

Coastal ecosystems are facing drastic environmental changes due to many human pressures that threat the integrity of communities and may have major impacts on ecosystem functioning and its ability to sustain ecosystem services (Halpern et al. 2008, Cardinale et al. 2012). In shallow costal ecosystems, benthic communities play a central ecological role in benthic-pelagic coupling through their feeding and movements that promote nutrient and oxygen exchange throughout the sediment layer. Thus, any alteration of these communities may have implications for ecosystem structure and performance (Thrush et al. 2006, Kristensen et al. 2014) and understanding how they respond to disturbance is crucial to predict future changes and help maintain and improve the quality of marine ecosystems. To date, most studies have focused on the response of benthic macrofaunal communities to
disturbances (Pratt et al. 2015, Dimitriou et al. 2017, Ellis et al. 2017). Recently, interest has been growing in the study of meiofauna, since it has been shown that these organisms may modulate biological interactions in the sediment layer (Bonaglia et al. 2014, Lacoste et al. 2018a) and play a significant role in benthic ecosystem processes and services (Schratzberger and Ingels 2017). Due to their widespread distribution, short life and reproductive cycles, high abundance and diversity, meiofauna are thus beginning to be used as biological indicators for the assessment and monitoring of aquatic ecosystems (Semprucci et al. 2015, Zeppilli et al. 2015, Bianchelli et al. 2018).

Bivalve aquaculture has various environmental impacts (McKindsey et al. 2011, Brenner et al. 2014) including the accumulation of biodeposits on the bottom under culture sites that may result in local organic enrichment. Such disturbance typically affect benthic communities according to the Pearson and Rosenberg (1978) model of organic enrichment with a progressive increase in the abundance of enrichment-tolerant species in the vicinity of farms, whereas communities directly under the farming structures are dominated by a few opportunistic species (Callier et al. 2007, 2009, McKindsey et al. 2011). Several studies have reported lower richness and/or abundance of organisms, as well as differential community composition of macrofauna (Christensen et al. 2003, Harstein and Rowden 2004, Lacoste et al. 2018b), or meiofauna (Mirto et al. 2000, Mahmoudi et al. 2008, Netto and Valgas 2010) under farming structures compared with reference sites. One notable feature of these studies is the focus on structural attributes of the benthos (e.g. taxonomic composition, number of species) whereas functional changes are disregarded. Only a few studies have used a functional approach to investigate the impact of interactions between the environment and fish (Papageorgiou et al. 2009, Dimitriadis and Koutsoubas 2011), or mussel (Lacoste et al. 2019) aquaculture.
Growing awareness that changes in biodiversity may modify ecosystem functioning (e.g. Loreau et al. 2001, Naeem et al. 2009) has led to the recognition that impacts on the functional diversity of benthic assemblages, as opposed to their structural features, should be better quantified (e.g. Strong et al. 2015, Cernansky 2017). Due to functional redundancies in communities, observed changes in species composition may be buffered (Walker 1992, Snelgrove 1998) such that structural impacts on benthic assemblages are not always matched by their functional counterparts (Bolam 2012). Otherwise, trophic pathways (indicator of ecosystem functioning) may be impacted without apparent changes of the overall benthic diversity (Dubois et al. 2007). Thus, functional-based approaches, including biological trait analysis and the computation of functional diversity (FD) indices, provide useful proxies to investigate the effect of human disturbances at the ecosystem-functioning level (Mouillot et al. 2006, 2013) and to anticipate the potential loss of ecosystem services associated with biodiversity erosion (Cardinale et al. 2012). Moreover, this approach can be used to compare communities that have no species in common but do share traits.

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

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

2. Materials and Methods

2.1. Study area and sampling strategy

Thau Lagoon is a French Mediterranean semi-enclosed shallow lagoon (mean depth 4 m) covering an area of 68 km² (Fig. 1). It is the most important French Mediterranean shellfish farming zone with an annual production of about 7 330 tons of oysters in 2016 (DDTM34 2017) reared on spatially organized structures, called “tables”. Each table is 50 × 12 m long and supports between 800 and 1200 suspended nets or ropes holding juvenile or adult oysters. Sixteen tables form a ‘block’ and the blocks are separated from each other by a distance of 150m. Annual variations in water temperature and salinity in the lagoon are large, varying from 3 °C to 30 °C and from 33 to 40 PSU respectively (Messiaen et al. 2019).

The study was conducted in a dedicated shellfish-farming zone in the western part of the lagoon (Fig. 1). As a part of a research program (see Richard et al. 2017, 2019), the study focused on juvenile production, whose impact on ecosystem has never previously been evaluated in Thau Lagoon. Samples were collected at two sites in the farmed zone directly below juvenile tables (TO and TE sites) and at two additional sites located at a distance of
200 m outside the farming zone (CO and CE sites) (Fig. 1). Some characteristics of the four sites are given in Table 1, the main difference being algal cover (*Halopytis* sp.) which was greater at TO and CO sites. Sampling was done during three surveys in 2015 (September 29–October 2; October 28–November 4 and December 1–December 4, hereafter referred to as October, November and December) covering the whole juvenile production period. Each date, three replicate sediment cores (9 cm diameter, 10 cm depth) were randomly collected by scuba divers at each site. The samples were preserved in 10% buffered formalin and later transferred into 70% alcohol.

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

Table 1. Summary description of the 4 sampled sites

<table>
<thead>
<tr>
<th></th>
<th>Reference sites</th>
<th>Farm sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO</td>
<td>CE</td>
</tr>
<tr>
<td>Depth</td>
<td>3.7 m</td>
<td>4.2 m</td>
</tr>
<tr>
<td>Bottom observation</td>
<td><img src="image1" alt="Reference bottom" /></td>
<td><img src="image2" alt="Reference bottom" /></td>
</tr>
<tr>
<td>Culture system</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Calculation from initial measurements on a sub-sample
2.2. Abundance and species richness

Back to the laboratory, the content of each core sample was passed through a 1mm mesh sieve. Macrofauna in the > 1mm fraction was sorted and identified to the lowest taxonomic resolution possible, up to species in most cases. The < 1 mm fraction was decanted over a 40-µm sieve. All nematodes were sorted under a stereo-microscope, transferred to glycerol through a series of ethanol-glycerine solutions, mounted on glycerine slides (Somerfield et al. 1994) and identified to the lowest taxonomic level possible using the generic keys of Platt and Warwick (1983, 1988) and Warwick et al. (1998) and species descriptions in the literature.

Abundance (N) and species richness (S) of macrofauna and nematodes were characterized. The Shannon-Wiener diversity index (H’) and Pielou’s evenness (J’) were calculated for each site. Taxa that were observed only once were removed from analyses (Clarke and Warwick 2001); all results are expressed per core (63 cm²).

2.3. Functional analysis

The common step in all functional ecology studies is to characterize the functional strategy of each species of interest by identifying a relevant combination of functional traits (Violle et al. 2007). There is currently no consensus on the methodology for selecting the most appropriate traits for a given study (Marchini et al. 2008). As the aim of the present study was to use traits as a proxy for assemblage response to shellfish culture, we decided to focus on the biological traits and ecological groups of macrofauna that have already been shown to be good indicators of organic enrichment in this specific context (Borja et al. 2000, Lacoste et al. 2019). To our knowledge, this is the first study to simultaneously evaluate the functional response of both macrofauna and meiofauna (nematodes) to bivalve aquaculture. We thus selected nematodes biological traits that have been shown to respond to organic enrichment.
(Alves et al. 2013, Kandratavicius et al. 2018), with the assumption that they are comparable with those of macrofauna. Thus, selected traits for both assemblages were related to trophic guilds, ecological strategy, and interaction with sediments (Table 2). Each selected trait was subdivided into several modalities (Table 2). Organisms identified up to species level were classified in functional groups based on existing literature (e.g. Wieser 1953, Thistle et al. 1995, Queiros et al. 2013, WoRMS Editorial Board 2018). When reliable information was not available, data from the closest phylogenetic neighbor were used or the taxon was excluded from subsequent analysis. All nematodes and 44 macrofaunal (accounting for 91% of all individuals identified) taxa were thus retained for functional analysis. The subsequent ‘trait per taxon’ matrix was combined with the ‘taxa per sample’ matrix (abundance) to create a ‘trait per sample’ matrix. The relative abundance of the functional groups was then calculated by summing abundance across all taxa allocated to each functional group at a given site per sampling date. Finally, the two matrices were used to calculate functional indices (functional richness: FRic, functional eveness: FEve and functional dispersion: FDis) as outlined by Villéger et al. (2008) and Laliberté and Legendre (2015) for both macrofauna and nematodes. Using only categorical traits, FRic was defined as the number of unique trait combinations (equivalent to singular species).

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

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

2.4. Statistical analysis

The effect of Date × Condition (Farm vs Reference) on univariate measurements of benthic communities (taxonomic and functional indices) was tested using linear mixed effect models (lMEM; modelling normally distributed responses) with site as a random factor nested in
Condition. The effect of factors was considered as significant when the null vs main effect models (using maximum likelihood estimation) differed significantly (ANOVA, $p < 0.05$). Assumptions were assessed visually by examination of residual plots (Quinn and Keough 2002, Bates et al. 2015). All mixed effect models were constructed in R using lme4 (Bates et al. 2015) and Tukey’s HSD tests as implemented in library emmeans (Lenth 2019) were used as post hoc tests to compare conditions at each date if lMEM showed a significant effect of the interaction. Variation in multivariate assemblage structure (abundance data) was visualized using model-based ordination (generalized linear latent variable model) with the R package gllvm (Niku et al. 2019) and evaluated using multivariate linear modelling with the R package mvabund (Wang et al. 2012), assuming a negative binominal distribution. Species that contributed significantly to differences among conditions were identified using the p.uni argument that allows univariate ‘species-by-species’ comparisons in mvabund. Variation in assemblage functional composition between dates and conditions (‘traits by site’ matrix) was also compared using the same multivariate approach.

3. Results

3.1. Taxonomic indices

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

A total of 778 nematodes belonging to 41 taxa were identified. Two taxa, Paracomesma dubium and Spirina gerlachi, represented 34% of the organisms recorded and 6 taxa were
represented by a single individual. As this is the first time the nematodes have been described for Thau Lagoon, a complete list of taxa is provided in Table S1.

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

A similar pattern was observed for nematodes, with the greatest abundance and richness values observed at reference sites (Table 3). lMEM revealed a significant effect of Date × Condition on S, H’ and J’, with contrasts indicating higher values of S and H’ in December at reference sites (S = 11 ± 3 and H = 2.1 ± 0.1) compared to farm sites (S = 4 ± 1 and H = 1.3 ± 0.2).

Table 3. Macrofauna and nematodes characteristics (mean ± SE, n = 3) under juvenile oyster tables (Farm sites) and beyond the farming zone (Reference sites). Abundance (number of individuals, N) and richness (number of taxa, S), Shannon diversity (H’) and Pielou’s eveness (J’). Statistical effect of factors (Fc) Condition (Cd: Farm vs Reference) or Date × Condition interaction (D × Cd). *Significant difference at the 0.05 level.

<table>
<thead>
<tr>
<th></th>
<th>Farm</th>
<th>Reference</th>
<th>Fc</th>
<th>df</th>
<th>L-ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrofauna</td>
<td>40±8</td>
<td>106±16</td>
<td>Cd</td>
<td>3</td>
<td>5.592</td>
<td>0.133</td>
</tr>
<tr>
<td></td>
<td>13±1</td>
<td>21±2</td>
<td>Cd</td>
<td>3</td>
<td>2.941</td>
<td>0.401</td>
</tr>
<tr>
<td></td>
<td>2.1±0.1</td>
<td>2.3±0.1</td>
<td>Cd</td>
<td>3</td>
<td>2.740</td>
<td>0.480</td>
</tr>
<tr>
<td></td>
<td>0.8±0.1</td>
<td>0.8±0.1</td>
<td>Cd</td>
<td>3</td>
<td>5.791</td>
<td>0.122</td>
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<tr>
<td>Meiofauna</td>
<td>12±1</td>
<td>31±5</td>
<td>Cd</td>
<td>3</td>
<td>5.962</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td>6±1</td>
<td>10±1</td>
<td>D×Cd</td>
<td>5</td>
<td>12.356</td>
<td>0.030*</td>
</tr>
<tr>
<td></td>
<td>1.5±0.1</td>
<td>1.9±0.1</td>
<td>D×Cd</td>
<td>5</td>
<td>20.704</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>0.9±0.1</td>
<td>0.8±0.1</td>
<td>D×Cd</td>
<td>5</td>
<td>12.538</td>
<td>0.028*</td>
</tr>
</tbody>
</table>

3.2. Community structure

11
The model-based projection of macrofauna community structure (Fig. 2A) showed a clear distinction between conditions. The multivariate test confirmed the significant difference between communities at the farm vs at the reference sites (Table 4). Univariate comparisons were significant for five taxa; three of which were more abundant at the farm sites (Chaetozone gibber, Notomastus sp. and Neanthes acuminata) and two that were observed only at reference sites (Phascolion strombus and Pusillina lineolata).

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

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

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Dev.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macrofauna</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condition</td>
<td>1</td>
<td>341.3</td>
<td>0.001*</td>
</tr>
<tr>
<td>Date</td>
<td>2</td>
<td>214.7</td>
<td>0.109</td>
</tr>
<tr>
<td>Condition × Date</td>
<td>2</td>
<td>121.2</td>
<td>0.089</td>
</tr>
<tr>
<td><strong>Meiofauna</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condition</td>
<td>1</td>
<td>141.83</td>
<td>0.001*</td>
</tr>
<tr>
<td>Date</td>
<td>2</td>
<td>125.58</td>
<td>0.019*</td>
</tr>
<tr>
<td>Condition × Date</td>
<td>2</td>
<td>78.07</td>
<td>0.055</td>
</tr>
</tbody>
</table>
Figure 2. Model based biplot (unconstrained ordination) of abundance data for (A) macrofauna and (B) nematodes. Samples (cores) are shown in colors indexed by Site and Date (symbols).

3.3. Functional analysis

3.3.1 Macrofauna functional composition

Figure 3 shows the relative abundance of each biological trait. Although there was some variability within conditions and between dates, macrofauna at the reference sites was mainly characterized by filter-feeder organisms (ca 50%: Fig. 3), which are mostly sensitive to organic enrichment (ecological group I, Fig. 3). The proportion of filter-feeders was lower in the farming zone, whereas the proportion of deposit feeders and grazers increased (Fig. 3). These communities were dominated by animals that are mostly tolerant to organic enrichment or first-order opportunistic (ecological groups III and IV: Fig. 3). Mobility and reworking types did not allow us to clearly distinguish farming and reference sites (Fig. 3), except that upward-downward conveyors (UC and UDC: Fig. 3) appeared to be more abundant at the farm sites. The high abundance of surficial reworkers (S) at site TO (Fig. 3) was mainly due to the presence of many Arthropoda, also belonging to grazers (Gr) and ecological group I, and the the bivalve Musculus subpictus. These taxa were mainly found in association with the algae Halopithys sp. The GLM indicated a significant effect of Condition on the functional
composition of assemblage (df = 1, Dev. = 225.45, \( p < 0.001 \)), with no significant effect of the interaction (Date × Condition, df = 2, Dev. = 48.39, \( p = 0.393 \)).

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

3.3.2. Nematode functional composition

The most representative feeding group at reference sites was epigrowth feeders (2A) whereas the percentage of detritivores (1B) was increased at the farm sites (Fig. 4). Overall, the community at the two reference sites was dominated by taxa with a cp score of 3 (Fig. 4) and amphids with a spiral shape (Sp: Fig. 4). At the two farm sites, the percentage of opportunistic nematodes (cp score of 2: Fig. 4) with circular amphid (Cr) increased (Fig. 4). The adult shape did not clearly differ between farming zone sites and those outside the farming zone (Fig. 4). The high percentage of long/thin adults (It) at site TO in September was mainly due
to the presence of *Metalinhomoeus longiseta*. The GLM indicated a significant effect of Condition on assemblage functional composition (df = 1, Dev. = 154.49, $p < 0.001$), with no significant effect of the interaction (Date × Condition, df = 2, Dev. = 48.22, $p = 0.212$).

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

### 3.3.2. Functional indices

The lowest FRic values of both macrofauna and nematodes were recorded under the tables (TE and TO) (Table 5). The Date × Condition interaction had a significant effect for nematodes, with contrasts indicating significantly higher FRic values at reference sites in December (9.2 ± 1.6) compared with the farm sites (4 ± 0.6). Macrofauna FEve was higher under the tables in November (0.72 ± 0.06) and December (0.72 ± 0.04) than at the reference sites (0.57 ± 0.04 and 0.56 ± 0.04 respectively in November and December) whereas no
difference was found for nematodes. The Date × Condition interaction had a significant effect on nematodes FDis but with no difference between conditions each date. The ITD for macrofauna was significantly lower at the farm sites (Table 5) indicating that these sites presented a higher trophic diversity than did reference sites. For nematodes, the Date × Condition interaction had a significant effect on ITD but no difference was found between conditions at each date.

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

<table>
<thead>
<tr>
<th></th>
<th>Farm</th>
<th>Reference</th>
<th>Fc</th>
<th>df</th>
<th>L-ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrofauna FRic</td>
<td>8±1</td>
<td>12±1</td>
<td>D×Cd</td>
<td>5</td>
<td>10.657</td>
<td>0.05</td>
</tr>
<tr>
<td>FEve</td>
<td>0.7±0.1</td>
<td>0.5±0.1</td>
<td>D×Cd</td>
<td>5</td>
<td>11.805</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>FDis</td>
<td>0.4±0.1</td>
<td>0.4±0.1</td>
<td>Cd</td>
<td>3</td>
<td>1.520</td>
<td>0.677</td>
</tr>
<tr>
<td>ITD</td>
<td>0.4±0.1</td>
<td>0.7±0.1</td>
<td>Cd</td>
<td>3</td>
<td>9.237</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Meiofauna FRic</td>
<td>6±1</td>
<td>9±1</td>
<td>D×Cd</td>
<td>5</td>
<td>12.326</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>FEve</td>
<td>0.7±0.1</td>
<td>0.7±0.1</td>
<td>Cd</td>
<td>3</td>
<td>2.481</td>
<td>0.479</td>
</tr>
<tr>
<td>FDis</td>
<td>0.3±0.1</td>
<td>0.4±0.1</td>
<td>D×Cd</td>
<td>5</td>
<td>13.69</td>
<td>0.017*</td>
</tr>
<tr>
<td>ITD</td>
<td>0.5±0.1</td>
<td>0.5±0.1</td>
<td>D×Cd</td>
<td>5</td>
<td>13.043</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

4. Discussion

4.1. First description of nematodes in Thau Lagoon

With the exception of marine benthic ecosystems in Italy and Tunisia (Semprucci et al. 2008, Boufahja et l. 2014), there is a serious gap in knowledge concerning the Mediterranean meiofauna. This is particularly true for coastal lagoons, for which the spatial and temporal distribution of meiofauna, including free-living nematodes, is not well documented or is underestimated, despite they often greatly contribute to the biodiversity of coastal areas (Balsamo et al. 2012). Nematodes can provide critical information on the functioning of ecosystems and have already been proposed as potential indicators of anthropogenic
disturbance (Schratzberger et al. 2004, Danovaro et al. 2008, Moreno et al. 2011, Pusceddu et al. 2011). Our study is the first to provide a complete list of nematode taxa observed in the French Mediterranean Thau lagoon. Compared to the few studies that have documented other Mediterranean coastal areas, the number of taxa reported here from Thau Lagoon is similar but abundances were in the lower range (Mirto et al. 2000, Danovaro et al. 2004, Mahmoudi et al. 2008). The community structure showed many similarities with checklists of Italian and Tunisian lagoons, published respectively by Semprucci et al. (2008) and Boufahja et al. (2014). Ten of the common species found in Thau Lagoon have also been found in Italy (e.g. Anticoma eberthi, Longicyatholaimus longicaudatus) and 18 species were recorded in both Thau Lagoon and Tunisia (e.g. Spirinia gerlachi, S. parasitifera, Thalassironus britanicus, for details, see Table S1). These shared records could be linked to common environmental parameters among areas and to water exchanges between the two Mediterranean basins, which make large-scale dispersal between Tunisia, Italy and France possible. The checklist of nematodes resulting from this study could be the basis to further documenting meiobenthic diversity in Mediterranean coastal areas, with the aim to advance our understanding of its spatio-temporal variability.

4.2. Response of macrofaunal and nematode assemblages to shellfish culture

Overall, community structure (taxonomic and functional) differed between conditions, with lower abundance and richness (some significant, some not) at farm sites, as expected from previous studies (Mirto et al. 2000, Christensen et al. 2003, Callier et al. 2008). The presence of opportunistic species under the farm tables (e.g. Neanthes acuminata, Prionospio fallax, Daptonema sp.) whose abundance is favored under organically enriched conditions, showed that biodeposition by juvenile oysters impacts the benthic environment below farm structures in the study area. However, the high and similar species evenness values (J) at the farm and
reference sites revealed the absence of strongly dominant species that would be expected in an organically enriched environment (Pearson and Rosenberg 1978). The organic enrichment due to the presence of juvenile oysters is likely low compared with that of adult oysters, but the continuous exploitation of the area for farming may have modified community characteristics compared with outside of the farming zone.

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

In this study, macrofaunal taxonomic indices failed to detect a significant effect of shellfish culture whereas the multivariate approach did, as already reported in other studies (Grant et al. 2012, Lacoste et al. 2018a). Previous studies in Thau Lagoon produced conflicting results on the impact of aquaculture on macrofauna, ranging from low (Mazouni et al. 1996, Duport et al. 2007) to severe with a completely defaunated site in a deep shellfish area (Thouzeau et al. 2007). These authors highlighted the concomitant role of depth, algal cover and aquaculture in community composition. Our study also suggests that algal cover played an
important role, at least for the macrofauna community. Some arthropods, (e.g. *Gammarella* sp., *Cymodoce truncata*) were found attached to the algae *Halopithys* sp., so the TO and CO sites had specific assemblages structure due to these taxa. The general trend thus supports the hypothesis that juvenile oysters slightly influenced both macrobenthic and nematode communities, although the variability between sites within conditions may have masked statistical differences in some indicators. Although this may require a labor-intensive sampling effort, it is recommended to sample several farm and reference sites to disentangle the influence of aquaculture on benthic communities, if such an influence exists, from the natural variability. This is particularly important in transitional waters as coastal lagoons where high natural variability may exist due to rapid environmental changes.

4.3. Insights from the functional approach

To date, the effect of aquaculture on benthic communities has usually been evaluated using traditional taxonomic approaches (Lacoste et al. 2020). Here, we demonstrate that some species traits can be used to predict the response of macro- and meiofauna to disturbances caused by shellfish culture since the distribution of traits and functional indices performed well in distinguishing between the farm and reference sites. For both assemblages, trophic group and ecological group/life history were the traits that best responded to the presence of oyster tables. As reported in other studies, the presence of aquaculture facilities often decreases the abundance and diversity of filter-feeding organisms (Forrest and Creese 2006, Dubois et al. 2007, Fabi et al. 2009) whereas increased organic supply in farm zones favors the presence of non-selective deposit feeders. Following the classification of Grall and Glemarec (1997) and Borja et al. (2000), farming zones favored the presence of tolerant and opportunistic taxa whereas reference sites were dominated by
sensitive organisms. Among the meiofauna, epigrowth feeders were the most abundant organisms at reference sites and were replaced by non-selective deposit feeders in the farming zone. The reduction in the abundance of epigrowth feeders at the farm sites may be due to excluding competition with other groups but we can also hypothesize a lower abundance of diatoms under oyster tables due to a shading effect (McKindsey et al. 2011).

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

Functional diversity (FD) indices were computed for macrofauna and nematodes to evaluate the relevance of these indices in the case of aquaculture-induced disturbances. Nematode FRic was reduced at the farm sites and FD indices revealed the influence of aquaculture on macrofauna assemblages that was not detected using taxonomic indices. This confirms the usefulness of the functional approach, and more generally of multi-index approaches to detect the influence of farming (Borja et al. 2009, Lacoste et al. 2019). Interestingly, macrofauna FD indices indicated a higher trophic diversity and functional evenness at farm sites whereas no changes were detected at the richness level. The actual level of disturbance may therefore have shifted community composition that promoted a more even use of the trophic niche, which, in turn, may have increased the productivity of the system (Mason et al. 2005). Compared with the taxonomic approach, functional analysis not only offers the opportunity to
study the response of communities to the environment but also the subsequent impact of the
changes on ecosystem properties (Lavorel and Garnier 2002, Lacoste et al. 2020), which is
essential to evaluate the sustainable exploitation of aquaculture systems.

4.4 Limitation and future outlook of the functional approach

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

The number of taxa and the completeness and accuracy of the species trait data play a
significant role in the estimation of functional trait metrics (Pakeman and Quested 2007,
Pakeman 2014). In this exploratory study, we chose to use the maximum number of taxa for
which traits were available. However, the inclusion of arthropods, for example, is
questionable since it appears that these organisms were mostly associated with Halopithys sp.
algae and are very mobile, so they could also have been classified as epifauna instead of
infauna and disregarded. When we excluded them from the functional analysis (data not
shown), we obtained differences in the representation of relative abundances based on traits, but no difference with respect to FD indices.

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

Conclusion and Perspectives

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

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