Assessing the ecological status of an estuarine ecosystem: linking biodiversity and food-web indicators

Camille Le Guen, Samuele Tecchio, Jean-Claude Dauvin, Gwenola de Roton, Jérémy Lobry, Mario Lepage, Jocelyne Morin, Géraldine Lassalle, Aurore Raoux, Nathalie Niquil

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4   **linking biodiversity and food-web indicators**

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26   **Highlights**

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28   The Seine estuary ecosystem was described before and after the Port2000 extension.

29   A toolbox of various indices was used, describing complementary characteristics.

30   Port2000 effects were complex to separate from other human-induced and natural effects.

31   Ecological Network Analysis was useful in defining functional indicators.
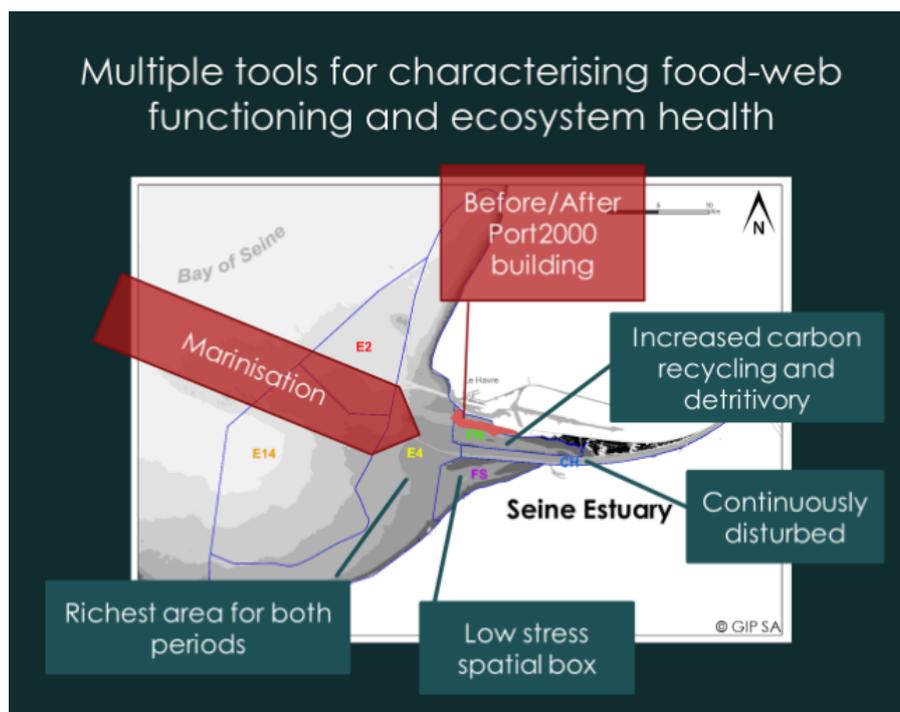
32   The need to continue long-term monitoring and to use multiple tools is emphasized.

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35   **Graphical abstract**

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42 **Abstract**

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44 During the last decades, the highly-anthropized Seine estuary has been impacted by

45 modification of its habitats (building of a major extension of Le Havre harbour, i.e. Port2000)

46 and a significant natural decrease in freshwater discharge. A Before/After analysis, using a

47 toolbox of indicators, was applied to characterize the effects of both events on the estuarine

48 ecosystem status. We selected from existing tool boxes several indicators derived from food

49 web modelling or community composition data, such as biodiversity indicators, a guild-based

50 index (*i.e.* Estuarine and Lagoon Fish Index ELFI) and ecological network analysis (ENA)

51 indices. ENA and biodiversity indicators were applied on six spatial boxes describing the Seine

52 estuary and its outlet. Results showed an increase in taxonomic and functional richness over

53 time, mainly due to marinisation, and significant changes in food-web properties in relation to

54 Port2000. ENA indices appeared as a promising method in ecological status assessment,

55 especially for estuaries considered as inherently disturbed.

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57 **Keywords:** ecology, biodiversity, food web, ecosystem functioning, ecosystem health

58 indicators

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60 **1. Introduction**

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62 Areas at the land-sea interface are often strongly impacted by human activities (e.g. Borja *et*

63 *al.*, 2012). Because estuaries are of great economic importance, they are commonly subject to

64 physical modifications such as port development and channelization to enhance harbour and

65 industrial activities (Marmin *et al.*, 2014). In addition, pollution and other anthropogenic

66 disturbances may cause modifications in aquatic communities (i.e. group of species or taxa

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67   present in a given area that can be characterised by the distribution of individuals or biomasses

68   among those species (Borja *et al.*, 2012)), that could in turn influence the whole ecosystem

69   functioning. A stressed ecosystem is here defined as an ecosystem undergoing changes in its

70   structure (e.g. productivity of its species) or functioning (e.g. food-web dynamics) following

71   physical, chemical or biological constraints or perturbations, either of a natural or an

72   anthropogenic origin. A stressed ecosystem is therefore expected to show modifications in both

73   its structure (e.g. species assemblages) and functioning (e.g. food-web properties), compared to

74   a defined baseline. Estuarine ecosystems are constituted of a mosaic of habitats linked to natural

75   (*e.g.* tide, freshwater input) and anthropogenic factors (McLusky and Elliott, 2004; Elliott and

76   Whitfield, 2011). However, it is difficult to distinguish between natural and human-induced

77   disturbances in estuaries due to their naturally high stress level and inherent variability: a

78   concept known as estuarine quality paradox (Dauvin, 2007; Elliott and Quintino, 2007; Dauvin

79   and Ruellet, 2009).

80   Multiple tools exist to derive various indicators describing and reflecting the structure and

81   functioning of ecosystems (Rombouts *et al.*, 2003; de la Vega *et al.*, 2018). Biodiversity and

82   guild-based indices are the most commonly used and were largely developed for application to

83   coastal and transition water masses in Europe under the EU Water Framework Directive (WFD)

84   (EU, 2000; Hering *et al.*, 2010). Biodiversity indices aim to describe communities'

85   characteristics and enable comparisons at different spatial scales (e.g. species richness, *alpha*,

86   *beta* and *gamma*, diversities). Guild-based indicators are generally developed to assess the

87   response of ecosystems to specific or more general disturbances (*i.e.* the comparison between

88   the Ecology Quality Status of an estuary or a part of an estuary to a reference ecosystem with

89   or without moderate disturbances) (Perez-Dominguez *et al.*, 2012; Lepage *et al.*, 2016).

90   Delpech *et al.* (2010) suggested using a fish-based indicator called Estuarine and Lagoon Fish

91   Index (ELFI) to assess the ecological status of French estuaries. ELFI was based on pressure-

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92 impact models and the purpose was to select relevant metrics that were known to be sensitive

93 to anthropogenic pressures (Tableau *et al.*, 2013; Pasquaud *et al.*, 2013). However, this

94 ecological quality indicator is mainly based on structural or taxonomic elements and do not

95 sufficiently reflect neither the functioning of ecosystems (de Jonge *et al.*, 2006; Rombouts *et*

96 *al.*, 2013) nor the biodiversity patterns between and within ecosystems (e.g. Vasconcelos *et al.*,

97 2013).

98 Ecological Network Analysis (ENA) corresponds to a collection of mathematical indices

99 describing the structure and functioning of a trophic network and characterise the overall

100 structural properties of food webs (Ulanowicz, 1997; Niquil *et al.*, 2014). Mass-balanced food-

101 web models such as Linear Inverse Modelling (Vézina and Platt, 1988; Niquil *et al.*, 2011;

102 Tecchio *et al.*, 2016) and Ecopath with Ecosim (Polovina, 1984) compute values of all carbon

103 flows in a given food web and then calculate ENA indices to assess the properties emerging

104 from the food-web architecture.

105 A common feature of both biodiversity indices and holistic ENA indicators is that they can

106 be related to concepts of stress, stability, resistance and resilience (Fath *et al.*, 2019; Safi *et al.*,

107 2019). The stability of an ecosystem corresponds to its ability to maintain a comparable

108 functioning in the presence of perturbations that drive it away from its original state

109 (Vasconcellos *et al.*, 1997; Lobry *et al.*, 2008; Saint-Béat *et al.*, 2015). Grimm & Wissel (1997)

110 defined resistance as the property of populations, communities or ecosystems to remain

111 "essentially unchanged". Resilience is the ability or the time taken by a system to recover from

112 a change due to perturbations and is linked to stability (Pimm, 1984; Mathevet and Bousquet,

113 2014). Although ENA indices have great potential to inform management decisions in estuaries

114 and large tidal areas (de la Vega *et al.*, 2018), their implementation in ecosystem health

115 assessment is not sufficiently established yet.

116   The objective of the present study was to investigate the contribution of ENA indices to the

117   assessment of the Seine estuarine ecosystem quality by confronting them to some more

118   commonly used indicators (i.e. biodiversity and guild-based indices). The Seine estuary is a

119   good example of a highly anthropogenic transitional area (Dauvin and Desroy, 2005; Dauvin

120   *et al.*, 2006). For more than 150 years, the Seine estuary has undergone many transformations,

121   such as shores linearization and calibration of the navigation channel from Le Havre (i.e.

122   entrance of the estuary) to port of Rouen (120 km upstream). Port2000 is an extension of the

123   existing commercial port of Le Havre, located in the Northern Channel of the Seine estuary,

124   that was constructed between 2002 and 2005 and that modified the morphological and

125   hydrological characteristics of the estuary (Dauvin *et al.*, 2006; Tecchio *et al.*, 2016). This

126   extension aimed to optimise the access of long-distance trade ships and to expand the available

127   area for those vessels in the harbour. Moreover, the navigation channel is also continuously

128   dredged to ensure maritime access of ships up to the port of Rouen (Marmin *et al.*, 2014). A

129   recent study using Ecopath models has shown that before the construction of Port2000, the

130   northern and navigation channels of the Seine estuary already presented signs of disturbance

131   (i.e. an effect, either biotic or abiotic, inducing a perturbation such as stress in an ecosystem;

132   relative to a specified reference state, (Rykiel JR, 1985)) due to maritime traffic, dredging, and

133   building works leading to a lack of connectivity between estuarine areas (Tecchio *et al.*, 2015).

134   Later, Tecchio *et al.* (2016) used a statistically more-advanced modelling framework (LIM-

135   MCMC) to estimate flows in the Seine estuary food web and compute ENA indices describing

136   its functional properties before and after Port2000. They showed that the northern channel

137   evolved towards a shorter food web and increased its resistance property. This was considered

138   as a potential impact of the Port2000 construction. Results from ENA indices also evidenced

139   that the food web in the navigation channel was constantly changing, probably due to

140 continuous dredging activities, and that the southern channel could be seen as the least disturbed

141 area for both periods (Tecchio *et al.*, 2016).

142 In addition to these anthropogenic disturbances, the Seine Estuary is also subject to an

143 increase of salinity in the lower part of the estuary, called marinisation. Marinisation is due to

144 a decrease in freshwater input coming from the river, associated with intrusion of marine water

145 in the lower part of the estuary. Marinisation is a common evolution in estuaries receiving water

146 from rivers and influenced by human activities (e.g. irrigation, dams) (David *et al.*, 2007;

147 Goberville *et al.*, 2010; Chaalali *et al.*, 2013).

148 We used here an original combination of methods to assess the ecosystem health of the Seine

149 estuary before and after the construction of Port2000. These analyses were performed on six

150 spatial boxes previously defined to describe the estuary (Tecchio *et al.*, 2016).

151 We first addressed the methodological question "Do the different indicators used in this

152 study (species-based, guild-based and ecosystem-based indices) bring similar, complementary

153 or different information on the ecological status of this estuarine ecosystem?" We then

154 addressed two ecological questions using this set of indicators: (i) How did physical distinct

155 areas of the estuary (spatial boxes) react to perturbations? (ii) Can we distinguish the effects

156 due to Port2000 from global change effects in the context of the estuary's natural marinisation?

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158 **2. Materials and Methods**

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160 *2.1. Study site and periods*

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162 Six spatial boxes, based on habitat characteristics and sediment types of the Seine estuary

163 and the adjacent eastern part of the Seine Bay, previously defined in Tecchio *et al.* (2015) were

164 considered: (a) three estuarine boxes: the navigation channel (CH), the northern channel (FN),

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165   and the southern channel (FS); (b) an intermediate box located at the mouth of the estuary (E4),

166   and (c) two marine spatial boxes (E2 and E14) (Fig. 1). Hydro-sedimentary and salinity

167   conditions were considered homogenous within each spatial box (Tecchio *et al.*, 2015). For

168   each zone, two sets of data were gathered corresponding to two different periods: 1996–2002,

169   representing the situation before the Port2000 construction, and 2005–2012, corresponding to

170   the recent situation, after the Port 2000 construction.

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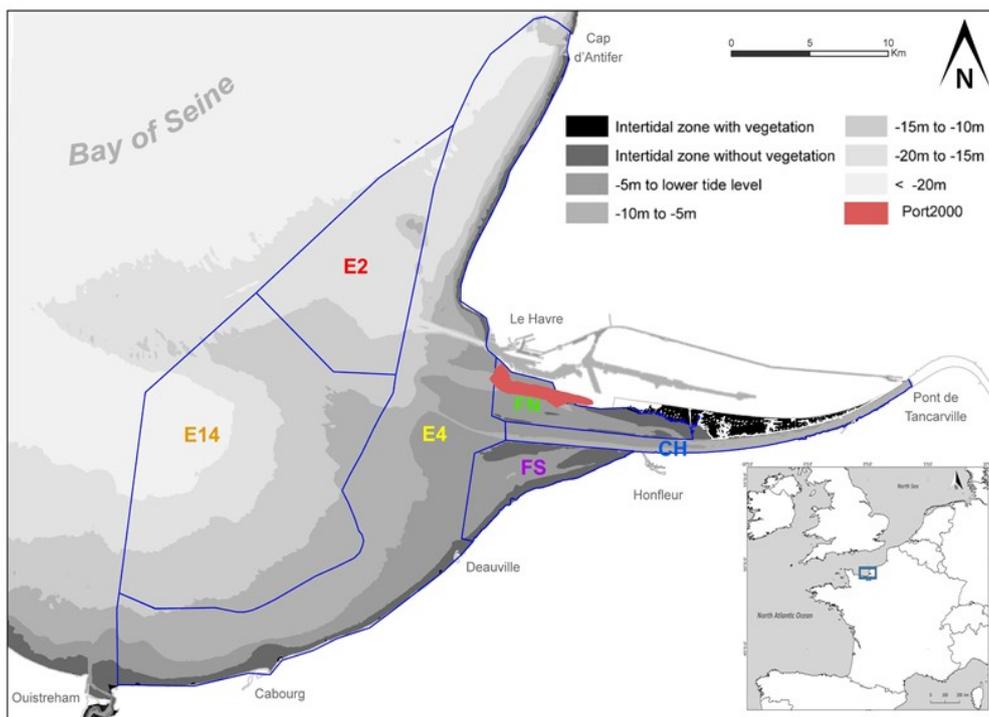
172   **Fig. 1.** Map showing the geographical position of the Seine estuary in France. The spatial boxes

173   are composed of the navigation channel (CH), the northern channel (FN), and the southern

174   channel (FS) as estuary boxes; an intermediate box located at the mouth of the estuary (E4),

175   and two marine boxes (E2 and E14). Source: GIP Seine-Aval and SHOM.

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178 *2.2. Indicators based on taxonomic diversity*

179 *2.2.1. Data*

180   For the subsequent indices' calculations, both fish data (from beam trawl) and
181 macrozoobenthos data (from 0.1 m² Smith McIntyre or Van Veen grabs) were collected
182 following identical sampling protocols between both periods, as results were standardised to
183 take into account differences in sampling effort. This allows quantitative and qualitative
184 comparisons to be made.

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186 *2.2.2. Indicators*

187   The taxonomic richness is defined as the total number of taxa sampled at a given scale. It
188 was computed by pooling all sampled taxa by spatial box and period (Before Port2000 = 1996-
189 2002 *vs* After Port2000 = 2005-2012). To limit bias due to sampling design, taxonomic richness
190 was standardized with relation to the sampling effort by dividing it by the log-transformed total
191 number of samples realised for a given space-time unit (Nicolas *et al.*, 2010).

192   Alpha (α), beta (*β*) and gamma (*γ)* diversity indices give information on changes in
193 community composition and the processes driving them, between spatial units or periods
194 (Barros *et al.*, 2014). α-diversity describes the taxonomic diversity on a local scale (calculated
195 for each spatial box), *γ*-diversity corresponds to the taxonomic diversity of the entire region of
196 interest (all six spatial boxes considered in the study) and *β*-diversity represents the variation in
197 taxonomic composition among the spatial boxes of the studied area (Barros *et al.*, 2014). The
198 mean α-diversity (ᾱ) of a region or area comprising several spatial boxes was calculated as the
199 average of α-diversity in each spatial box (Eq. 1).

200 $\bar{\alpha} = \sum_{i=1}^{N} \alpha^i / N$ (1); with α_i the taxonomic richness in the spatial box *i*, and *N* the number of
201 spatial boxes.

202   β-diversity is the ratio between γ and $\bar{\alpha}$ (Eq. 2) (Anderson *et al.*, 2011) and represents the

203 number of times by which the taxonomic richness in the whole region considered (*i.e.* the entire

204 estuary) is greater than the average taxonomic richness in the different spatial boxes. In other

205 words, it corresponds to the ratio between regional and local species diversity and is related to

206 compositional heterogeneity.

207 $\beta = \gamma/\bar{\alpha}$ (2)

208 with γ-diversity = taxonomic diversity of the entire region of interest and $\bar{\alpha}$ = mean α-diversity.

209   Biodiversity analyses were performed using the software package PRIMER-E v.6 (Clarke

210 & Gorley, 2006). A two-dimensional non-metric Multi-Dimensional Scaling (nMDS)

211 ordination was implemented for the six spatial boxes for each of the two periods, based on

212 square-root-transformed biomasses to reduce the asymmetry in species distributions, and a

213 Bray-Curtis similarity index computed on a species composition matrix. The nMDS plot gives

214 a visual representation of the proximity in species composition between samples: the closer

215 they are on the plot, the more similar they are in their species composition (Clarke and Warwick,

216 2001). The 'stress' index calculated by the routine in PRIMER reflects how well the ordination

217 is capable of preserving the rank of similarities in a bi-dimensional space, providing information

218 on the quality of the representation.

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220 *2.3. Multimetric fish-based indicator ELFI*

221 *2.3.1. Data*

222   Fish data 'After Port2000' were standardized data, collected from surveys following a

223 normalized protocol especially designed for fish monitoring in the French estuaries for the

224 WFD (Appendix A). Fish data used for multimetric fish-based indicator ELFI (Delpech *et al.*,

225 2010) calculation 'Before Port2000' were collected from surveys designed for various other

226 monitoring purposes but were obtained using similar sampling gear (i.e. beam trawls with

227 similar net features). For standardization and comparison, we accounted for sampling effort

228 (trawled surface area and time spent trawling) and considered that the spatial design was

229 relevant regarding WFD fish sampling protocol (> 6 samples by haline zone, defined by salinity

230 level) following the Venice estuarine system: oligohaline (0-5); mesohaline (5-18), polyhaline

231 (18-30) and euhaline (<30) zones (Venice System, 1958; Elliott & McLusky, 2002). This

232 allowed for comparison of ELFI values between both periods.

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234 *2.3.2. Indicator calculation*

235   ELFI has already been used to assess the ecological status of French estuaries (Tableau *et*

236 *al.*, 2013; Teichert *et al.*, 2018) (ecological status categories described in Appendix B). The

237 ELFI indicator is composed of seven metrics: density of (1) benthic fish (DB), (2) diadromous

238 migrant species (DDIA), (3) marine juveniles migrants (DMJ), (4) freshwater species (DFW),

239 (5) resident species (DER), (6) total density of fish (DT) and (7) standardised taxonomic

240 richness (RT/lnS) (Pasquaud *et al.*, 2013). Indeed, taxonomic richness (RT) was divided by the

241 log-transformed total number of samples in each spatial box (lnS) to limit the bias due to

242 sampling design, defining RT/lnS as the standardised taxonomic richness. These metrics are

243 proxies that reflect the physical quality of the habitat (DB, DER), the chemical quality of water

244 and sediment (DB, DMJ, DFW), but also the habitat connectivity (i.e. the degree to which

245 separate patches of habitat are connected; DDIA) (Hall *et al.*, 2011) and the general degradation

246 level, which reflects a general dysfunction in ecosystem health due to accumulation of negative

247 impacts (DT, RT/lnS). Contrary to the diversity indices calculated at the spatial box level, ELFI

248 represents the estuary level due to the high number of samples required to calculate this index.

249 ELFI values were computed using R (R Development Core Team, 2015). This fish index was

250 inter-calibrated at the European level with seven other fish indices in use in the North East

251 Atlantic region of Europe (Lepage *et al*., 2016) in order to ensure that assessments provided by

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252 these indices are consistent. Data were provided by '*Agence de l'Eau Seine Normandie*' for the

253 'After Port2000' period whereas values were computed from Ifremer and CSLN (*Cellule de*

254 *Suivi du Littoral Normand*) fish surveys for the 'Before Port2000' period.

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2.4. Indicators based on food-web models: Ecological Network Analysis (ENA)

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258 *2.4.1. Data sources and model construction*

259 We used the LIM model from Tecchio *et al.* (2016) for the three estuarine spatial boxes (FN,

260 FS and CH; Fig. 1) for both periods, using 13 functional groups. A summary of data sources is

261 given in Appendices C and D. Biomasses are expressed in $gC.m^{-2}$. We constructed three

262 additional LIM models representing the spatial boxes E2, E4 and E14 based on the same

263 functional groups (Appendix C) and the same types of input data, including production, food

264 conversion efficiency (i.e. ratio of the biomass produced by one compartment compared to its

265 food intake (Christensen & Walters, 2004)), respiration, and excretion ratios. Data sources and

266 references used for the construction of the LIM-MCMC models are presented in detail in

267 Tecchio et al. (2015, 2016). Briefly, a system of linear equations was set up, linking species

268 metabolic ratios (such as production/biomass, respiration/biomass, excretion/consumption

269 ratios), species biomass, and diet compositions. In these equations, the unknowns were the

270 flows connecting the compartments. The flow constraints were established using data from

271 literature or other modelling works (Appendix C). Known equalities such as those including

272 the biomass stocks were included to constrain the model into a finite multi-dimensional space.

273 Metabolic parameters as well as dietary constraints (i.e. proportion of prey in predator diet)

274 were implemented as "inequalities": minimum and maximum values were included in the linear

275 equations to partially constrain the related flows. This approach produces solutions that are

276 robust to the effect of parameterisation uncertainty (Hines *et al.*, 2015; Hines *et al.*, 2018).

277 Then, this space was sampled by using a Markov-Chain Monte Carlo routine to obtain 200,000

278 solutions of the set of flow values. Ecological Network Analysis was applied to these 200,000

279 solutions, using the R software packages *NetIndices* (Soetaert *et al.*, 2015) and *enaR* (Borrett

280 & Lau, 2014)*,* along with scripts developed for the present study [see Tecchio *et al*. (2016) for

281 additional methodological details].

282 To assess statistical differences in ENA indices between both periods, the non-parametric

283 effect size statistic introduced by Cliff (1993) was applied [see Tecchio *et al*. (2016) for

284 methodological details] as we were dealing with a situation of large amount of data (each ENA

285 index was calculated 200,000 times before Port2000 and the same number after Port2000)

286 which renders the estimation of classical parametric test statistics unfeasible (Mulholland &

287 Jones, 1968).

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289 *2.4.2. Indices*

290 We calculated six relevant ENA indices: the Total System Throughput (T..), the System

291 Omnivory Index (SOI), the Finn's Cycling Index (FCI), the Ascendency (A), the relative

292 Redundancy (R/DC) and the Detritivory/Herbivory ratio (D/H). This set of indices was used by

293 Tecchio *et al*. (2016) to characterize the inner part of the Seine estuary and most of them have

294 been proposed as management tools in the OSPAR Convention context (Safi *et al.*, 2019). The

295 Total system throughput (T..) corresponds to the sum of all flows occurring in the system, and

296 acts as an indicator of system size and activity (Rutledge *et al.*, 1976; Aoki, 1988; Latham,

297 2006). The Finn's Cycling Index (FCI) represents the fraction of the flows in the system that is

298 generated by recycling and was calculated as the proportion of T.. generated by cycling (Finn,

299 1976; Finn, 1980). Although other FCI definitions exist, this particular one was chosen to

300 remain consistent with previous work and another common modelling approach, *Ecopath with*

301 *Ecosim*. The Ascendency was calculated as the product of the Total system throughput (T..)

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302 and the Average Mutual Information (AMI), where AMI expresses the degree of organisation

303 of exchanges between the different functional groups (increasing AMI indicates higher dietary

304 specialisation; Hirata & Ulanowicz, 1984). High ascendency values mean that the system is

305 more actively channelling flows along more specific pathways, whereas low values have been

306 linked to system immaturity (Ortiz & Wolff, 2002). Relative redundancy (R/DC) was calculated

307 as the ratio between the internal flows overhead and the total development capacity of the

308 ecosystem (Ulanowicz, 1986; Ulanowicz, 2001) and measures the amount of parallel trophic

309 pathways connecting the trophic compartments. R/DC acts as an indicator of inefficiency of the

310 network (Hirata & Ulanowicz, 1984; Bondavalli *et al.*, 2000) but it is also a way to increase

311 ecosystem resilience because parallel pathways can replace each other (Ulanowicz, 1997). The

312 System Omnivory Index (SOI) was calculated as the mean of the omnivory indices of each

313 consumer compartment, weighted by the logarithm of their consumption (Christensen &

314 Walters, 1993; Libralato, 2008). Omnivory is defined as the variability of prey trophic levels

315 where high SOI values correspond to a food web with a web-like structure (i.e. with several

316 pathways between compartments) whereas low SOI values reflect a chain-like structure with

317 fewer pathways (Dimitrios *et al.*, 2018). Therefore, SOI is an indicator of the overall dietary

318 adaptation of the consumers, and an increase in SOI generally corresponds to a stabilizing

319 response to an external disturbance (Fagan, 1997; Libralato, 2008). The Detritivory/Herbivory

320 ratio (D/H) was calculated as the ratio between the sum of all predation flows on the detritus

321 compartment (detritivory, flows from detritus to consumers) and the sum of all predation flows

322 on primary producers (herbivory, flows from phytoplankton and microphytobenthos to

323 consumers). An increase in D/H would indicate that the ecosystem shifts towards a more

324 detritus-based food web, depending less on plant material for trophic interactions (Ulanowicz,

325 1992; Luong *et al.*, 2014; Niquil *et al.*, 2014).

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3. Results

Results concern "species-based" (species diversity and biomasses), "community-based" (fish index ELFI) and "ecosystem-based" (Ecological Network Analysis) indicators, which provide information at different levels.

3.1. Species-based indices related to diversity and biomasses

3.1.1. Biomass trend

In the estuary boxes CH and FN, fish and macrozoobenthos biomasses increased between periods (Fig. 2, Table 1). For the southern channel (FS), while macrozoobenthos biomass increased, fish biomass remained stable, and it appeared that this was the case for all fish groups (Fig. 2). For instance, the biomass of piscivorous fish in FS averaged 0.057 g C.m^{-2} in the first period and approached 0.055 g C.m^{-2} in the second one. In CH, while total fish biomass increased, the biomass of planktivorous fish decreased (Fig. 2). This was mainly related to two pelagic species: *Clupea harengus* and *Sprattus sprattus*. In the same way, for FN, while the total fish biomass increased, the biomass of piscivorous and planktivorous fishes slightly decreased (Fig. 2). This was quantitatively compensated by a strong increase in benthos feeders' biomass. The decrease in planktivorous fish biomass in FN was due to four species *Clupea harengus, Gasterosteus aculeatus, Osmerus eperlanus* and *Sprattus sprattus*. The overall biomass (including fish, macrozoobenthos and the suprabenthos) increased for the three estuarine spatial boxes. Among the three estuarine boxes, the ratio of fish biomass on benthic invertebrate biomass (BFish/BMacrozoobenthos) was the highest in CH (0.09 in the first period and 0.03 in the second one). Nonetheless, this ratio was divided by three in CH congruently to the sharp increase in benthic macrofauna biomass between periods that was multiplied by 7 (Fig. 2, Table 1).

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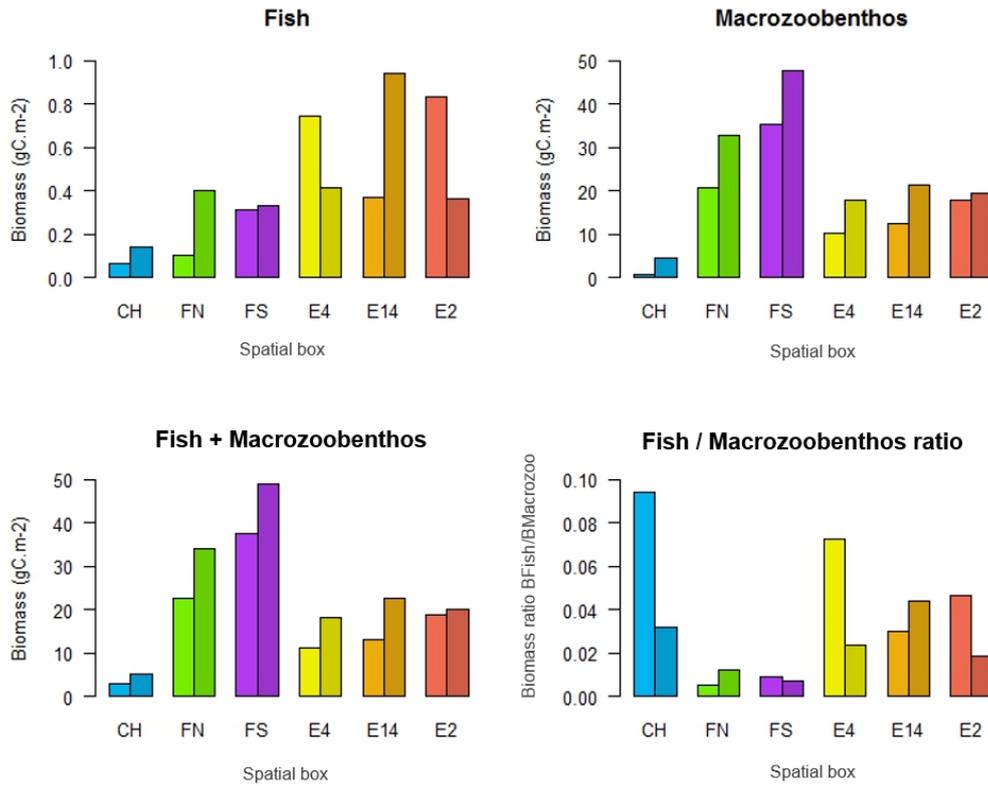
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Fig. 2. Barplots representing fish biomass, macrozoobenthos biomass, the sum of fish and macrozoobenthos biomasses, and the ratio BFish/BMacrozoobenthos, for all spatial boxes before the construction of Port2000 (first bars) and after (second bars). The spatial boxes are composed of the navigation channel (CH), the northern channel (FN), and the southern channel (FS) as estuary boxes; an intermediate box located at the mouth of the estuary (E4), and two marine boxes (E2 and E14).

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361 **Table 1.** Summary of comparisons between periods (Before and After Port2000 construction)

362 for biomass values, biodiversity indices, and the ELFI index. See text for details on the indices.

363 < indicates that the value of the index is lower Before than After Port2000; > indicates that the

364 value of the index is greater Before than After Port2000.

			CH	FN	FS	E4	E14	E2
			Spatial boxes					
Biomass		Fish	<	<	<	>	<	>
		Benthos	<	<	<	<	<	<
Diversity	TR	Fish	<	<	<	<	>	>
		Benthos	<	<	<	<	<	<
	α	Fish	<	<	<	<	>	>
		Benthos	<	<	<	<	<	<
	□ *	Fish				<		
		Benthos				<		
	β *	Fish				<		
		Benthos				>		
	γ *	Fish				<		
		Benthos				<		
ELFI*						<		

365 * □, β and γ diversity indices are computed for the whole estuary

366

3.1.2. Taxonomic richness and species composition

368 The taxonomic richness increased between periods, from 16 to 47 fish taxa and from 5 to 29

369 macrozoobenthos taxa in the navigation channel (CH) (Fig. 3). The same trend was observed

370 in the Northern and Southern channels (FN and FS; Fig. 3). The estuary box CH showed

371 particularly low values of macrozoobenthos taxonomic richness compared to the other spatial

372 boxes (Fig. 3). The intermediate box E4 was the richest spatial box in terms of fish (with 51

373 taxa during the first period and 59 taxa during the second one) and benthic macrofauna (with

374 177 taxa during the first period and 307 during the second one). No strong changes were

375 observed for the fish taxonomic richness in the marine spatial boxes E14 and E2. All taxa that

376 were gained between periods, both for fish and macrozoobenthos, were exclusively of marine

377 origin. Results on standardised taxonomic richness are given in Figure 3.
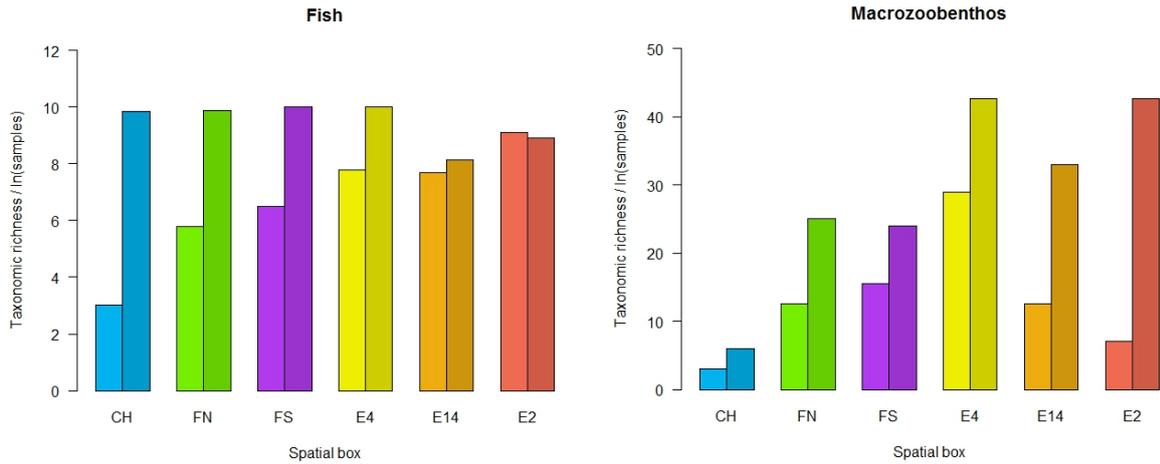
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Fig. 3. Barplots representing taxonomic richness divided by the log-transformed total number of samples realised within the six spatial boxes before (first bars) and after (second bars) the construction of Port2000 for fish and macrozoobenthos. The spatial boxes are composed of the navigation channel (CH), the northern channel (FN), and the southern channel (FS) as estuary boxes; an intermediate box located at the mouth of the estuary (E4), and two marine boxes (E2 and E14).

3.1.3. Diversity indices

The α, β and γ taxonomic diversity analyses between periods showed that the average number of fish species □ increased from 32.8 to 41 species, and from 65.7 to 177.2 for macrozoobenthos. γ-diversity increased from 54 to 71 taxa for fish and from 195 to 353 taxa for macrozoobenthos, and β-diversity increased from 1.65 to 1.73 for fish and decreased from 2.97 to 1.99 for macrozoobenthos (Table 1).

The MDS ordination based on fish composition data showed a remarkable spatial segregation according to the salinity gradient between the estuarine and the marine spatial boxes (Fig. 4 top). The same pattern was observed with the MDS ordination based on macrozoobenthos species (Fig. 4 bottom). The 'stress' value of 0.05 was very low, underlying the good quality of the representation.

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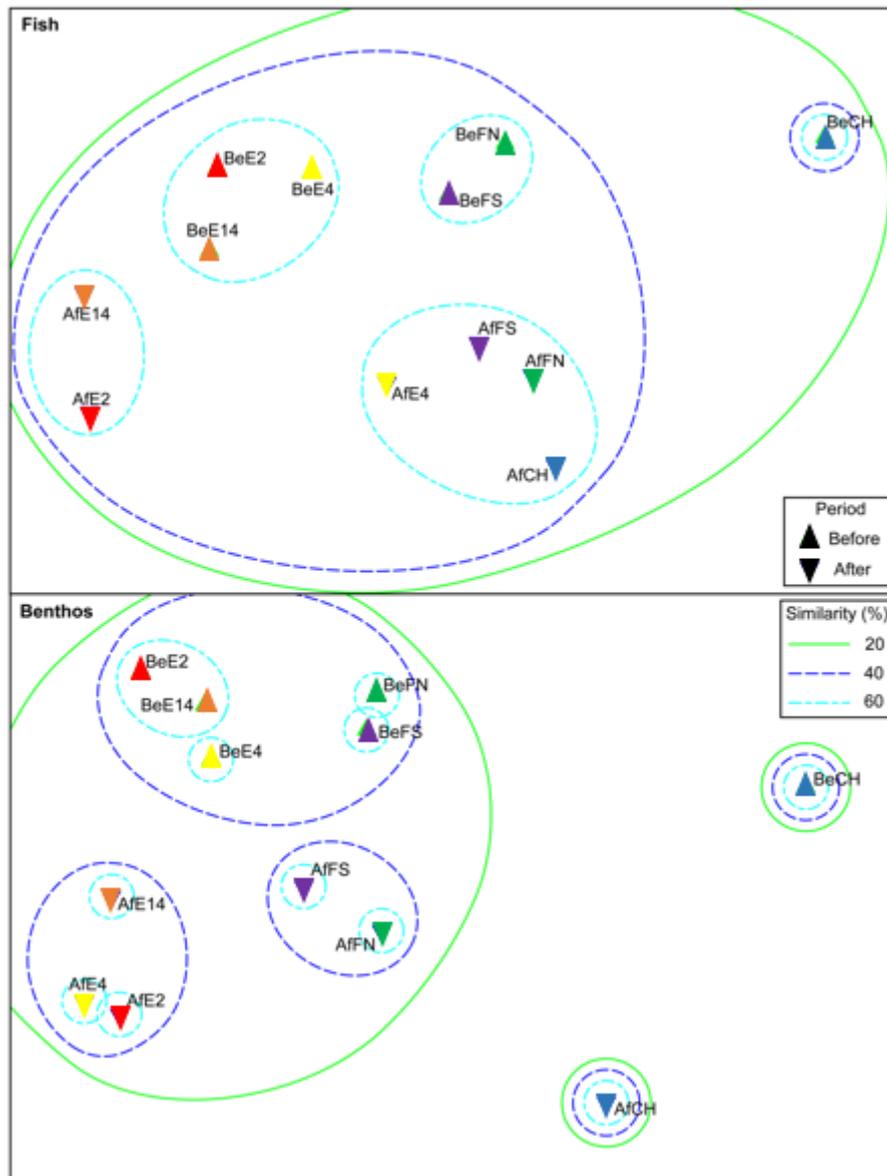
Fig. 4. MDS ordination based on Bray-Curtis similarity matrices from biomass data (square-root transformed) for fish (on the upper part) and for macrozoobenthos (on the lower part), for all spatial boxes during the first (first bars), before Port2000, and the recent (second bars) periods.

CH appeared as the spatial box that has experienced the greatest change in fish composition between periods, with less than 20% similarity in the community composition before and after Port2000. In addition, the benthic macrofauna composition in this estuary box demonstrated few similarities with the other spatial boxes, and this for both periods. For fish in the 'Before' period, marine boxes E2, E4 and E14 formed a group well separated from the inner estuary,

410 where FN and FS were grouped and CH isolated from these two other estuarine boxes. Similar

411 results were obtained for macrozoobenthos. But in the 'After' period, the marine box E4 was

412 grouped, for fish, with not only the estuary boxes FN and FS, but also CH (Fig. 4). This

413 similarity in biodiversity composition was only limited to fish compartment; the benthos

414 remained structured in a very similar way, in both periods.

415

416 3.2. *A community-based index for fish: ELFI*

417   During the period before Port2000, the Seine estuary showed lower values of ELFI (from

418 0.1 to 0.14) compared to the period after Port2000, the highest value recorded being 0.68 in

419 2006 (Fig. 5). The metric DDIA, indicator of habitat connectivity related to diadromous species,

420 showed strong changes in the recent period (after Port2000), with a value of 0.88 in 2006, 0.70

421 in 2010 and 0.71 in 2012 (missing value in 2011), meaning that this metric gradually decreased

422 after the Port2000 construction.
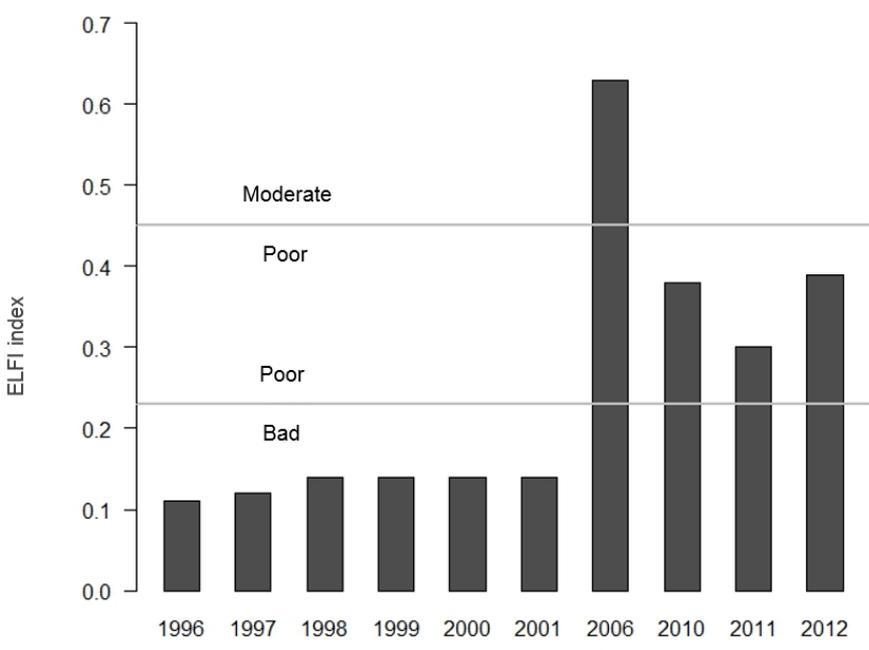
423

424 **Fig. 5.** ELFI values for the whole estuary for 10 years, between 1995 and 2012, with the class

425 boundaries of the Water Framework Directive.

426

3.3. Ecosystem-based indicators: food webs and ENA

427

428 ENA indices showed that all indices in the two marine zones (E2 and E14) remained similar

429 between the two periods (Table 2). R/DC and SOI decreased between before and after "Port

430 2000" in the estuary box (FS). All indices, except SOI, increased between the two periods in

431 the intermediate box E4 and in the estuary boxes CH and FN.

432 Total System Throughput (T..), representing the size and activity of the system, significantly

433 increased in the estuary boxes CH and FN as well as in the intermediate box E4 and remained

434 stable in all the other spatial boxes (Table 2); Cliff's delta; Fig. 6).

435 The SOI, reflecting the omnivory in the system, significantly decreased in the estuary box

436 FS between the two periods. (Fig. 6). The D/H ratio significantly increased in the estuary boxes

437 CH, FN and in the intermediate box E4, while remaining stable in the marine boxes E14, E2

438 and in FS (Table 2, Fig. 6).

439 The FCI reflecting the recycling in the system, significantly increased between the two

440 periods in the estuary boxes CH, FN and in the intermediate box E4 (Table 2, Fig. 6).

441 The ascendency (A) and relative redundancy (R/DC) indices significantly increased in CH

442 between the two periods. The ascendency also significantly increased in the intermediary box

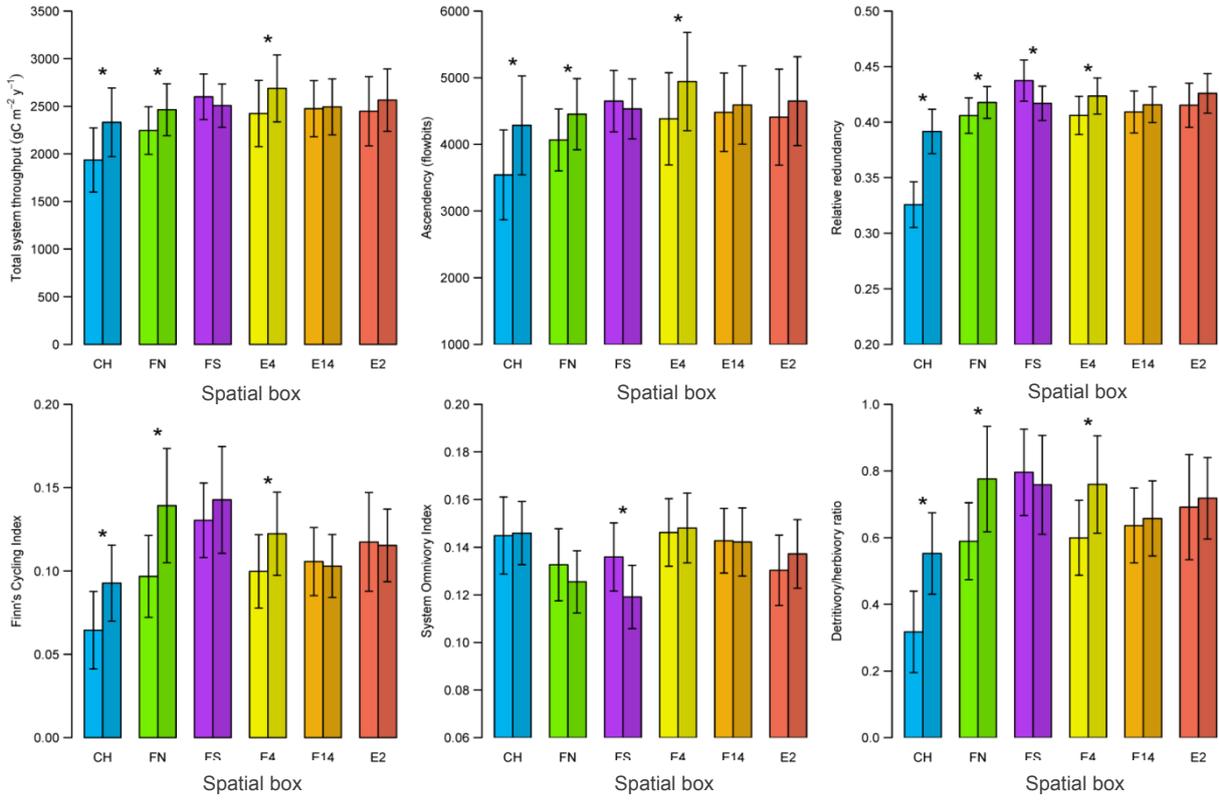443 E4 between the two periods (Table 2, Fig. 6).

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445

446 **Table 2.** ENA means and standard deviations, derived from LIM-MCMC models, for all spatial

447 boxes before (1996-2002) and after (2005-2012) Port2000 construction. < indicates that the

448 value of the index is lower Before than After Port2000; > indicates that the value of the index

449 is greater Before than After Port2000. Only statistically significant results based on Cliff's delta

450 are listed. ns: non-significant.

451

ENA	Period	CH	FN	FS	E4	E14	E2
T..	Before	1935 ± 337	2244 ± 251	2600 ± 240	2424 ± 347	2475 ± 296	2447 ± 364
T..	After	2332 ± 361	2463 ± 273	2507 ± 227	2687 ± 352	2494 ± 294	2564 ± 328
Evolution T..		<	<	ns	<	ns	ns
FCI	Before	0.087 ± 0.03	0.124 ± 0.03	0.163 ± 0.03	0.128 ± 0.03	0.135 ± 0.02	0.149 ± 0.04
FCI	After	0.119 ± 0.03	0.175 ± 0.04	0.178 ± 0.04	0.153 ± 0.03	0.131 ± 0.02	0.145 ± 0.03
Evolution FCI		<	<	Ns	<	ns	ns
Ascendency	Before	3542 ± 675	4066 ± 465	4648 ± 461	4382 ± 692	4480 ± 589	4408 ± 721
Ascendency	After	4285 ± 742	4453 ± 534	4532 ± 450	4942 ± 737	4590 ± 588	4649 ± 667
Evolution Ascendency		<	<	Ns	<	ns	ns
R/DC (%)	Before	32.6 ± 2	40.6 ± 2	43.7 ± 2	40.6 ± 2	40.9 ± 2	41.5 ± 2
R/DC (%)	After	39.2 ± 2	41.8 ± 1	41.7 ± 2	42.4 ± 2	41.6 ± 2	42.6 ± 2
Evolution R/DC(%)		<	<	>	<	ns	ns
SOI	Before	0.145 ± 0.02	0.133 ± 0.02	0.136 ±0.01	0.146 ± 0,01	0.143 ± 0.01	0.130 ± 0.01
SOI	After	0.146 ± 0.01	0.125 ± 0.01	0.119 ± 0.01	0.148 ± 0.01	0.142 ± 0.01	0.137 ± 0.01
Evolution SOI		ns	ns	>	ns	ns	ns
D/H	Before	0.317 ± 0.12	0.589 ± 0.12	0.796 ± 0.13	0.600 ± 0.11	0.637 ± 0.11	0.692 ± 0.16
D/H	After	0.553 ± 0.12	0.776 ± 0.16	0.758 ± 0.15	0.760 ± 0.15	0.658 ± 0.11	0.718 ± 0.12
Evolution D/H		<	<	ns	<	ns	ns

Fig. 6. Ecological network analysis (ENA) indices for all spatial boxes for the period before (first bars) and after (second bars) Port2000. The spatial boxes are composed of the navigation channel (CH), the northern channel (FN), and the southern channel (FS) as estuary boxes; an intermediate box located at the mouth of the estuary (E4), and two marine boxes (E2 and E14). Means and standard deviations are represented. Asterisks represent significant changes assessed from Cliff's delta statistics (level 0.05; between before and after construction).

4. Discussion

4.1. Limitations

Fish species were sampled using beam trawls, which target the first 50cm above the seafloor. The fish assemblage was therefore biased, the pelagic species likely being underestimated. In addition, the edges of each habitat were not sampled. Therefore, a few fish species might not have been sampled. However, the sampling gears and methods were similar between periods

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469   and among spatial boxes, allowing comparison between time periods and spatial boxes. ELFI

470   values, for both periods, need to be considered with some caution, given the differences in

471   sampling protocol between periods that may slightly affect the comparability.

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473   *4.2. Signs of estuarine marinisation*

474   The biomasses of marine species increased between both periods in the Seine estuary. For

475   example, *Pomatoschistus minutus* biomass increased in two estuarine boxes (FN, FS), and in

476   the intermediate box (E4), and *P. microps* was newly observed in these areas after Port2000.

477   These two fish species, especially *P. minutus* are mostly marine species (Leitão *et al.*, 2006),

478   suggesting a tendency of marinisation. Previously termed by David *et al.* (2007) and later used

479   in several papers (Pasquaud *et al.*, 2012; Chevillot *et al.*, 2016, 2017), « marinisation »

480   describes the process whereby marine waters flow far upstream into the basin along with a

481   higher abundance of marine species in the estuarine transitional zone. The Seine estuary, as

482   many north-eastern Atlantic Ocean estuarine ecosystems (Goberville *et al.*, 2010), is going

483   through a process of 'marinisation'. The time period 2005-2012 was characterised by a

484   significantly lower discharge of the Seine river (Dauvin and Pezy, 2013), resulting in an

485   increase of salinity up to 5 in the estuarine boxes (Bacq *et al.*, 2013). The salinity in the marine

486   boxes remained stable between periods. The arrival of marine species finding newly suitable

487   conditions in most spatial boxes might explain the increase in taxonomic richness. This

488   marinisation tendency was supported by the MDS ordination showing a clear spatial (along the

489   salinity gradient) and temporal (before and after Port2000) segregation, indicating that

490   marinisation modified the ecological structure of communities. This is also supported by the

491   higher ELFI values in the Seine estuary after Port2000 which is likely a consequence of the

492   marinisation enhancing the taxonomic richness and biomass of fish in the estuarine spatial

493   boxes. The same trend has already been observed in the Gironde estuary where changes in fish

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494   assemblages and an increase of the ELFI index were attributed to an increase in temperature

495   and salinity indicating marinisation (Pasquaud *et al.* 2012; Chevillot *et al.*, 2016, 2017).

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497   *4.3. Signs of anthropogenic impacts*

498   *4.3.1. The global estuary*

499   In the Seine estuary, the ELFI indicator increases with ecosystem quality (Le Pape *et al.*,

500   2015). The ecological status of the Seine estuary therefore improved from a 'bad' status before

501   Port2000 to a 'poor' status after Port2000. Diadromous species abundance was associated to

502   habitat connectivity, as opposed to habitat fragmentation (barriers to migratory fish) (Pasquaud

503   *et al.*, 2013). After Port2000, the highest DDIA value recorded was 0.88 in 2006 and then the

504   general pattern of this metric for that period was characterized by an overall decrease of DDIA,

505   with values dropping down to 0.71 in 2012, likely due to a lower connectivity for migrant

506   species such as eel *Anguilla anguilla* or grey mullet *Liza ramada,* associated with the extension

507   of the breakwaters between FN and CH during the Port2000 construction. As an example, smelt

508   *O. eperlanus* is a diadromous species which was formerly abundant in the upper part of the

509   northern channel for reproduction (Morin *et al.*, 2015). Extending the breakwaters between FN

510   and CH during the Port2000 construction might have impacted the connectivity between these

511   two zones, and *O. eperlanus* has been negatively affected by this rupture of connectivity.

512   Moreover, it seemed that the differences in fish assemblages between the spatial boxes widened

513   (β-diversity increased from 1.65 to 1.73), which might be related to availability of new habitats

514   for marine species inside the estuary. Concerning benthic species, the diversity trend between

515   spatial boxes showed the reverse trend, showing signs of standardisation (β-diversity decreased

516   from 2.97 to 1.99).

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518   *4.3.2. Estuarine disturbed boxes*

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Comparatively to the rest of the estuary, strong effects of perturbations were expected in the navigation channel (CH) because of the continuous dredging and the high hydrodynamics. The estuarine box FN was the spatial box the most directly affected by the construction of Port2000 and by the mitigation measures such as the artificial formation of meanders, which might have modified the hydrodynamics (Cuvilliez *et al.*, 2015). In addition, an increase in water turbidity was noted in this box (S. Lesourd, personal communication).

The increase in detritivory/herbivory ratio (D/H) observed in both CH and FN between the two periods suggests an ecosystem more stressed after the Port2000 construction (Niquil *et al.*, 2014), probably reacting to perturbations by enhancing opportunistic behaviours such as detritivory. The higher carbon recycling (FCI) observed after Port2000 in these two estuarine spatial boxes can also be a sign of increasing stress (Vasconcellos *et al.*, 1997). However, increasing FCI and D/H values could also be interpreted by a sign of increasing maturity (Odum, 1969; Christensen, 1995; Allesina and Ulanowicz, 2004; Vasconcelos *et al.*, 2007) but other ENA indices support the statement of increasing stress. Indeed, the relative redundancy (R/DC) significantly increased across periods in CH and FN, highlighting the fact that the system was enduring a higher level of stress (Hirata & Ulanowicz, 1984; Bondavalli *et al.*, 2000) but was also more able to resist to disturbances as the energy transfers could be maintained through the trophic network via other pathways (Ulanowicz, 1997; Rybarczyk & Elkaïm, 2003).

4.3.3. Estuarine reference box

The FS, which was initially considered as the least varying spatial box (Tecchio *et al.*, 2015) and thus a reference, showed a reduction in redundancy (R/DC), omnivory (SOI) and detritivory (D/H), while maintaining a stable system activity (T..) and carbon recycling (FCI). The trophic specialisation was evidenced by the congruent reduction in omnivory (leading to a more chain-

like structure of the food web) and redundancy (reduction of parallel pathways), which can be a sign of maturity. Results for FS therefore suggested that the reduced disturbance conditions here favoured an expected ecological succession.

4.3.4. Intermediate box

E4 is the transitional area between the inner estuary and the marine environment. Results from ENA indices show an increase in system activity (T..), redundancy (R/DC), carbon cycling (FCI), omnivory (SOI) and detritivory (D/H). Therefore, as for CH and FN, following the same interpretation framework, we could assume that the system in E4 was increasing its stabilizing mechanisms when facing stronger disturbances.

4.3.5. Marine boxes

Results from ENA indices show no significant differences between the two periods for both spatial boxes E14 and E2. Therefore, we can assume that the construction of Port2000 has no negative effect on the food web dynamics of these two marine boxes. However, the spatial box E2 contains the dumping site of Octeville for dredged sediments from the Le Havre harbour (Marmin *et al.*, 2014). For these two spatial boxes, we noticed that the fish taxonomic richness decreased whereas the benthic macrofauna richness increased. The relative importance of benthic communities had intensified in E2, as shown by the decrease in the BFish/BMacrozoobenthos ratio between both periods, contrary to what was noticed in E14. This might be explained by a potential increasing turbidity in E2 due to the dumping of sediments that could affect fish more than the benthic communities. However, the effect of the Port2000 construction on these two marine boxes cannot be evidenced.

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4.4. Relative effects of marinisation and port construction

4.4.1. The global estuary

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570   The higher taxonomic diversity and ELFI index values observed after Port2000 in a context

571   of marinisation suggest a decrease of ecosystem stress after Port2000, as taxonomic richness is

572   considered a key factor in ecosystem stability in response to anthropogenic pressures (e.g.

573   McCann, 2000; Chapin *et al*., 2000). However, investigations in the different spatial boxes were

574   necessary to detect potential effects of the Port2000 construction.

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4.4.1. Estuarine disturbed boxes

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577   The food web in CH is continuously disturbed due to the dredging activity, greatly limiting

578   the colonisation of the seafloor by benthic organisms, as seen in other locations (e.g. in the

579   Venice lagoon: Pranovi *et al*., 2003). In CH, the community was characterized by an extremely

580   low benthos richness and biomass compared to the other spatial boxes. Nonetheless,

581   communities subjected to regular disturbances are also more adapted, less sensitive, and recover

582   more quickly than undisturbed communities (Dernie *et al*., 2003; Marmin *et al*., 2014). Results

583   from ENA indices in the two boxes CH and FN suggest an ecosystem more stressed after the

584   Port2000 construction. Species-based indices were very useful to detect the effects of dredging

585   activities which directly affect the settlement of benthic communities. This effect was also

586   reflected in the ENA indices.

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4.4.2. Estuarine reference box

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589   In FS, both fish and macrozoobenthos taxonomic richness increased. However, the ratio

590   BFish/BMacrozoobenthos remained stable. The MDS analysis showed that the fish

591   composition in this spatial box did not deeply change between periods. ENA indices suggest a

592   mature system for FS for both periods. Therefore, this estuarine spatial box could be confirmed

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as the least stressed area of the Seine estuary, as previously assessed by Tecchio *et al*. (2016),

and that species-based indicators, ELFI and ENA indices bring similar information for this

spatial box.

4.4.3. Intermediate box

We assumed that E4 should exhibit the highest taxonomic richness and biomasses given its

intermediate position between marine and estuarine waters (Dauvin & Desroy, 2005). It had the

highest fish and benthic macrofauna taxonomic richness of all spatial boxes. Similar results

were found by Martino and Able (2003), who showed that in the Mullica river-Great Bay

(USA), fish species richness increased from the marine stations to the ones at the mouth of the

estuary, and then strongly decreased towards the estuarine sampling stations. The MDS analysis

put forward that E4 could be seen as an intermediate spatial box for fish, but as a marine box

for the macrobenthos community. Indeed, this confirmed that E4 itself can be considered as an

'ecocline', i.e. a boundary of progressive changes (spatial and ecological) between two different

systems, the ocean and the estuary (Attrill and Rundle, 2002; Basset *et al*., 2013). Finally,

results from ENA indices demonstrated an increase in system activity, redundancy, cycling,

omnivory and detritivory between the two periods. Therefore, as for CH and FN, ENA indices

enabled us to identify that the system was increasing its stabilizing mechanisms, probably to

face stronger disturbances.

4.4.4. Marine boxes

Finally, in the bay, E14 and E2 marine boxes appeared similar in terms of fish and

macrozoobenthos taxonomic composition between the two periods. Results from ENA indices

show no significant differences between the two periods for both spatial boxes. Indices based

617 on diversity and biomasses showed the importance of benthic communities has intensified in

618 E2 but the origin of this change remains unknown.

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4.4.5. Summary

621 The estuarine marinisation effects are potentially stronger than the effects of the Port2000

622 construction. Indeed, on the one hand, the marinisation of the Seine estuary mostly changed the

623 species assemblages, therefore affecting the ecosystem structure (i.e. biological and physical

624 architecture of an ecosystem), due to the arrival of numerous marine species. On the other hand,

625 the anthropogenic pressures affected habitat connectivity and food-web dynamics (i.e. transfer

626 of energy from one part of the food web to another, Lindeman, 1942), assessed with ENA

627 indicators such as system activity, omnivory, recycling or detritivory. The main information

628 brought by the combination of indices was summarized in Table 3.

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632 **Table 3.** Main information about the functioning and the status of the six spatial boxes.

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Method	Main results	Conclusions
Biotic indices based on diversity and biomasses => **give information about the structure of communities**	Increase of the taxonomic richness between periods for the estuarine spatial boxes (CH, FN, FS) and the biomass of fish and macrozoobenthos increased	**Arrival of marine species** such as *P. minutus* in relation to the marinisation of the estuary (lower river discharge in the second period)
	CH: low values of macrozoobenthos richness	Low benthos biomass due to dredging and high hydrodynamics in this part of the estuary
	E4: the richest area for both periods	This corresponds to the rich *Abra alba-Lagis koreni* macrobenthic community and the feeding zone for fish (mainly flatfish).
	The MDS ordination showed a spatial segregation between CH and the other zones, and a temporal change between periods, both for fish and macrozoobenthos	**Evolution in salinity plays a determinant role in the spatial gradient of the fauna; both periods were clearly distinguished**
Biotic indices based on the classification of species in trophic groups => **give information about the functioning of the food web**	FN: higher carbon recycling and detritivory (increase in detritus input) + higher redundancy (more species)	**Stress increased in the estuary mainly for CH and FN zones. The other zones were less disturbed.**
	CH: increase in system activity, carbon cycling, ascendency, redundancy and detritivory	
Biotic index based on the classification of species in ecological groups => **give information about the health status of ecosystems**	FS: still in 'high ecological status'	Reference box
	Increase in ELFI values between periods	The ecological status was supposed to have improved but it is likely due to the arrival of marine species only
	Decrease of the metric DDIA (diadromous species) in the second period, indicator of a lower habitat connectivity	**Lower connectivity due to the extension of the breakwaters** between FN and CH during the Port2000 construction

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635   ELFI was sensitive to the arrival of marine species and therefore was highly relevant in a

636 context of marinisation of the estuary. ENA indices appeared as good 'surveillance indicators',

637 that Shephard *et al.* (2015) proposed as indicators that would be a good complement to

638 operational indicators, for example in the context of the Marine Strategy Framework Directive

639 application. ENA indices are also useful to take ecosystem-based management measures for

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640   such ecosystems (de la Vega *et al.*, 2018; Safi *et al.*, 2019). In addition, their association with

641   biodiversity indicators made the analysis more robust as suggested by Rombouts *et al.* (2013).

642   For instance, the arrival of new species and the higher biomasses observed in some spatial boxes

643   between periods can also explain increases in some ENA indicators such as system activity,

644   omnivory or redundancy but this cannot be evidenced and requires further investigations.

645

646   **5. Conclusions**

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648   The effects of the Port2000 construction were not as evident as expected due to the presence

649   of other natural (e.g. marinisation) and anthropogenic (e.g. continuous harbour activities)

650   perturbations, which makes the interpretation of ecological indicators difficult. ELFI was

651   particularly relevant in a context of marinisation, as this index is sensitive to the arrival of new

652   marine species. But the different metrics used to calculate ELFI are also indicative of

653   anthropogenic impacts (e.g. DDIA and habitat connectivity affected by the construction of

654   Port2000), making ELFI a very powerful tool to assess the health status of this estuary. The use

655   of ENA indices alone was not sufficient to describe changes in ecosystem functioning between

656   the two periods, distinguishing the effects of the Port2000 construction and the process of

657   marinisation, but they appeared as an interesting complementary set of tools for characterising

658   the evolution of food-web dynamics. Indeed, ENA indices allowed us to observe changes that

659   occurred in specific ecosystem processes and to identify how an ecosystem becomes more

660   stable (e.g. by increasing omnivory) under increasing stress. We showed that detecting changes

661   related to anthropogenic disturbances alone in the Seine requires using ecological indicators at

662   different levels (species, communities and ecosystem) and at multiple scales of observations.

663   This work emphasizes the need to use several metrics to assess ecosystem health status and to

664   continue long-term monitoring of such complex and reactive ecosystems.

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937 **Figure legends**

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939 **Fig. 1.** Map showing the geographical position of the Seine estuary in France. The spatial boxes
940 are composed of the navigation channel (CH), the northern channel (FN), and the southern
941 channel (FS) as estuary boxes; an intermediate box located at the mouth of the estuary (E4),
942 and two marine boxes (E2 and E14). Source: GIP Seine-Aval and SHOM.

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944 **Fig. 2.** Barplots representing fish biomass, macrozoobenthos biomass, the sum of fish and
945 macrozoobenthos biomasses, and the ratio BFish/BMacrozoobenthos, for all spatial boxes
946 before the construction of Port2000 (first bars) and after (second bars). The spatial boxes are
947 composed of the navigation channel (CH), the northern channel (FN), and the southern channel
948 (FS) as estuary boxes; an intermediate box located at the mouth of the estuary (E4), and two
949 marine boxes (E2 and E14).

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951 **Fig. 3.** Barplots representing taxonomic richness divided by the log-transformed total number
952 of samples realised within the six spatial boxes before (first bars) and after (second bars) the
953 construction of Port2000 for fish and macrozoobenthos. The spatial boxes are composed of the
954 navigation channel (CH), the northern channel (FN), and the southern channel (FS) as estuary
955 boxes; an intermediate box located at the mouth of the estuary (E4), and two marine boxes (E2
956 and E14).

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958 **Fig. 4.** MDS ordination based on Bray-Curtis similarity matrices from biomass data (square-
959 root transformed) for fish (on the upper part) and for macrozoobenthos (on the lower part), for
960 all spatial boxes during the first (first bars), before Port2000, and the recent (second bars)
961 periods.

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963 **Fig. 5.** ELFI values for the whole estuary for 10 years, between 1995 and 2012, with the class
964 boundaries of the Water Framework Directive.

965

966 **Fig. 6.** Ecological network analysis (ENA) indices for all spatial boxes for the period before
967 (first bars) and after (second bars) Port2000. The spatial boxes are composed of the navigation
968 channel (CH), the northern channel (FN), and the southern channel (FS) as estuary boxes; an
969 intermediate box located at the mouth of the estuary (E4), and two marine boxes (E2 and E14).
970 Means and standard deviations are represented. Asterisks represent significant changes assessed
971 from Cliff's delta statistics (level 0.05; between before and after construction).

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Appendices

Appendix A. Data sources used in the calculation of ELFI index

Indicator	Before Port2000	After Port2000	Boundary classes
ELFI index	Biomasses from Ifremer and CSLN. Use of an R script provided by Mario Lepage (Irstea, UR EABX)	Index values from Agence de l'Eau Seine-Normandie	Delpech et al., 2010

Fish data 'After Port2000' are standardized data from surveys designed for ELFI calculation following normalized protocol. Fish data used for ELFI calculation 'Before Port2000' were obtained using similar sampling gear (beam trawls with similar net features), accounting for sampling effort (trawled surface area) and considering a relevant spatial design regarding WFD fish sampling protocol (> 6 tows by haline zone)

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Appendix B. Range values between the five 'Ecological Quality Status' levels considered for the ELFI indicator (Delpech et al., 2010). Five levels of EQS classified as 'high', 'good', 'moderate', 'poor', and 'bad' were used. 'High status' means very low human pressure. 'Good status' means a 'slight' deviation from this condition, 'moderate status' means moderate deviation, 'poor status' means high deviation and 'bad status' means very high human pressure.

	Range values for
EQS level	ELFI
High	$0.90 \leq \text{ELFI} \leq 1.00$
Good	$0.68 \leq \text{ELFI} < 0.90$
Moderate	$0.45 \leq \text{ELFI} < 0.68$
Poor	$0.23 \leq \text{ELFI} < 0.45$
Bad	$0.00 \leq \text{ELFI} < 0.23$

Appendix C. Data sources used in the calculation of the different indicators

Group	Compartment	Biomass data	Biodiversity indicators	ENA indices	ELFI
1	Seabirds	-		X	
2	Fish, piscivores	Ifremer, CSLN, Le Havre port authority sampling programs (Liteau, Port2000, Grand Canal, COLMATAGE 2008-2009-2010), and GIP Seine-Aval	X	X	X
3	Fish, planktivores		X	X	X
4	Fish, benthos feeders		X	X	X
5	Benthic predators	PECTOW, BENTHOSEINE, and COLMATAGE projects, Le Havre port authority, and GIP Seine-Aval	X	X	
6	Benthic filter feeders		X	X	
7	Benthic deposit feeders		X	X	
8	Suprabenthos	National Natural History Museum Paris, Wimereux marine station, Caen University, Le Havre port authority, and GIP Seine-Aval sampling programs		X	
9	Zooplankton	From Rybarczyk and Elkaïm (2003)		X	
10	Meiofauna	Le Guellec and Bodin (1992); Ratsimbazafy, (1998); Spilmont *et al.* (2005)		X	
11	Bacteria	Chardy (1987); Chardy and Dauvin (1992)		X	
12	Microphytobenthos	Spilmont *et al.* (2005), Napoleon *et al.* (2012)		X	
13	Phytoplankton	SURVAL database (http://envlit.ifremer.fr/resultats/surval)		X	
14	Detritus	Seine-Aval sampling program		X	

Appendix D. Biomasses entered in LIM-MCMC models for all trophic compartments and for both periods, before (1996-2002) and after (2005-2012) Port2000 construction. The values of biomass are mean over the years. Data sources are presented in Appendix A. More information can be found in Tecchio et al. 2016. For the spatial boxes, see Fig.1.

	Trophic compartments		Period	Biomass (gC m^{-2} y^{-1})					
				CH	FN	FS	E4	E14	E2
1	OIS	Seabirds	1996-2002						
			2005-2012						
2	FPI	Fish, piscivorous	1996-2002	0.019	0.060	0.057	0.143	0.103	0.264
			2005-2012	0.044	0.048	0.055	0.061	0.543	0.004
3	FPV	Fish, planktivorous	1996-2002	0.040	0.037	0.025	0.043	0.026	0.016
			2005-2012	0.012	0.027	0.028	0.047	0.013	0.004
4	FBF	Fish, benthos feeders	1996-2002	0.003	0.005	0.230	0.560	0.244	0.554
			2005-2012	0.088	0.327	0.252	0.307	0.387	0.358
5	IPR	Invertebrates, predators	1996-2002	0.163	17.15	9.830	1.000	2.590	0.981
			2005-2012	1.118	26.88	41.65	1.109	14.69	2.817
6	IFF	Invertebrates, filter feeders	1996-2002	0.442	1.897	22.32	3.08	3.81	12.71
			2005-2012	1.963	4.340	5.262	9.425	3.123	10.82
7	IDF	Invertebrates, deposit feeders	1996-2002	0.052	1.587	3.140	6.134	6.134	4.255
			2005-2012	1.420	1.466	0.865	7.200	3.705	5.923
8	SUP	Suprabenthos	1996-2002	2.208	2.030	2.010	0.101	0.101	0.101
			2005-2012	0.542	0.960	0.930	0.020	0.020	0.020
9	ZOO	Zooplankton	1996-2002						
			2005-2012						
10	NEM	Meiofauna	1996-2002	0.427	0.119	0.119	0.377	0.402	0.167
			2005-2012	0.427	0.119	0.119	0.377	0.402	0.167
11	BAC	Bacteria	1996-2002						
			2005-2012						
12	MPB	Microphytobenthos	1996-2002	0.050	1.725	1.720	0.300	0.350	0.380
			2005-2012	0.050	1.725	1.720	0.300	0.350	0.380
13	PHY	Phytoplankton	1996-2002						
			2005-2012						