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7 **Estimating the minimum suitable catch size of two clam species (*Ruditapes***
8 ***decussatus* and *Ruditapes phillipunarum*) on the northern coast of Spain**
9 **(Cantabrian Sea) using a kernel-based nonparametric model**

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38 **Abstract**

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40 The present study analyzes the weight gain patterns per unit of size and estimates the minimum
41 suitable catch size of *Ruditapes decussatus* and *Ruditapes phillipinarum*. For this purpose, data
42 from the two largest estuaries along the northern coast of Spain (Cantabrian Sea) were used. The
43 length-weight relationship of both studied species was estimated using two models: a classic
44 allometric model and a nonparametric regression model based on local linear kernel smoothers.
45 Additionally, first derivatives were used to estimate a minimum capture size for this species,
46 corresponding to the size at which the first derivative reached the maximum. Within this context,
47 the models application showed (a) the nonparametric model resulted in a better fit of data for both
48 species (b) different minimum catch sizes for each species based on maximum length (49.5 mm
49 for *R. decussatus* and 44.7 mm for *R. phillipinarum*), both considerably larger than the currently
50 established in EU and (c) an effect of estuaries and zones on individuals weight gain patterns.
51 This confirmed the nonparametric model as an alternative approach to analyze the length-weight
52 relationship for the studied species and to estimate a minimum suitable catch size of capture in
53 order to obtain, in the long run, the maximum yield in weight from the fishery.

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58 **Keywords:** *Ruditapes decussatus*, *Ruditapes phillipinarum*, length-weight, catch size, non
59 parametric, kernel, bootstrap

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72 **1. Introduction**

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74 The grooved carpet shell clam *Ruditapes decussatus* (Linnaeus, 1758) is a commercial species
75 native to Europe, being found along the NE Atlantic coasts and in the Mediterranean (Gosling,

76 2003). The Manila clam *Ruditapes philippinarum* (Adams and Reeve, 1850), endemic in Indo-
77 Pacific, was introduced in Europe at the beginning of the 1970s for culture purposes and
78 naturalized rapidly in estuaries and lagoons occupying a habitat that overlaps that of the native
79 species *R. decussatus* (Jensen et al., 2004). In a short number of years, the Manila clam became a
80 commercially exploited resource due to its considerable commercial value (Usero et al., 1997)
81 and its higher productivity and resistance to unfavourable conditions compared to the native
82 species (Melia et al., 2004). It is known that *R. philippinarum* is more hardy and resistant than
83 *R. decussatus* (Breber, 1985;1991) and it also grows faster over a wide range of temperatures
84 (Laing et al., 1987; Jensen et al., 2004 and references therein) or under the influence of a potential
85 competitor (Mistri, 2004)

86

87 In the northern coast of Spain, Bahía de Santander and Marismas de Santoña are considerably
88 productive estuaries in terms of standing stocks of these species, with 58 t of dry weight for *R.*
89 *decussatus* and 90 t for *R. philippinarum* and 18 t for *R. decussatus* and 16 for *R. philippinarum*,
90 respectively (GESHA, 2005a). Besides the shellfishing conducted on the natural resources, some
91 *R. philippinarum* farming areas are located on the central south-eastern sand flat of Bahía de
92 Santander. In 2005 and 2010 clam populations of these species were evaluated in both estuaries
93 showing a considerable decrease in the relative abundance of *R. decussatus* in the Bahía de
94 Santander (Juanes et al, 2012). The management of clam fishery in these estuaries has been based
95 on setting a minimal size of capture and closing areas through regional annual regulations (e.g.
96 Orden DES/25/2011). Regardless of the existing important biological differences between these
97 species the minimum catch size of individuals established in these estuaries and across all the
98 north coast of Iberian Peninsula is the same (40 mm) for both species. However, the minimum
99 legal size established by the European Union was 40 mm for both species to 2007 and after that
100 it was decreased to 35 mm for Manila clam (Dang, 2010).

101

102 A fishery management model without a solid base in scientific knowledge could lead to an
103 overexploitation of the target species. This knowledge is even more relevant when introducing a
104 fast-growing and resistant species such as Manila clam which must coexist with native ones, due
105 to an actual risk of dislodgment of this last species to very restricted areas. This occurred in other
106 European estuaries (e.g. Arcachon Bay, Lagoon of Venice) (Auby, 1993; Marin, 2003; Mistri,
107 2004). The estimation and respect of a specific suitable minimum catch size could have important
108 positive effects on the conservation of the native species and on the yield of the clam fishery,
109 which in turns, in the medium term might also benefit a great variety of macropredators such as
110 crabs, birds or fishes (Toba et al., 1992; Jamieson et al., 2001; Byers, 2005, Caldow et al., 2007;
111 Lum, 2011).

112

113 In this regard, setting the minimum differential catch size of exploited clam species is one of the
114 most important and widely used measures in fishery management strategies (e.g. Berthou et al.,
115 2005; Gilbert et al., 2006). Bald et al. (2009) developed a dynamic model, capable of predicting
116 clam population evolution in response to different management measures. Based on that work
117 they suggested that best management actions were in order of efficiency: (1) the establishment of
118 an appropriated minimum catch size, (2) the reduction in the capture season and (3) the increase
119 of non fishing zones. Freire and García-Allut (2000) also considered the use of protected areas
120 and minimum sizes as key regulation tools in European artisanal fisheries management models.

121

122 The estimation of adequate catch sizes for commercial marine invertebrates such as bivalves or
123 crustaceans integrate several biological aspects such as individual size at sexual maturation,
124 growth rate and length-weight relationship (Donaldson and Donaldson, 1992; XUNTA, 1992;
125 Coutures and Chauvet, 2001; Camacho-Mondragon et al., 2012). The length-weight relationship
126 has been studied in various marine species such as fishes (Froese, 2006; Nieto-Navarro et al.
127 2010) and crustaceans (Pinheiro and Fiscarelli, 2009) using different parametric models. For the
128 grooved carpet shell clam and Manila clam, this relationship has usually been defined by the
129 classic allometric model (Bald and Borja, 2001, 2004; Caill-Milly et al., 2003, 2006; Bradbury et
130 al., 2005); where clam weight (DW, g) is related to shell length (ML, cm) by the equation $DW =$
131 $a (ML)^b$, being a and b constants. In other bivalve species such as *Macoma baltica* or *Mercenaria*
132 *mercenaria* this relationship has also been analyzed using the same model (Bachelet, 1980;
133 Hoffman et al., 2006). Moreover, Sestelo and Roca-Pardiñas (2011) analyzed the potential of
134 benefit of using this relationship to objectively estimate a suitable catch size of the gooseneck
135 barnacle *Pollicipes pollicipes*, in order to obtain, in the long run, the maximum yield from the
136 fishery. They proposed the minimum catch size as the one at which the first derivative of the
137 length-weight relationship regression curve reached the maximum, ensuring that over this point
138 weight gain from one size to the next decreases. For this purpose, they used a nonparametric
139 model considering the absence of a maximum in the first derivative of the allometric model.

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141 The success in the application of this nonparametric model to other marine commercial species
142 (e.g. bivalves) could confirm this technique as an alternative method to study the length-weight
143 relationship and to estimate the minimum size of capture. Within this context, the aims of this
144 study were to (a) to assess the suitability of the non parametric model to study the length-weight
145 relationship of *Ruditapes decussatus* and *Ruditapes phillipinarum* (b) to analyze the applicability
146 of this model to estimate a minimum catch size for each species taking into account the
147 environmental conditions integrated in different estuaries and zones of each estuary.

148

149

150 **2. Materials and methods**

151

152 2.1. Study site

153 This study was conducted in the intertidal areas of the two most important estuaries in northern
154 Spain (Gulf of Biscay): Bahía de Santander (22.7 km²) and Marismas de Santoña (18.7 km²)
155 (Figure 1). Galvan et al. (2010) classified both transitional waters as morphologically complex
156 and dominated by intertidal areas and tidal dynamics. The intertidal area represents 67 % and 57
157 % of the total area of Bahía de Santander and Marismas de Santoña, respectively. In these
158 intertidal sandflats shellfishing of *Ruditapes phillipinarum* and *Ruditapes decussatus* bivalves
159 is conducted using traditional techniques (i.e. hand rakes, knives). The substratum of this area
160 varies from sandy (open and more oceanic areas) to muddy sediments (inner and more estuaric
161 areas) (Puente et al., 2002 and references therein). Subtidal zones are dominated by shallow
162 waters (< 5m) and hydrodynamic conditions are controlled by a semidiurnal tidal regime and 3 m
163 mean tidal range, interacting with variable freshwater inputs. Further details of these estuaries and
164 sand flats are provided elsewhere (e.g. Puente et al., 2002; Irabien et al., 2008; Galván et al.,
165 2010).

166

167 **(Figure 1)**

168

169 2.2. Data collection and laboratory procedures

170

171 Specimens of *R. decussatus* and *R. phillipinarum* were collected from different sites in both
172 estuaries where commercial operation of these resources is conducted. Sampling was performed
173 during spring low tides in April 2010. All the individuals were extracted in stations (i.e. transects
174 of 1m x 10m) located in the intertidal sand flats by means of the hand raking of the sediment
175 (upper 15 cm). This operation was similar to the artisanal shellfishing technique used to gather
176 clams and it was conducted by an experienced shellfisherwoman supervised by scientific
177 personnel (Juanes et al., 2012). The sediment was turned over by the rake and clams were
178 collected by hand as they appear to the surface.

179

180 In the Bahía de Santander individuals were collected from 18 stations located on three zones:
181 Central zone, considered as a more oceanic area, and northern and southern zones considered as
182 more estuarine or inner areas. In Marismas de Santoña individuals were gathered from 30 stations
183 located on four zones: Central zone, considered as more oceanic open zone, and northern,
184 southern and western zones considered as inner areas (Figure 2). This grouping of stations by
185 inner and open areas was made by GESHA (2005b) based on spatial proximity and similar
186 environment characteristics according to the requirements of the Water Framework Directive for

187 the classification of superficial water bodies (Vincent et al., 2002; Borja et al., 2004). Taxonomic
188 determination of each individual was carried out in the laboratory, followed by maximum shell
189 length (ML , mm) and dry weight (DW , g) measurements. These measurements were made using
190 a digital calliper with a precision of 0.1 mm and a 0.01 g precision balance after oven drying of
191 individuals for 72 h at a temperature of 60°C till constant weight. A total of 2693 individuals were
192 measured (1605 individuals of *R. decussatus* and 1088 individuals of *R. phillipinarum*).

193

194 **(Figure 2)**

195

196 2.3. Statistical background

197 In this section we report the applied statistical methodology. Firstly, we present both the used
198 models and the introduced test and secondly, we describe with detail the estimation algorithms
199 and the inference procedures.

200 2.3.1. Length-weight relationship

201 The length-weight relationship of both species was estimated using two models: a parametric
202 model and a more generalized model, according to Sestelo and Roca-Pardiñas (2011). Firstly, the
203 dependence between DW and ML was estimated using a classic allometric model. As we
204 mentioned before, this parametric model is one of the most widely used models to estimate this
205 relationship in fish biology and fisheries. The regression curve takes the following form

$$206 \quad E[DW|ML] = a ML^b \quad (1)$$

207 being a and b constants. This model is usually converted into its logarithmic expression, and this
208 conversion, which is quite simple, both conceptually and mathematically, facilitates the
209 estimation of its parameters by linear regression.

210 However, in certain circumstances, the assumption of a given curve on the effects of the covariate
211 is very restrictive and it is not supported by the data at hand. Therefore and secondly, to ascertain
212 the cited relationship, we propose the use of a more generalized nonparametric model of the type

$$DW = m(ML) + \varepsilon \quad (2)$$

213 where m is an unknown smooth function and ε is the error that is assumed to have mean zero and
214 variance as function of the covariate ML . It should be note that, in contrast to allometric model,
215 in this type of model there is no need to establish a parametric form of m .

216 It is important to highlight that in practice a bad specification in the model could suppose incorrect
217 conclusions. Hence, we also propose a procedure that will help us to compare and select an
218 adequate model to fit the data. To this end, consideration will be given to a test for the null
219 hypothesis of an allometric model vs. a general nonparametric model. The objective is to test

$$H_0: m(ML) = a ML^b \quad (3)$$

220 versus H_1 with m being an unknown function.

221 Note that if H_0 is not rejected, then the parametric model will be suitable to the data and we will
222 reject the use of a more general model. By contrast, if H_0 is rejected, the conclusion to be drawn
223 is that the specified form is not correct and it will be necessary to fit the nonparametric model
224 proposed above.

225 *Factor-by-curve interactions*

226 In some circumstances, it might be interesting to compare the estimated curves between the
227 different levels of a factor (e.g. species, estuary or zone). In this framework, a generalization of
228 the “pure” model in (2) is the regression model with factor-by-curve interactions. In this type of
229 model, the relationship between DW and ML can vary among subsets defined by levels of a
230 categorical covariate F .

231 Particularly, in our study, we were interested in comparing first the length-weight relationship
232 between the two species of the study. To this end, we considered the following model

$$DW = m_0 + \begin{cases} m_1(ML) + \varepsilon_1 & \text{if } F = 1 \\ m_2(ML) + \varepsilon_2 & \text{if } F = 2 \end{cases} \quad (4)$$

233 where ε_1 and ε_2 are the mean zero errors for each factor’s levels, m_0 represents global effect of
234 ML on the response; and m_1 and m_2 are the specific effects of ML associated with the level 1 (*R.*
235 *decussatus*) and 2 (*R. philipinarum*) of the factor F (species).

236 Secondly, in order to detect a possible effect of the estuary in the gain weight pattern, two
237 alternative models were proposed, one for each species. These models are analogues to the
238 presented in (4) but taking into account the factor F as estuary, being level 1 Bahía de Santander
239 and level 2 Marisma de Santoña.

240 Thirdly, to know the effect of the zone we propose again a similarly model for each species, being
241 F the factor zone and inner zones and open zones the levels 1 and 2, respectively.

242 *Minimum suitable catch size*

243 Based on the model in (2), we could suggest a possible size of capture for these species. The ideal
244 size, named ml_0 , will be given for the maximizer of the first derivative of m . This point could be
245 define as

246
$$ml_0 = \arg \max_{ml} m^1(ml)$$

247 In practice, the true $m^1(ml)$ is not known, and decisions will be taken on the basis of its estimate
248 $\hat{m}^1(ml)$. A natural estimator of ml_0 can be defined as the maximizer of

249
$$\hat{m}^1(ml_1), \dots, \hat{m}^1(ml_N)$$

250 with ml_1, \dots, ml_N being a grid of N equidistant points in a ranger of the ML values. We have taken
251 an $N = 10000$ points, so the distance between consecutive nodes is less than 0.01 mm of ML .
252 Analogously, we can obtain ml_{01} and ml_{02} as the maximizer of $ml_1^1(ml)$ and $ml_2^1(ml)$, being ml_1^1
253 (ml) and being $ml_2^1(ml)$ the regression curves of DW on ML for the level 1 and 2 of the factor,
254 respectively.

255 Additionally, to make inference about this size and compare it between the two levels of the factor
256 (e.g. species, estuary or zone), we propose the use of a measure of association as statistical test.
257 The proposed measure could be considered as the following difference

258
$$dif = ml_{02} - ml_{01}$$

259 where ml_{01} and ml_{02} are the maximizer of the first derivatives for *R. decussatus* and *R.*
260 *phillipinarum*, or for Bahía de Santander and Marismas de Santoña or for inner and open zones,
261 respectively. The confidence interval constructed for this measure will help us to know with
262 statistical significance if the size for the two levels is the same. The general rule is that the point
263 where the clams reach their maximum weight gain is different between levels if the zero value is
264 not within the interval.

265 Additionally to the size where the first derivative reached a maximum, differences in weight gain
266 patterns between species, estuaries and zones were analyzed descriptively by comparing the value
267 of the curve at several sizes (e.g. 20, 30, 40, 50 mm).

268 The procedure that enables the confidence intervals for ml_0 or for dif to be constructed is outlined
 269 below.

270 *2.3.2. Estimation and inference procedures*

271 Here, we describe briefly the estimation of the allometric and nonparametric model. In the case
 272 of the first one (eq.1), it was converted into its logarithmic expression in (5) to estimate its
 273 parameters by linear regression

$$\log DW = \log a + b \log ML = a^* + b^* \log ML + \varepsilon \quad (5)$$

274

275 Once \hat{a}^* and \hat{b}^* have been obtained by fitting the model in (4), the parameters' original scale was
 276 returned to, $\hat{a} = \exp(\hat{a}^*)$ and $\hat{b} = \hat{b}^*$, and the estimated model, $\widehat{DW} = \hat{a}ML^{\hat{b}}$, was obtained. In
 277 addition, the estimation of the derivative of DW was then given by $\widehat{DW}' = \hat{a}\hat{b}ML^{\hat{b}-1}$.

278 In the case of the nonparametric model in (2), to date, several approaches to estimating the
 279 regression curve m and its first derivative m^1 have been suggested in the statistical literature, e.g.
 280 methods based on penalized regression splines (Eilers and Marx, 1996) or the Bayesian versions
 281 of these (Lang and Brezger, 2004). This paper is focused on local linear kernel smoothers (Wand
 282 and Jones, 1995). These smoothers have been chosen since, among other advantages, they enable
 283 the use of binning type acceleration techniques (Fan and Marron, 1994) to reduce computational
 284 time and so ensure that the problem can be adequately addressed in practical situations. Based on
 285 this approach and for simplicity of notation, from now on, we denote this model as KNP model
 286 (kernel-based nonparametric model).

287 Given the original sample $\{ML_i, DW_i\}_{i=1}^n$, the local linear kernel estimator of $m(ml)$ and its first
 288 derivative $m^1(ml)$ at a location ml are defined as $\hat{m}(ml) = \hat{\beta}_0(ml)$ and $\hat{m}^1(ml) = \hat{\beta}_1(ml)$,
 289 where $\hat{\beta} = (\hat{\beta}_0, \hat{\beta}_1)$ is the minimizer of

290

$$\sum_{i=1}^n (DW_i - \beta_0 - \beta_1(ML_i - ml))^2 h^{-1} K\left(\frac{ML_i - ml}{h}\right)$$

292

293 where $K(u) = 1/\sqrt{2\pi} \exp(-0.5u^2)$ is the Gaussian kernel function (a symmetric density), and h
 294 > 0 is the smoothing parameter or bandwidth. The nonparametric estimates obtained of m and m^1
 295 are known to depend heavily on the bandwidth, h , which controls the trade-off between the bias
 296 and the variance of the resulting estimates. Various proposals based on some error criterion for

297 an optimal selection have been suggested, yet the difficulty of asymptotic theory means that
 298 nowadays optimal selection is still a challenging open problem. As a practical solution to this
 299 problem, in this paper we consider that the smoothing bandwidth, h , can be selected automatically
 300 by minimizing the following cross-validation error criterion (Stone, 1977)

$$301 \quad CV = \sum_{i=1}^n \left(DW_i - \hat{m}^{(-i)}(ML_i) \right)^2$$

302 where $\hat{m}^{(-i)}(ML_i)$ indicates the estimate at ML_i , leaving out the i^{th} element of the sample.

303

304 *Bootstrap Based Confidence Intervals*

305

306 The wild bootstrap procedure (see Härdle and Mammen (1993); Härdle and
 307 Marron (1991); Mammen (1992)) was used again for the construction of pointwise confidence
 308 intervals (*CI*). This resampling method is valid for heterocedastic models where variance of ε is
 309 a function of ML . The steps for constructing these *CI* for a Z value obtained from the model in (2)
 310 (for instance, $Z = ml_0$, $Z = dif$, or $Z = m(ml)$ for a given ml) are the following:

311

312 Step 1. Obtain the estimated \hat{Z} from the original sample.

313

314 Step 2. For $b = 1 \dots B$ (e.g. $B = 1000$), generate bootstrap samples $\{ML_i, DW_i^{*b}\}_{i=1}^n$ generated
 315 the same way as in Step 2 from testing procedure presented earlier, but using, in this case, the
 316 estimations and errors of the nonparametric model, and obtain the bootstrap estimates \hat{Z}^{*b} the
 317 same way as in Step 1.

318

319 Finally, the $100(1 - \alpha)\%$ limits for the confidence interval of Z are given by

320

$$321 \quad I = (\hat{Z}^{\alpha/2}, \hat{Z}^{1-\alpha/2})$$

322

323 where \hat{Z}^p represents the percentile p of bootstrapped estimates $\hat{Z}^{*1}, \dots, \hat{Z}^{*B}$.

324

325 *Testing for the allometric model*

326 The objective is to test the null hypothesis in (3), $H_0: m(ML) = a ML^b$ versus general hypothesis
327 H_1 being m an unknown nonparametric function. To test H_0 , we propose the use of the likelihood
328 ratio test given by

$$329 \quad T = \sum_{i=1}^n (DW_i - \hat{a}ML_i^{\hat{b}})^2 - \sum_{i=1}^n (DW_i - \hat{m}(ML_i))^2$$

330

331 where $\hat{a}ML_i^{\hat{b}}$ and $\hat{m}(ML_i)$ are the estimates of $m(ML_i)$ under H_0 and H_1 respectively.

332

333 The test rule based on T consists of rejecting the null hypothesis if $T > T^{1-\alpha}$, where T^p is the
334 p -percentile of T under H_0 . Nevertheless, it is well known that, within a nonparametric
335 regression context, the asymptotic theory for determining such percentiles is not closed, and
336 resampling methods such as bootstrap introduced by Efron (1979) (see also Efron and
337 Tibshirani, 1993; Härdle and Mammen, 1993; Kauermann and Opsomer, 2003) can be applied
338 instead.

339

340 In this section we have used the wild bootstrap for determining the critical values of test T . The
341 steps of the procedure are as follows:

342

343 Step 1. Obtain from the sample data $\{ML_i, DW_i\}_{i=1}^n$ the estimates $\hat{a}ML_i^{\hat{b}}$ and $\hat{m}(ML_i)$ obtained
344 under H_0 and H_1 respectively, and compute the T value.

345

346 Step 2. For $b = 1 \dots B$ (e.g. $B=1000$), generate bootstrap samples $\{(ML_i, DW_i^{\bullet b})\}_{i=1}^n$ with

347 $DW_i^{\bullet b} = \hat{a}ML_i^{\hat{b}} + \hat{\varepsilon}_i^{\bullet b}$ being

348

$$349 \quad \hat{\varepsilon}_i^{\bullet b} = \begin{cases} \hat{\varepsilon}_i \cdot \frac{(1 - \sqrt{5})}{2} & \text{with probability } p = \frac{5 + \sqrt{5}}{10} \\ \hat{\varepsilon}_i \cdot \frac{(1 + \sqrt{5})}{2} & \text{with probability } p = \frac{5 - \sqrt{5}}{10} \end{cases}$$

350

351 where $\hat{\varepsilon}_i = DW_i - \hat{a}ML_i^{\hat{b}}$ are the errors of the allometric model, and compute $\hat{T}^{\bullet b}$ the same way
352 as in Step 1.

353

354 Finally, the test rule based on T consists of rejecting the null hypothesis if $T > T^{1-\alpha}$, where T^p
355 is the empirical p –percentile of values $T^{\bullet b}$ ($b = 1, \dots, B$) obtained before.

356

357

358 **3. Results**

359

360 *3.1. Length-weight relationship models*

361 The fitted allometric model was $\widehat{DW} = 4.24 \times 10^{-5} ML^{3.26}$ for *R. decussatus*, whilst
362 the model resulted for *R. phillippinarum* was $\widehat{DW} = 7.34 \times 10^{-5} ML^{3.19}$ (Figure 3). Under
363 the KNP model, the initial regression curves were increasing functions for both species, very
364 similar to the obtained with the allometric model. However, the KNP model detected variations
365 in the final part of the regression curve which were more pronounced for *R. phillippinarum*
366 (Figure 3). In the case of the KNP model the expression or formulae is omitted since in this
367 framework there are no parameters to be estimated. Both models estimated very similar DW
368 values until a ML value of 40 mm for *R. decussatus* and 45 mm for *R. phillippinarum* was reached.
369 Thereafter, for an ML size of 50 mm, the allometric model estimates, for *R. decussatus*, a mean
370 DW value (95 % confidence interval) of 14.92 g (14.50, 15.53), versus 13.86 g (13.52, 14.18)
371 estimated by the KNP model. Similarly, for *R. phillippinarum*, the allometric model estimates a
372 DW value of 19.80 g (18.97, 20.43), versus 17.92 g (17.24, 18.57) estimated by the KNP model.

373

374 **(Figure 3)**

375

376 The results obtained from the likelihood ratio test (Table 1) to compare the fit of the models
377 showed a better fit of data was achieved using the KNP model. The same result was obtained for
378 both species, independently of using all data set, separating data by estuaries or by inner and open
379 zones.

380

381 **(Table 1)**

382

383 *3.2. Estimation of the minimum size of capture*

384

385 Under the allometric model, the first derivatives of the initial curves displayed increasing
386 monotonous functions. However, under the KNP model, the first derivatives showed a maximum
387 at a specific size after which it began to decrease (Figure 4). Therefore, the first derivative of the
388 regression curve displayed by the nonparametric model was used to estimate the minimum suitable
389 catch size.

390

391 **(Figure 4)**

392

393 Thus, for *R. decussatus* this minimum size (49.5 mm) was significantly larger than the one
394 estimated for *R. phillipinarum* (44.7 mm) (Table 2 and 3). The analysis carried out for estimating
395 a possible size of capture in each estuary and in different studied zones (i.e. inner and open
396 zones), showed for *R. decussatus* that this size was significantly higher in Marismas de Santoña
397 and inner estuarine zones. On the contrary, for *R. phillipinarum* this size of capture was the same
398 in both estuaries and also in both inner and open zones (Tables 2 and 3, Figures 5 and 6).

399

400 **(Table 2) (Table 3)**

401 **(Figure 5) (Figure 6)**

402

403 *3.3. Weight gain patterns*

404

405 The descriptive analysis of weight gain patterns by exploring the value of the curve of the first
406 derivative at several sizes showed that the weight gain per unit of size increased for both species
407 to a specific size after which it began to decrease. The weight gain per unit of size was considerably
408 higher for *R. phillipinarum* to the size beyond which it began to decrease. The decreasing pattern
409 was much pronounced for *R. phillipinarum* than for *R. decussatus* (Figure 4).

410

411 On the other hand, this descriptive analysis was done to explore the weight gain patterns in each
412 estuary (Figure 5) and in open and inner zones (Figure 6). For *R. decussatus* in Marismas de
413 Santoña and in inner estuarine zones, the decrease after reaching the maximum is hardly
414 appreciable. Besides, although the weight gain pattern of *R. decussatus* was similar in both
415 estuaries and also in both zones, the maximum weight gain per unit of size was reached at
416 significantly smaller sizes in Bahía de Santander and in open zones. However, for *R.*
417 *phillipinarum* the weight gain per unit of size was appreciably higher to 30 mm in Marismas de
418 Santoña and inner zones while the maximum was reached at similar sizes in both estuaries and
419 zones (Figures 5-6 and Tables 2-3).

420

421

422 **4. Discussion**

423 The KNP model applied in this paper to analyze the length-weight relationship of *Ruditapes*
424 *decussatus* and *Ruditapes phillipinarum* is a suitable tool to estimate the minimum catch size of
425 both species, considering this as the optimum size to obtain in the long term the maximum yield
426 in weight from the fishery.

427

428 This model resulted in a better fit of data when studying the length-weight relationships of *R.*
429 *decussatus* and *R. phillipinarum* in the two major estuaries in northern Spain, indicating that the
430 KNP model can be considered a viable alternative to the classic allometric model for both species.
431 The results coincide with those reported recently by Sestelo and Roca-Pardiñas (2011) for the
432 gooseneck barnacle *Pollicipes pollicipes*, which is also a species that displays a differentiated
433 weight gain pattern throughout its development.

434

435 Furthermore, the KNP model was able to record specific patterns in the data at the end of the
436 regression curve that could not be detected by the allometric model. This suggests that some
437 valuable biological information is lost using the allometric model (Rabaoui et al., 2007). This
438 may be due to the fact that the nonparametric models allow for a more flexible fit of the data than
439 the parametric regression techniques because they do not specify in advance any function that
440 links the covariates to the response. However, the detected variations in the final part of the
441 regression curve were slightly more pronounced for *R. phillipinarum* than those of *R. decussatus*.
442 Although this distinction could be linked to biological differences, there might be at least two
443 possible explanations for this pattern in need of further investigation: (1) The sampling dates of
444 this study could coincide with the beginning of the gonad development phase in *R. decussatus*
445 (Urrutia et al., 1999; Rodrigues-Carballo et al., 1992) and consequently, some individuals might
446 be contributing to skew the curve as they have a higher weight linked to their corresponding size
447 than they have during the resting period; (2) the reduction in the expected number of large
448 individuals of *R. decussatus* due to a higher fishing pressure on this species might be removing
449 information at the last part of the curve. Therefore, in further studies it is essential a long term
450 monitoring and sampling effort covering different seasons of the year in order to assess the
451 influence of the gonad development on the body growth and to explore the effect of the lack of
452 large sizes. On the other side of the curve, the absence of individuals < 20 mm is linked with the
453 sampling technique (i.e. traditional raking of sediments carried out by fishermen, Juanes et al.,
454 2012) but, due to the intrinsic features of the kernel estimator which use only the neighboring
455 points to the target point to fit the model, this fact does not affect to any of the objectives of this
456 work.

457

458 The demonstrated ability of the KNP model to analyze the length-weight relationship of this
459 species makes it a suitable model to estimate the weight gain patterns and therefore, to estimate a
460 minimum suitable capture size (Sestelo and Roca-Pardiñas, 2011). The first derivative of this
461 model was analyzed for both species. The minimum suitable catch size was given by the
462 maximizer of this derivative of the regression curve. Beyond these point the increase of weight
463 per unit of size decreases. Thus, this size ensures that individuals smaller than this size had not
464 yet attained the maximum yield in weight. In accordance with FAO guidelines for other species

465 with allometric length-weight relationship, such as fishes, individuals should be not captured until
466 they reach the “maximum yield size” (Sparre and Venema, 1997).

467

468 Based on that approach, the estimated minimum sizes were significantly higher for *R. decussatus*
469 (49.5 mm) than for *R. phillipinarum* (44.7 mm). But they were both larger than those established
470 by both the current UE normative (40 mm and 35 mm respectively) and that one in force until
471 2007 (40 mm for both species) and by the actual regulations of the Regional Government in the
472 studied Marismas de Santoña and Bahía de Santander estuaries (40 mm for both species). Size
473 limits are intended to protect exploited marine populations by allowing individuals to grow larger
474 and spawn at least once before removal from the fishery and thereby increase long-term yield
475 (Goodyear, 1996). In this line, considering their respective maturity sizes the specific minimum
476 catch sizes enforced by UE and the Regional Government, allow *R. decussatus* to spawn at least
477 once and *R. phillipinarum*, with high probability, at least twice. Sexual maturation phase can
478 begin between 10-20 mm shell lengths in *R. decussatus* (Lucas, 1968; Urrutia et al., 1999) while
479 in *R. phillipinarum* it can start at a 5-10 mm (Ponuvorovsky and Yakolev, 1992 and references
480 therein). However, the major contribution to reproduction is done by large individuals since
481 fecundity increases with size for both species (Holland and Chew, 1974; Ponuvorovsky and
482 Yakolev, 1992; Robert et al., 1993; Laruelle et al., 1994; Urrutia et al., 1999). Therefore, in this
483 study estimated catch sizes could avoid the exploitation of large individuals that still have a high
484 reproduction activity allowing clams to have more spawns than currently before they are fished.
485 It is worth noting that growth rate decreases considerably for larger clams (>30 mm) and
486 consequently individuals might take approximately 0.5-1 years (*R. phillipinarum*) to 2-2.5 years
487 (*R. decussatus*) (Arnal and Fernández-Pato, 1977, 1978; Spencer et al., 1991; Solidoro et al.,
488 2000; Chessa et al., 2005; Dang, 2009) to reach the estimated minimum catch sizes starting from
489 40 mm (i.e. the current legal size in the region). Thus, the setting of these higher minimum legal
490 sizes might lead to a substantial decrease of captures during the first years after the establishment
491 of the measure and, afterwards, the available annual commercial stock together with the spawning
492 activity and recruitment success could be considerably higher than the current ones for both
493 species. This prediction of higher yields for the fishery is assumed based on results obtained by
494 Bald and Borja (2002) for *R. decussatus* after modelling its exploitation and extrapolating them
495 to our findings and to *R. phillipinarum* which is a faster growing and hardier species (Breber,
496 1985;1991, Spencer, 1991). These authors simulated several exploitation scenarios and analyzed
497 the evolution of the population stock including biological variables at different size classes such
498 as fertility rate and natural mortality (Pérez-Camacho, 1979). When a scenario of exploitation of
499 legal sizes > 40 mm was compared with a scenario of illegal fishing (21-40 mm) they found that
500 the stock had decreased dramatically from ~3000 t to ~500 t. Regarding this result it seems that
501 contribution of large size clams (>40 mm) to the standing stock due their high fertility rates, might

502 be considerably more important than the negative effect associated to the increase of natural
503 mortality at this sizes. Taking to account this author's results and the fertility and mortality rates
504 of different size classes (Perez-Camacho, 1979) higher standing stocks and yields in the fishery
505 could be expected in the medium term in a scenario where the minimum catch size ensures that
506 individuals smaller than this size had not yet attained the maximum yield in weight.

507

508 This expected higher clam density scenario would be in line with the conservation of the European
509 native clam *R. decussatus*. However, it could also lead to a dramatic expansion and predomination
510 of the introduced Manila clam as it occurred in other European estuaries (e.g. Arcachon Bay,
511 Lagoon of Venice) (Auby, 1993; Marin, 2003; Mistri, 2004). However, in this hypothetic scenario
512 a great variety of macropredators suchs as crabs, birds or fishes (Toba et al., 1992; Jamieson et
513 al., 2001; Byers, 2005, Caldow et al., 2007; Lum, 2011) might mitigate the high *R. phillipinarum*
514 densities preying on them differentially more than *R. decussatus*, because crabs, birds and fish
515 excavate the shallowly burrowing Manila clam more easily (Seitz et al., 2001). This differential
516 mitigation effect could be highly probable in the studied estuaries both regarding to the significant
517 higher mortality due to predation found for *R. phillipnarum* comparing with *R. decussatus* in the
518 Bay of Santander by Bidegain and Juanes (2012) and considering the high concentration of
519 predators in Marismas de Santoña wetlands since it is an area of international importance for the
520 passage of migratory birds. The habitat suitability for the Manila clam could also play an
521 important role regulating a potential drastic expansion of the introduced clam (Bidegain et al.,
522 2012)

523

524 With respect to the study conducted by Sestelo and Pardiñas (2011), both the identification of
525 area-specific minimum catch sizes and the statistical analysis conducted to compare these sizes
526 are innovative aspects. The significant differences observed for *R. decussatus* when the catch size
527 was estimated by estuary and zone could suggest the feasibility of establishing estuary or zone-
528 specific minimum legal sizes. However, although this measure should be effective in obtaining
529 the maximum yield in weight for each estuary or zone, it is hardly applicable in a shellfishery
530 management plan due to the potential difficulties regarding both the control of illegal fishing and
531 the shellfishers. Thus, other less complicated measures could include more viable zone-based and
532 specific management measures related with capture seasons, no-fishing zones, rotations strategy
533 or sustainable locations for aquaculture (Morsan, 2007; Longdill, 2008).

534 Regarding the weight gain patterns analyzed by the first derivatives, higher dry weights of both
535 soft tissue and shell documented for Manila clam (Pazos et al., 2005; Dincer, 2006) may be
536 contributing to the observed differences between studied species. Moreover, several
537 environmental factors governing estuarine or zonal conditions (e.g. substrate, food availability,

538 salinity, fluctuations of mean conditions, etc.) could be, in part, responsible for the growth, weight
539 gain and shell structure variability of the clams (Robert et al., 1993; Gosling, 2003; Kanazawa
540 and Sato, 2007). In line with this, the higher weight gain per unit of size observed for *R.*
541 *phillipinarum* (to 30-35 mm) and for *R. decussatus* (around its maximum) in Marismas de
542 Santoña and inner zones might be related to the higher availability of an alternative source of food
543 avoiding decreases in dry weight associated to a loss in reserve tissue when phytoplankton is
544 scarce (Delgado and Pérez-Camacho, 2005). Page and Lastra (2003) documented that intertidal
545 suspension-feeding bivalves primarily fed on resuspended microbenthos or detritus during
546 periods of low phytoplankton concentration. This resuspended materia is more abundant in
547 estuaries with high organic matter inputs (river inflows) and within them in inner muddy zones
548 (Junoy and Viéitez, 1990; Mendes et al., 2006). Thus, the higher river inflows in Marismas de
549 Santoña estuary (twice the flow observed in Bahía de Santander) (Galván et al., 2010), which
550 result in higher concentrations of organic matter in the water as well as a higher capacity of inner
551 muddy zones to keep the incoming organic matter (Middelburg and Herman, 2007), might have
552 an effect in food availability and consequently, in individuals weight gain patterns. Moreover, the
553 waste water treatment plan is still in the last phases of construction in this estuary, which leads to
554 have an extra input of organic matter.

555 Although the above mentioned lack of larger sized data may be affecting the weight gain pattern
556 results, the food availability and therefore, better growth efficiency also in large sizes could be
557 involved in the hardly appreciable decreasing pattern observed for *R. decussatus* in Marismas de
558 Santoña and in inner estuarine zones.

559 Besides, differences in growth between oceanic and inner stations resulting from differences in
560 fluctuations of environmental conditions (e.g. temperature and salinity) observed by Robert et al.
561 (1993) may also be contributing to these variations in weight gain, although the high water
562 renovations rates for both estuaries (Galvan et al., 2010) are likely to minimize this potential
563 effect. Moreover, the incorporation rates of carbon sources into shell carbonate, from dissolved
564 inorganic carbon as well from food, has an important role in the shell weight gain (Poulain et al.,
565 2010) and it is expected to be different between estuaries or zones according to the environmental
566 differences above commented which could be affecting this rate. Finally, clams higher burying
567 capacity linked to smaller sediment grain size (i.e. in inner zones) (Nair and Ansell, 1968) leads
568 to larger and heavier siphons development (Zwarts and Wanink, 1989). This may be another
569 factor contributing to the differences observed in weight gain, especially for *R. decussatus* which
570 has considerably larger siphons and burial depth than *R. phillipinarum* (Mistri et al., 2004).

571

572 **5. Conclusions**

573 The results demonstrate the feasibility of using nonparametric techniques based on local linear
574 kernel smothers to analyze the length-weight relationship and to estimate the minimum capture
575 size of commercial species that display differentiated weight gain patterns throughout their
576 development. The estimated minimum suitable catch sizes are different between these species and
577 larger than those currently considered in the normative (UE and regional). These results are in
578 line with the biological differences between this species and may provide considerably higher
579 yields in weight from the fishery.

580

581 This study provides a preliminary study and a starting point to consider the revision of the
582 minimum legal size of the studied species in order to improve the current management models
583 and obtain, in the long run, an increase in available commercial stocks. Moreover, the differences
584 in weight gain patterns observed between estuaries and zones provide insight into the growth
585 patterns of these species and their link to the environmental conditions governing estuaries.
586 Finally, it is important to underscore that the potential effect of gonadal development of some
587 individuals and the effect of the lack of large individual remain to be determined.

588

589

590

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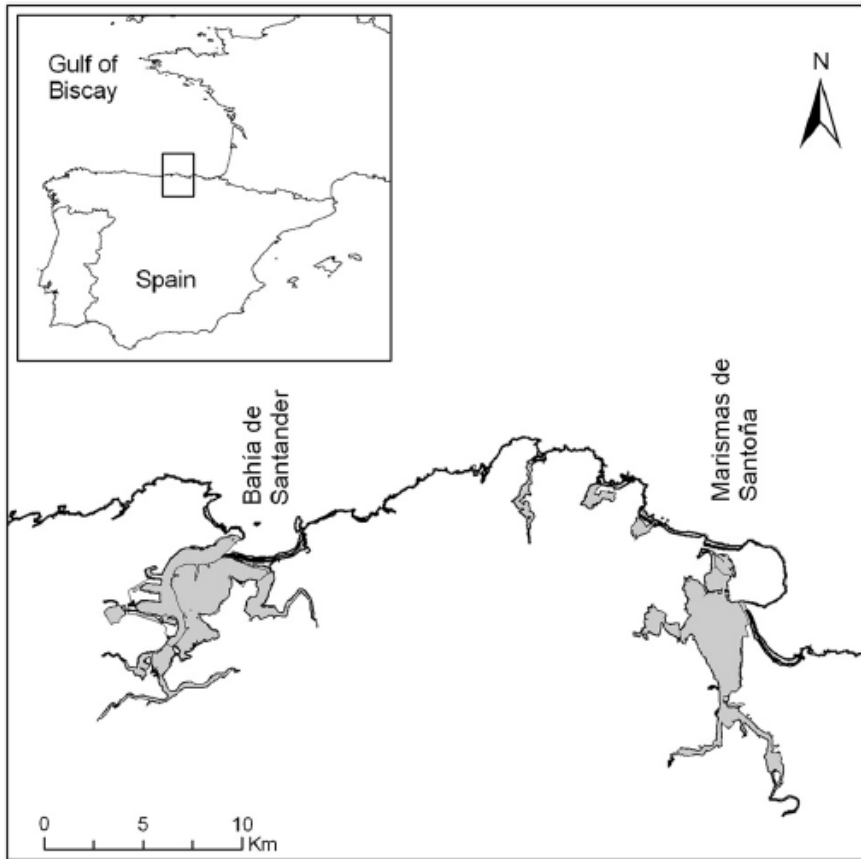
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854 **Figure footnotes**

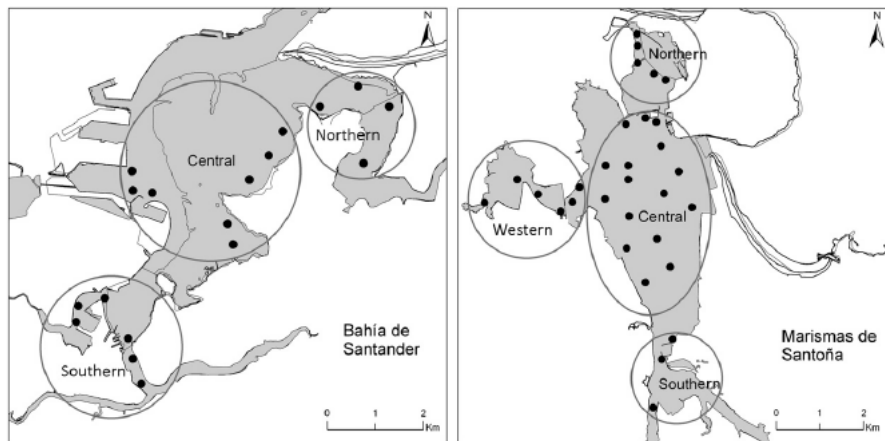
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857 Figure 1. Study area: Bahía de Santander and Marismas de Santoña estuaries located in the
 858 northern coast of Spain.

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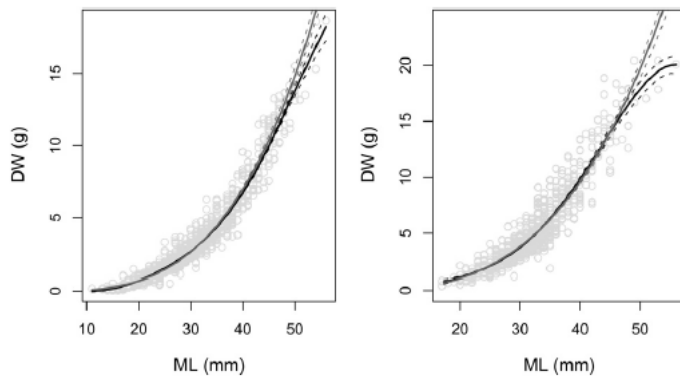


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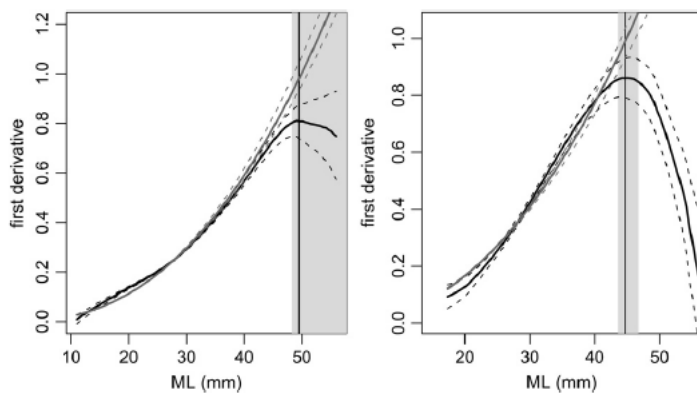
861

862 Figure 2. Location of the sampling stations and zones where *R. decussatus* and *R. phillippinarum*
 863 individuals were collected in Bahía de Santander (left) and Marismas de Santoña (right) estuaries.
 864 Stations are represented by black circles and zones are represented by grouping circles: in Bahía
 865 de Santander, Central zone, considered as a more oceanic area and northern and southern zones
 866 considered as more estuarine or inner areas; in Marismas de Santoña Central zone, considered as

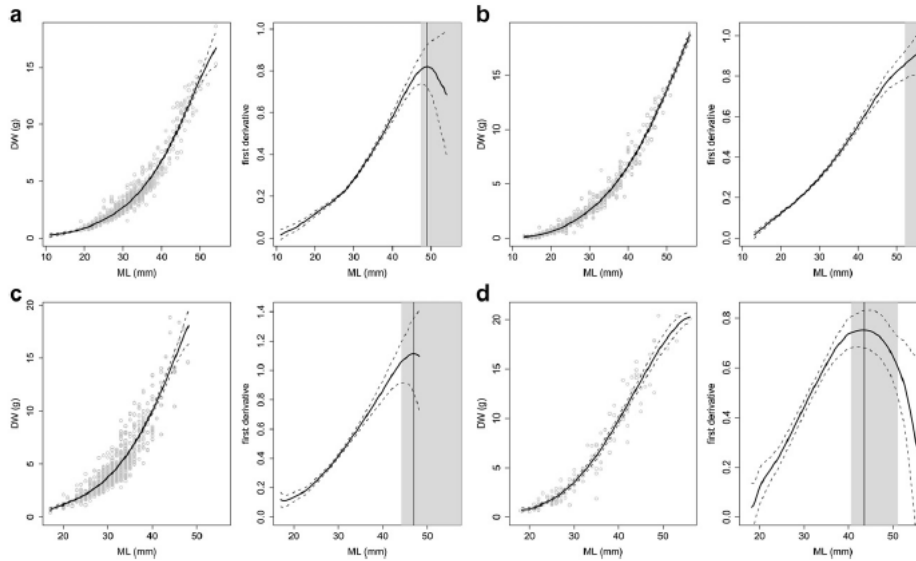
867 more oceanic open zone, and northern, southern zone and western zones considered as inner areas.
868



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870 Figure 3. Regression curves of the length-weight relationship (DW, dry weight and ML maximum
871 length) for *Ruditapes decussatus* (left) and *Ruditapes phillipinarum* (right). Grey solid lines refer
872 to the allometric model and black solid lines refer to the KNP model. Bootstrap-based 95%
873 confidence intervals are presented by broken lines. Both estuaries data set was used.

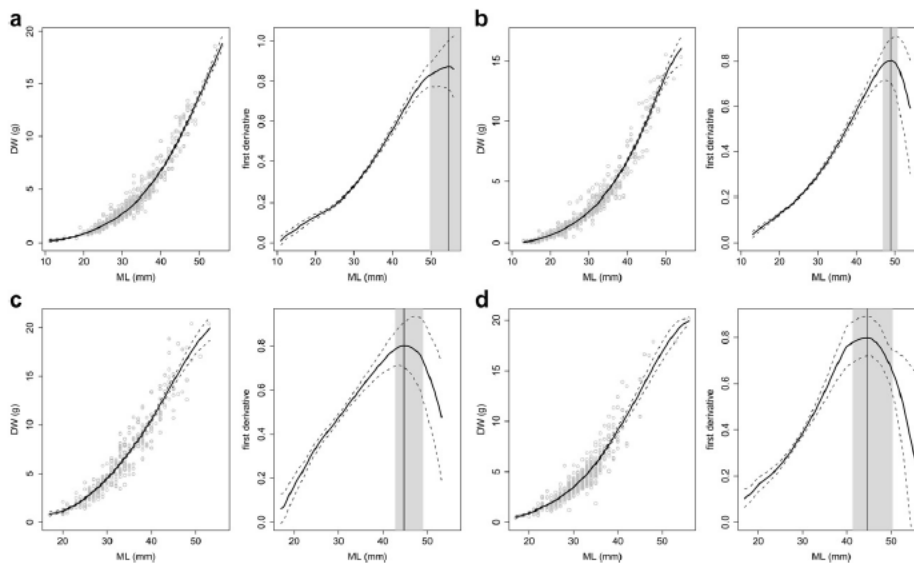


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875 Figure 4. First derivative of the initial regression curves for *Ruditapes decussatus* (left) and
876 *Ruditapes phillipinarum* (right). Grey solid lines refer to the allometric model and black solid
877 lines refer to the KNP model. For the KNP model, solid vertical line represents the estimated ml_0
878 (size where first derivative is maximum) and the grey area represents the confidence interval
879 constructed for the estimated ml_0 . Bootstrap-based 95% confidence intervals are presented by
880 broken lines. Both estuaries data set was used.



881

882 Figure 5. Non parametric model regression curve and first derivative (solid curved lines) with
 883 bootstrap-based 95% confidence intervals (broken lines) for dry weight (DW) and maximum
 884 length (ML) of *Ruditapes decussatus* in Bahía de Santander (a) and Marismas de Santoña (b), and
 885 for *Ruditapes philippinarum* in Bahía de Santander (c) and Marismas de Santoña (d). Solid
 886 vertical lines: estimated ml_0 (size where first derivative is maximum). Grey area: confidence
 887 interval constructed for the estimated ml_0 .



888

889 Figure 6. Non parametric model regression curve and first derivative (solid lines) with bootstrap-
 890 based 95% confidence intervals (broken lines) for dry weight (DW) and maximum length (ML)
 891 of *Ruditapes decussatus* on inner (a) and open zones (b) zones and for *Ruditapes philippinarum*
 892 on inner (c) and open zones (d). Solid vertical lines: estimated ml_0 (size where first derivative is
 893 maximum). Grey area: confidence interval constructed for the estimated ml_0 .

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Tables and footnotes

<u>Factor</u>	<u>n</u>	<u>Statistic</u>	<u>p-value</u>
Species			
<i>R. decussatus</i>	1605	0.06	0.02 *
<i>R. phillipinarum</i>	1088	0.20	0.01 *
Estuary			
<i>R. decussatus</i>			
Bahía Santander	926	0.08	0.01 *
Marismas Santoña	679	0.19	0.01 *
<i>R. phillipinarum</i>			
Bahía Santander	949	0.05	0.01 *
Marismas Santoña	139	1.74	0.01 *
Zone			
<i>R. decussatus</i>			
Inner	861	0.05	0.01 *
Open	744	0.09	0.02*
<i>R. phillipinarum</i>			
Inner	404	0.26	0.03 *
Open	684	0.14	0.04 *

900 Table 1. Comparison of the fit of the allometric and KNP models to the relationship between
901 length and weight using the likelihood ratio test for each of the analysis conducted: the first
902 analysis with species as factor (*R. decussatus* and *R. phillipinarum*) for both estuaries data, the
903 second one with estuary as factor (Bahía de Santander and Marismas de Santoña) for each species
904 and the last one with zone as factor (inner and open areas) for each species. For values *p*-
905 *value*<0.05, marked by an asterisk, the H_0 is rejected, so the nonparametric regression model
906 afforded better fit than the allometric model.

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Factor	n	\widehat{ml}_0	Lower CI	Upper CI
Species				
<i>R.decussatus</i>	1605	49.51	48.25	—
<i>R.phillipinarum</i>	1088	44.74	43.52	46.76
Estuary				
<i>R. decussatus</i>				
Bahía Santander	926	48.66	47.53	—
Marismas Santoña	679	56.00	52.71	—
<i>R. phillipinarum</i>				
Bahía de Santander	949	46.98	44.01	—
Marismas Santoña	139	43.58	40.69	48.46
Zone				
<i>R. decussatus</i>				
Inner zones	861	54.56	48.74	—
Open zones	744	48.88	46.90	50.77
<i>R. phillipinarum</i>				
Inner zones	404	44.79	42.73	49.05
Open zones	684	44.52	41.24	50.30

914

915 Table 2. Size \widehat{ml}_0 , which maximizes the first derivative of the regression curves and 95%
916 confidence interval, for each of the analysis conducted: the first analysis with species as factor
917 (*R. decussatus* and *R. phillipinarum*), the second one with estuary as factor (Bahía de Santander
918 and Marismas de Santoña) and the last one with zone as factor (inner and open areas)

919

Factor	dif	Lower CI	Upper CI
Species	- 4.77	- 12.03	- 2.34 *
<i>R.decussatus</i>			
Estuary	7.34	0.43	8.29 *
Zone	- 5.68	- 8.65	- 0.18 *
<i>R.phillipinarum</i>			
Estuary	- 3.4	- 7.11	3.31
Zone	- 0.27	- 6.01	5.14

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922 Table 3. Results of the statistical test used to compare the size \widehat{ml}_0 between species, estuaries and
923 zones. The difference between the maximizers of the first derivatives of the compared regression

924 curves (*dif*) together with the 95 % confidence intervals are presented. Significant differences (i.e.
925 zero is not included within the confidence interval) are presented by an asterisc.
926