1    Age and growth of the endangered fan mussel *Pinna nobilis* in the

2    Western Mediterranean Sea

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21   **Highlights**

22   • High variability is observed in growth models of 12 *Pinna nobilis* populations.

23   • Three general growth models are proposed for distinct environments.

24   • The models could be used to plan conservation strategies for *P. nobilis*.

25   • Populations surviving the die-off in paralic environments show low longevity.

26   • Oldest fan mussels were observed in marine protected areas.

27

28   **Abstract**

29   The present work, which is the first comparative study of the growth of the fan mussel

30   *Pinna nobilis* in the western Mediterranean, encompasses 12 populations of this species

31   living in different environments in France and Spain. Two hundred nine shells were

32   processed and used to obtain growth records from the posterior adductor muscle scar.

33   Size-at-age data were fitted to the Von Bertalanffy growth model. Considerable variability

34   in growth parameters and age was detected among the populations. The results show

35   that the only two fan mussel populations remaining in Spain, which live in an estuary and

36   a coastal lagoon, occupy habitats that are optimal for fast growth, but individuals show

37   low longevity, complicating the long-term conservation of the species. Multivariate

analyses groups the populations into three groups (SO, EO and LG), and a general model is proposed for each group; the model can be used as an approximation to calculate the ages of individuals living in similar environments.

## 1. INTRODUCTION

Age and growth are key features in population demography and relate the trophic and demographic aspects of a system (Margalef, 1998). Within this context, growth is also a tool that can be used to estimate age based on its relationship to measurable dimensions of the studied organism. Differences in size, age and growth among bivalve populations can be related to the environmental characteristics of their habitats, such as hydrodynamic conditions and seagrass cover (Garcia-March et al., 2007b; Hendriks et al., 2011; Irlandi, 1996), food availability and quality (Blicher et al., 2010; Fréchette and Bourget, 1985; Ortmann and Grieshaber, 2003; Wong and Cheung, 2001), population density (van Erkom Schurink and Griffiths, 1993), temperature (Blicher et al., 2010; Schwartzmann et al., 2011) and grain size (De la Huz et al., 2002) among other possible factors. Demographic features have been successfully used to estimate the best habitats for the reintroduction or protection of endangered species (Fariñas-Franco et al., 2016). Extensive demographic studies of the fan mussel *Pinna nobilis* that include measurement of age and growth have rarely been conducted because age and growth estimations were costly and/or unreliable until recently (Basso et al., 2015). The methodology proposed by Garcia-March et al. (2011), which uses the growth records of the posterior adductor muscle scar (PAMS) observed in radial sections of the shell, enabled the development of more precise and less costly age and growth estimations of this species (Kersting and Garcia-March, 2017). For years, the fan mussel has been considered an endangered Mediterranean endemic species, and it is included in the 'Habitats Directive' and in the ANNEX II of the Barcelona Convention. A recent mass mortality event (MME) that resulted in almost 100% mortality of the species along the Spanish Mediterranean coasts (Vázquez-Luis et al., 2017) (García-March et al., *in revision*) resulted in its reclassification to "endangered with extinction" in Spain (Orden TEC/596/2019, Ministerio para la transición Ecológica, 8 April, 2019). This MME was very likely caused by a recently discovered parasitic protozoan, *Haplosporidium pinnae* (Catanese et al., 2018), although Carella et al. (2019) also found a *Mycobacterium* in samples of diseased fan mussels that may have contributed to the die-off. The mortality

is presently spreading through the Mediterranean with lethal consequences (Katsanevakis et al., 2019; Panarese et al., 2019), leaving the species in a critical situation; only isolated populations remaining unaffected in specific reservoirs such as coastal marine lagoons and deltas remain unaffected (García-March et al., *in revision*). *P. nobilis* is the largest Mediterranean bivalve mollusk, reaching a size of up to 120 cm (Vicente, 1990; Zavodnik, 1991). It has a long life span that can exceed 45 years (Rouanet et al., 2015). Furthermore, it displays the fastest shell growth rate reported for any bivalve (Richardson et al., 2004). This growth is especially noticeable during the first months of life (Hendriks et al., 2012; Kersting and Garcia-March, 2017). Shell growth in this species is highly variable among populations (Richardson et al., 1999) and within the same population living at different depths (Garcia-March et al., 2007a). Oceanographic differences among sites (e.g., depth, temperature, hydrodynamics and food availability) may have a great influence on the species' growth rate (Garcia-March et al., 2007a; Garcia-March et al., 2007b; Hendriks et al., 2011; Katsanevakis, 2007). An understanding of the age and growth parameters of fan mussel populations inhabiting different conditions and their relationship to environmental variables such as hydrodynamics will improve the quality of demographic studies and the implementation of protection measures (Basso et al., 2015; Garcia-March et al., 2011; Richardson et al., 2004).

The present work represents the first comparative study of 12 fan mussel statistical populations (referred to hereinafter as populations) (Ludwig and Reynolds, 1988) living under various environmental conditions and located in protected and unprotected areas of the western Mediterranean (France and Spain). The relationship between growth parameters estimated using the method of Garcia-March et al. (2011) and the animals' habitat conditions is evaluated on the basis of differences in the site (lagoon, estuary or open sea), depth (shallow or deep) and hydrodynamic regime (sheltered and exposed) of the habitat. The protection status of the marine areas (protected or unprotected) was also considered. The results of this study will permit a better understanding of fan mussel ecology in relation to environmental factors such as wave exposure, especially considering that the IPCC (2018) panel predicts that increased weather extremes will occur in the future. The results will also help in the planning of effective restocking actions, the evaluation of the resilience of remaining populations and the creation of new marine protected areas specifically designed for the recovery of *P. nobilis* populations.

## 2. MATERIAL AND METHODS

## 2.1. Shell collections and study

The study was conducted using 12 populations of *P. nobilis* shells from the Spanish and French coasts (western Mediterranean) (**¡Error! No se encuentra el origen de la referencia.**); the shells had been stored in various laboratories. The empty shells were gathered from locations that were subject to different hydrodynamic and environmental conditions and various levels of governmental protection (5 of the locations have protected status), although all of the locations are presently included in the Natura2000 Network.

A total of 209 shells were used for growth parameter calculations. When possible, 20 shells, including shells that represented the entire size range available, were chosen from each population for analysis. However, some collections included many small individuals less than 3 years old; shells from these individuals were not used in the growth parameter calculations. Therefore, the final sample size ranged from 8 to 21 shells per population (

).

Populations 1 (Freus, N = 16) and 2 (Gandulf, N = 16) were obtained at the Marine Protected Area of Cabrera National Park (Balearic Islands) from exposed and sheltered sites, respectively, within *Posidonia oceanica* meadows and at a depth range of 5-10 m. Population 3 (Tabarca, N = 20) was obtained from the Tabarca Island Marine Protected Area, the first Marine Protected Area of Fishery Interest (RMIP) of Spain, created in 1986. The shells were sampled in a *P. oceanica* meadow located on the western part of the island in a site sheltered from main storms at 5-10 m depth within the *P. oceanica* meadow. Population 4 (Port-Cros, N = 20) was obtained from Port-Cros National Park (northwestern Mediterranean, Var, France), one of the oldest marine national parks in the Mediterranean Sea, created in 1963. Beginning in 1969, a monitoring program was initiated in the "Champ de La Palud" with the main purpose of controlling the evolution of fan mussels in this area (Vicente et al., 1980). Empty shells were sampled in this area; most were obtained from a dead matte of *P. oceanica* between 15 and 25 m in depth. The shells of population 5 (Olla, N = 19) were obtained at the southwest portion of a small islet in the 'Parque Natural Marítimo Terrestre Serra Gelada' near the town of Altea (Alicante, Spain) in a *P. oceanica* meadow in an exposed area at 5-10 m depth. Population 6 (Mar Menor, N = 17) was obtained from the Mar Menor hyperhaline coastal lagoon (Murcia, Spain), which is included in the RAMSAR Convention. It is one of the largest Mediterranean coastal lagoons. The maximum depth of the lagoon is 7 m; the empty shells were collected at 2-6 m depth from a muddy bed covered by *Caulerpa prolifera*. Shells of Population 7 (Moraira, N = 21) were obtained from a bay that is

149  oriented southwards, delimited by the capes of Moraira and Ifach (Alicante, Spain) and
150  exposed to southerly waves (Garcia-March et al. 2007). The shells were sampled within
151  a dense *P. oceanica* meadow at 5-7 m depth. Population 8 (Racó, N = 18) was obtained
152  at Calpe (Alicante, Spain) on the western side of the "Peñón de Ifach" at 5-10 m depth
153  in a *P. oceanica* meadow sheltered from the main waves by the crag. Population 9 (Diana
154  lagoon, N = 14) was obtained from the east coast of Corsica (France). Diana lagoon is
155  the deepest of the Corse lagoons (11 m depth); however, the densest *P. nobilis*
156  populations, from which the empty shells were sampled, occur in *Cymodocea nodosa*
157  meadows at a depth of 0.5-1 m (De Gaulejac and Vicente, 1990). Shells from Population
158  10 (Embiez, N = 8) were obtained from the Le Brusc lagoon located at the southern end
159  of the Embiez archipelago. This shallow lagoon is sheltered from the open sea by a *P.*
160  *oceanica* barrier reef (Trigos et al., 2014). The sampling site was covered by a disperse
161  *P. oceanica* meadow and has a maximum depth of 1.5 m. Population 11 (Balearia, N =
162  21) groups individuals from various areas around the Balearic Islands located at 20 m
163  depth. Population 12 (Alfacs, N = 20) was obtained from an estuarine bay in the southern
164  part of the Ebro Delta (Cataluña, Spain). This area features dispersed patches of
165  *Caulerpa prolifera* and *Cymodocea nodosa*, and the empty shells were sampled at
166  depths between 0.2 and 1.2 m.
167  With respect to their environmental characteristics, the sampled populations came from
168  shallow areas in the open sea that are mainly protected from hydrodynamics that are
169  harmful to fan mussels (Gandulf, Raco, and Tabarca), from areas sufficiently deep to be
170  unaffected by hydrodynamics harmful to fan mussels (Port-Cros and Balearia), from
171  shallow areas in the open sea that are exposed to hydrodynamics harmful to fan mussels
172  (Olla, Moraira and Freus), from coastal marine lagoons (Embiez, Diana and Mar Menor),
173  and from estuaries (Alfacs).
174

175  ## *2.2.  Shell processing*

176

177  The shells were treated according to the methodology described by Garcia-March et al.
178  (2011). The dorsal nacre lobe of one valve of each shell was embedded in epoxy resin
179  and cut into 3 to 5 8-cm-long dorsal-to-ventral sections (the portion of the shell lost in the
180  cut was ca. 0.4 mm). Each section was cut radially across the PAMS. One side of the
181  cross-section was polished to 1200 grit and mounted on a glass slide, and a thin sheet
182  (ca. 300 μm) was cut using a precision sectioning saw (Buehler Isomet low-speed saw).
183  The free surface of the slide was polished down to 1200 grit (Garcia-March et al., 2011).
184  The thin sheets produced in this way allow microstructural analysis of growth records
185  using a magnifying binocular lens and optical microscopy (Garcia-March and Marquez-
186  Aliaga, 2007).

187 To estimate growth parameters, the positions of the PAMS was related to the total size
188 of the shell (Ht) using linear regression analysis. Based on the good linear relationship
189 between Ht and the length of the dorsal nacre lobe (DNL), an equation was fitted to the
190 data for each population (Garcia-March and Marquez-Aliaga, 2007; Garcia-March et al.,
191 2011; Richardson et al., 1999; Vicente et al., 1980). The sizes of the individuals when
192 each growth record was deposited were also calculated.
193 As typically occurs with fan mussels, the calcite layer is incomplete in the anterior part of
194 the shell, especially in adult specimens. For this reason, some of the oldest annual
195 increments may be missing (Garcia-March et al., 2011). Given that the calcite width at
196 each annual increment is a function of the number of years over which calcite was
197 deposited (Garcia-March and Marquez-Aliaga, 2007), the number of missing records
198 could be obtained by comparing the calcite widths in the 3 or 4 oldest records for all
199 individuals within a population.
200
201 ## *2.3.  Growth model*
202
203 Size-at-age data were fitted to the Von-Bertalanffy growth function using the non-linear
204 mixed effects model (Vigliola and Meekan, 2009) considering $L\infty$ as random and $t_0$ and
205 k as fixed (Garcia-March et al., 2011). This method fits any nonlinear model to
206 longitudinal data with great flexibility in modeling the within-group correlations that are
207 often present in such data (Vigliola and Meekan, 2009).
208 Non-parametric multidimensional scaling (MDS) was used as the ordination method for
209 exploring affinities among populations according to maximum age, Max_Ht (maximum
210 individual size in the population), $L\infty$ and K. The similarity matrix, which was calculated
211 by the Bray–Curtis index based on square-root transformed data, was used to construct
212 bivariate MDS plots. The multivariate analysis was carried out using the PRIMER v.5
213 package (Clarke and Gorley, 2001).
214 The Z-test (Clogg et al., 1995) was used to determine the significance of the differences
215 in the parameters $L\infty$ and K among the groups, applying the Bonferroni correction ($\alpha$ =
216 0.0083). The groups were also compared with the population studied by Garcia-March
217 et al. (2011) in Moraira Bay, which was located in the same area as one of the
218 populations in the present study but at a different depth range (11-13 m depth).
219 The size differences among groups of different ages were tested by applying Tukey's
220 honestly significant differences (HDS) test to the data for size-at-age obtained previously
221 (see *2.2. Shell processing*). The ages compared ranged from 2 years (the first age for
222 which data were available for most individuals) to 11 years (when only SO and EO could
223 be compared). From age 7 onwards, there were insufficient data from LG for comparison

224 (only SO, EO and Alfacs could be compared), and from age 11 onwards there were
225 insufficient data from Alfacs (only SO and EO could be compared).
226

227 ## 3. Results

228

229 A remarkable variability in age and growth parameters was observed (**¡Error! No se**
230 **encuentra el origen de la referencia.**). The maximum age of empty shells ranged from
231 6 years in the Embiez lagoon to 38 years in Port-Cros. The maximum shell length
232 measured (Max_Ht) ranged from 44.7 cm in Freus to 79.1 cm in Balearia. In the Von-
233 Bertalanffy growth function, parameter K, the speed at which the asymptotic size is
234 reached, varied between 0.15 in Port-Cros and 0.37 in Mar Menor Lagoon, while L∞
235 varied between 39.5 cm in Olla and 75.0 cm in Alfacs. The data for each population are
236 presented in
237 .
238 Multivariate analyses revealed 4 groups within the studied populations with a 95% of
239 similarity (Figure 3): 1) Sheltered Open-sea –SO– (Gandulf, Raco, Tabarca, Balearia
240 and Port-Cros); 2) Exposed Open-sea –EO– (Olla, Moraira and Freus); 3) Lagoons –LG–
241 (Embiez, Diana and Mar Menor), located in coastal marine lagoons; and 4) Alfacs,
242 located in an estuary.
243 Except for the Alfacs group, which included only one population, a general model was
244 calculated for the groups identified by the multivariate analysis (SO, EO and LG; Figure
245 4 and Table 1): SO (N = 113) with K = 0.17 and L∞ = 63.1 cm (Eq. 1); LG (N = 39) with
246 K = 0.30 and L∞ = 56.5 cm (Eq. 2); and EO (N = 56) with K = 0.23 and L∞ = 43.0 cm
247 (Eq. 3). The standardized residuals in relation to size for each of the groups showed no
248 relevant trends and few outliers; most of the data fell within 2 standard deviations of the
249 mean (SO = 94.1%, EO= 95.3%, LG= 94.8%, and Alfacs= 92.62%), indicating good fit
250 of the models (Figure 5).
251

$$L_t = 63.1 + \left(1 - e^{-0.17 \cdot (t+0.67)}\right) \tag{1}$$

$$L_t = 56.5 + \left(1 - e^{-0.30 \cdot (t+0.05)}\right) \tag{2}$$

$$L_t = 43.0 + \left(1 - e^{-0.23 \cdot (t+0.47)}\right) \tag{3}$$

252

253 The two-sided p-values for the Z-test results (Table 2) showed significant differences in
254 L∞ between SO-EO, SO-Alfacs, SO-Mor, EO-LG, EO-Alfacs, EO-Mor, LG-Alfacs and
255 Alfacs-Mor and significant differences in K between SO-EO, SO-LG, EO-Alfacs, EO-Mor,
256 LG-Alfacs and LG-Mor.

257  Tukey's HDS found significant differences among the groups through the years. EO
258  shows significant differences for all groups and all years except Alfacs at age 2. LG
259  shows significant differences from SO in all years, and SO and Alfacs show significant
260  differences from ages 5 to 10. The results of Tukey's HDS analysis are presented in
261  Table 3.

262

263  ## 4. Discussion

264

265  The present work constitutes the first comparative growth study of the endangered species
266  *P. nobilis* in 12 different locations in the western Mediterranean. Considerable variability in
267  growth and longevity due to environmental conditions and protection status was detected.
268  Multivariate analysis grouped the populations into four different groups, each of which
269  shares common environmental characteristics. The groups are: 1) Sheltered and Shallow
270  Open-sea SO (Gandulf, Raco, Tabarca, Balearia and Port-Cros populations), located in
271  shallow and deep areas in the open sea but mainly protected from hydrodynamics that are
272  harmful to fan mussels; 2) Exposed open-sea EO (Olla, Moraira and Freus populations),
273  located in shallow areas in the open sea and exposed to hydrodynamics harmful to fan
274  mussels; 3) Lagoons LG (Embiez, Diana and Mar Menor populations), located in coastal
275  marine lagoons; and 4) Alfacs, separated from the other groups and the only population
276  inhabiting an estuary.

277  Three general growth models were established based on multivariate analysis. Although
278  calculation of specific models for each population would be advisable, in the absence of
279  specific population models, the general models proposed here could be used as a
280  reference for other *P. nobilis* populations living in similar habitats. Estuaries such as
281  Delta del Ebro could be grouped in a different general growth model; however, because
282  Alfacs was the only population sampled from an estuarine environment, further research
283  that includes more populations living in deltaic environments should be conducted to
284  support its singularity as a model.

285  EO populations show lower growth rates and L∞ than other populations. According to
286  Deudero et al. (2015); Garcia-March et al. (2007b), the effects of intermittent high
287  hydrodynamics or continuous moderate hydrodynamics could increase mortality and
288  limit growth by causing stress and shell breakage. Moreover, Garcia-March et al. (2016)
289  studied the in situ gaping activity of fan mussels and found that bimodal currents such
290  as those generated by waves cause greater disturbance to *P. nobilis* individuals than
291  unimodal currents such as tides, even at lower water speeds. The effect of these forces
292  decreases with increasing depth and with the presence of *Posidonia oceanica* and is
293  influenced by seabed topography (Garcia-March et al., 2007b; Hendriks et al., 2011).

Therefore, it is hypothesized that the maximum size of EO populations may be constrained by hydrodynamics, while SO and LG populations may grow to larger sizes because they are typically sheltered from detrimental hydrodynamics.

Environmental conditions tend to be more stable in deep areas. Although shallow areas are protected from hydrodynamics, they are more prone to anthropogenic impacts and climatic extremes. Therefore, it seemed reasonable to expect that deep populations in different areas of the western Mediterranean Sea would have more similarities in their growth parameters than more closely situated populations living in shallower sheltered areas. However, the populations within the SO group show similar growth patterns despite living at different depths. This supports the idea that, effectively, in the open sea, hydrodynamics may be a determinant of fan mussel growth, constraining shell size in populations that inhabit exposed sites. When the effect of hydrodynamics on fan mussels is low due because the populations are sheltered or are situated at greater depth, other environmental factors would exert a similar effect on the species independently of location.

In this regard, the population studied by Garcia-March et al. (2007a) in Moraira (Alicante, Spain), which is located at a depth of 11-13 m, shows a growth model with $L\infty$ higher than that of the EO population but lower than that of the SO population and k lower than that of the EO population but similar to that of the SO population. This population could be in a situation intermediate between those of the deep (20 m) and exposed populations and may be partially affected by hydrodynamics. On the other hand, $L\infty$ and growth rate appear to be independent of the legal protection of the area, considering that the multivariate analysis groups populations independently of such protection and no differences are found between protected and unprotected populations.

The Alfacs and LG populations inhabit confined waters. These populations are notable for their higher growth rate from 5 to 9 years of age and $L\infty$ (Alfacs) and their higher growth rate from 2 to 7 years of age (LG) compared to the other populations studied (**¡Error! No se encuentra el origen de la referencia.**). The specific conditions that exist in these paralic environments could be responsible for these extremes. Higher food availability compared with open sea, could explain this discrepancy as has been demonstrated for growth and survival differences of *P. nobilis* living in eutrophic versus oligotrophic environments (Alomar et al., 2015). Ebro Delta waters are nutrient-enriched by inputs from agricultural irrigation (Falco et al., 2010; Mañosa et al., 2001; Prado, 2018; Sierra et al., 2002). The same occurs for coastal lagoons, which are also affected by the increase in the population in coastal areas and by agriculture and industry. These impacts, in conjunction with environmental conditions such as low water circulation and long water residence, make these areas more susceptible to nutrient enrichment (Kennish and Paerl, 2010). This situation has been remarkable during recent years in

Mar Menor lagoon, which has undergone some eutrophication (Garcia-Ayllon, 2018; Pérez-Ruzafa et al., 2005b; Velasco et al., 2006). The reason that $L\infty$ for the Alfacs population is 18.5 cm larger than the value predicted by the general growth model for LG is unknown. The salinity regimes of paralic environments show higher fluctuations than those of open sea environments due to their environmental characteristics (Kennish and Paerl, 2010). These fluctuations, however, are not mirrored by the growth trends observed in the fan mussel populations living within these areas. Salinity in Alfacs is usually lower than that in the open sea due to precipitation and discharge of irrigation channels (Solé et al., 2009). Mar Menor is a hyperhaline lagoon that can reach salinity levels of up to 51 psu (Pérez-Ruzafa et al., 2005a). The Diana and Embiez lagoons show lower salinity levels than Mar Menor but often oscillate below and above open-sea levels following the wet and dry seasons (Burgeot et al., 1996; De Gaulejac and Vicente, 1990; Rouanet et al., 2009). Taken together, the data suggest that environmental factors other than salinity may have more weight in determining the growth trends observed in the paralic environments. Additional studies of more fan mussel populations living in paralic environments should be conducted, however, before definitely ruling out the possibility that fan mussel growth is affected by salinity.

Remarkable variation in survival and maximum age is also found among the studied populations. The EO, LG and Alfacs populations show the lowest maximum ages (17, 12 and 15 years) of the studied individuals. In exposed areas, the effect of hydrodynamic conditions, as previously noted, could be responsible for lower survival, but hydrodynamic conditions are usually gentle in lagoon/estuarine environments. Furthermore, lagoon/estuarine populations are the only populations in which $L\infty$ is higher than Max_Ht; this could indicate that in these locations individuals die before reaching maximum size and/or that the posterior part of the shell has been broken and reconstructed, making it appear smaller in size. Shell breakage caused by intense boat traffic, which often hits the individuals and breaks their shells (Prado et al., 2014) could be an explanation for the condition of the Alfacs population, in which 19 of 20 shells showed conspicuous reconstruction marks. Multiple factors could be affecting the lifespans of lagoon/estuarine populations. 1) Compared to open-sea ecosystems, lagoon/estuarine ecosystems present more stressful extreme conditions (Cañedo-Argüelles et al., 2018). During the winter and the rainy season, the temperature and salinity may approach the tolerance limit for the species. The same occurs during summer, when high temperatures and high salinity levels occur (except in the case of the Ebro Delta, where salinity decreases in summer due to agriculture discharges) and oxygen concentrations may reach dangerously low levels (Cataudella et al., 2015). 2) The presence of chemical contaminants produced by anthropogenic activities is also common in these environments (Kennish and Paerl, 2010), as reported for the Ebro

370 estuary (Köck et al., 2010; Mañosa et al., 2001; Solé et al., 2000), Mar Menor (Cañedo-
371 Argüelles et al., 2018; Pérez-Ruzafa et al., 2000) and the Diana lagoon (Burgeot et al.,
372 1996; Galgani et al., 2006). 3) In some taxa, rapid growth and large body size appear to
373 be related to shorter lifespan (Metcalfe and Monaghan, 2003), although this remains to
374 be demonstrated for *P. nobilis.* Either separately or together, these factors could limit the
375 life expectancy of fan mussel populations living in lagoon/estuarine environments.
376 Accordingly, the general LG model should be used with caution. The oscillations that
377 occur in coastal lagoons due to natural conditions and anthropogenic effects could
378 induce stochastic variations in fan mussel growth. The same could be true for estuarine
379 areas such as Alfacs.

380 Anthropogenic effects go beyond contamination, and other threats such as anchoring,
381 habitat loss and shell poaching have been proven to decimate fan mussel populations
382 (Basso et al., 2015; Deudero et al., 2015; Hendriks et al., 2013; Katsanevakis et al.,
383 2011; Vázquez-Luis et al., 2015; Vázquez-Luis et al., 2014). Accordingly, it should be
384 highlighted that the maximum ages detected, 38 and 34 years, were found in specimens
385 obtained from the Port-Cros National Park, which was created in 1963. The other marine
386 reserves, the National Marine Reserve of Tabarca and the Cabrera Archipelago
387 Maritime-Terrestrial National Park, are relatively recent (they were created in 1986 and
388 1991, respectively); these reserves hosted individuals 27 years old, similar to the age of
389 the reserves at the time of shell sampling. The maximum ages of the sampled
390 populations suggest a possible positive effect of the protection of marine areas on *P.*
391 *nobilis* longevity, although additional studies should be conducted to conclusively
392 determine the association of protection status with fan mussel longevity.

393 The current situation of *P. nobilis* is critical. The recent MME affecting the species is
394 devastating almost all fan mussel populations (Katsanevakis et al., 2019) (García-March
395 et al., *in revision*). Only some populations living in confined waters such as lagoons and
396 estuaries are surviving, and the reasons for this are unknown. Among the populations
397 addressed in the present study, only the populations at Mar Menor, Alfacs (García-March
398 et al., *in revision*), Embiez and Diana (Nardo Vicente, pers. com.) remain alive today,
399 whereas the other populations have experienced 100% mortality (García-March et al., *in*
400 *revision*). In the current situation, one strategy to ensure the future of the species would
401 be captive breeding and artificial reintroduction of juveniles. Of the studied populations,
402 Port-Cros, Gandulf and Tabarca appear to be the most optimal locations for *P. nobilis*
403 reintroduction based on the sizes and ages reached by the individuals and the protection
404 status of the sites. However, the lack of resistant individuals and the possible long-term
405 presence of disease could make these areas unavailable for the reintroduction of fan
406 mussels. This leaves lagoons and estuaries as the only hope for the short term survival
407 of individuals under natural conditions and for the reintroduction of juveniles. The growth

parameters of the populations living in these environments indicate that they may be good areas for the growth of the species during the first years of life, but populations living in lagoons and, to a lesser extent, in the Ebro Delta, appear to be unstable in the long term. The short lifespan of fan mussels in these environments suggests that these populations rely on abundant recruitment and that the survival of introduced individuals could be constrained in the long term. Furthermore, the instability of these ecosystems due to both natural and anthropogenic factors (Kennish and Paerl, 2010; Reizopoulou and Nicolaidou, 2007) could lead to sudden collapse of these populations. In the Mar Menor lagoon, eutrophication has been threatening the ecosystem for a long time, and it spiked during the summer of 2015 and the spring of 2016, resulting in the collapse of the lagoon (Garcia-Ayllon, 2018; Pérez-Ruzafa et al., 2019). Furthermore, natural resettlement of fan mussels in coastal lagoons or deltas recovered after a collapse would be impossible due to the lack of connectivity among populations unless manipulative reintroduction of fan mussels were undertaken (García-March et al., *in revision*). On the other hand, *Callinectes sapidus,* an invasive Mediterranean crab introduced from the Atlantic, is spreading throughout the Mediterranean, has been recently observed in Delta del Ebro (Fuentes et al., 2019), and has colonized Mar Menor for several years (Castejón and Guerao, 2013; Mancinelli et al., 2017). This voracious crustacean could also become a threat to *P. nobilis* juveniles in these reservoirs. Therefore, as also suggested by growth parameters and longevity, the survival of fan mussel populations living in these reservoirs could be endangered in the absence of connectivity with other populations. Urgent measures should be implemented to increase the long-term stability of these areas in the future and to preserve *P. nobilis* from extinction.

The data obtained in the present study can also be used to predict the resilience of fan mussels in the context of climate change, which may produce a scenario of weather extremes and associated wave action in the Mediterranean Sea (IPCC, 2018). It is expected that the surviving populations in exposed areas will experience increasing hydrodynamic stress in the future, probably resulting in individuals dying younger and growing to lower sizes.

Further research is necessary to expand the models to other environmental conditions and to adjust for the inherent morphological variations in *P. nobilis* shells. Shell shape appears to be related to the environmental conditions under which the individuals grow, and it could be used bidirectionally. On one hand, it might be possible to separate growth models within a population according to shell shape. It is hypothesized that more accurate growth rate and age estimations could be achieved in this way. On the other hand, the method could be used together with growth parameter estimations as an indicator of environmental conditions.

**Acknowledgments**

Figure 1: Locations of the populations used in the present study.

Figure 2: Growth models of the 12 populations studied. K, speed at which the asymptotic size is reached; L∞, maximum size according to the model; ma, maximum age detected among the individuals studied in the population.

Figure 3: Two-dimensional MDS plot for the 12 studied *P. nobilis* populations by exposure and protection based on square root transformed and Bray-Curtis similarity of maximum age, Max_Ht, L∞ and K. Groups are based in a 95% of similarity: SO: Sheltered Open-sea, EO: Exposed Open-sea, and LG: Lagoons.

Figure 4: General growth models for the three classifications according to multivariate analysis results (Sheltered Open-sea -SO-; Exposed and Shallow Open-Sea -EO-; Lagoon -LG-; Alfacs, the only population inhabiting an estuary).

482    Figure 5: Standardized residuals in relation to size for each of the groups resulting from
483    multivariate analysis.

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Table 1: Data for each population: N (number of shells); Depth (m) (depth at which shells were collected); Location (open sea, lagoon or estuary); S/E (whether the area is sheltered from (S) or exposed to (E) hydrodynamics); P/U (whether the area has special protection status (P) or is unprotected (U)); Max age (maximum age detected population); Min age (minimum age detected in the population). Max Ht (maximum individual size in the population); Min Ht (minimum individual size in the population); k (the speed at which the asymptotic size is reached); k SE standard error; $L\infty$ (maximum theoretical size of the population); $L\infty$ SE standard error; $t_0$ (the point in time when an individual has zero length. It has no biological meaning); $T_0$ SE standard error; SO (Sheltered Open-sea); EO (Exposed Open-sea); and LG (Lagoons).

| Population | N | Depth(m) | Location | S/E | P/U | Max age (years) | Min age (years) | Max Ht (cm) | Min Ht (cm) | k | k SE | $L\infty$ (cm) | $L\infty$ SE | $t_0$ | $t_0$ SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Freus | 16 | 5-10 | Open sea | E | P | 14 | 5 | 44.7 | 28.8 | 0.21 | 0.02 | 43.9 | 1.3 | -0.57 | 0.23 |
| Gandulf | 16 | 5-10 | Open sea | S | P | 27 | 5 | 65.6 | 38.5 | 0.19 | 0.01 | 62.4 | 1.5 | -0.05 | 0.13 |
| Tabarca | 20 | 5-10 | Open sea | S | P | 27 | 4 | 68.8 | 30.9 | 0.19 | 0.01 | 58.7 | 1.4 | -0.40 | 0.12 |
| PortCros | 19 | 10-25 | Open sea | S | P | 38 | 3 | 68.0 | 29.3 | 0.15 | 0.00 | 65.4 | 1.9 | -0.95 | 0.12 |
| Olla | 19 | 5-10 | Open sea | E | P | 11 | 4 | 51.5 | 18.7 | 0.29 | 0.02 | 39.9 | 1.9 | 0.24 | 0.13 |
| Mar Menor | 17 | 0-2 | Lagoon | S | U | 9 | 3 | 58.0 | 30.0 | 0.37 | 0.04 | 58.2 | 2.5 | -0.06 | 0.13 |
| Moraira | 21 | 5-7 | Open sea | E | U | 17 | 6 | 49.1 | 25.6 | 0.21 | 0.01 | 45.6 | 1.0 | -0.88 | 0.12 |
| Raco | 18 | 5-10 | Open sea | S | U | 21 | 4 | 68.2 | 20.9 | 0.24 | 0.01 | 60.7 | 1.7 | 0.12 | 0.10 |
| Diana | 14 | 0-2 | Lagoon | S | U | 12 | 3 | 47.8 | 26.8 | 0.24 | 0.04 | 56.9 | 3.9 | -0.04 | 0.21 |
| Embiez | 8 | 0-2 | Lagoon | S | U | 6 | 4 | 54.3 | 26.3 | 0.30 | 0.05 | 56.0 | 4.5 | 0.28 | 0.12 |
| Balearia | 21 | 20 | Open sea | S | U | 26 | 3 | 79.1 | 39.0 | 0.13 | 0.00 | 65.5 | 1.9 | -1.78 | 0.16 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alfaques | 20 | 0-2 | Estuary | S | U | 15 | 5 | 59.8 | 42.8 | 0.18 | 0.01 | 75.0 | 2.6 | -0.03 | 0.16 |
| SO | 113 | | | | | | | | | 0.17 | 0.00 | 63.1 | 0.8 | -0.67 | 0.06 |
| EO | 56 | | | | | | | | | 0.23 | 0.01 | 43.0 | 0.8 | -0.47 | 0.09 |
| LG | 39 | | | | | | | | | 0.30 | 0.03 | 56.5 | 2.3 | -0.05 | 0.11 |

493

494

495 Table 2: Z-test results for comparison of L∞ and k values among the groups established
496 by multivariate analysis and Mor (the population studied by Garcia-March et al. (2011) in
497 Moraira).

| | L∞ | | | | |
|---|---|---|---|---|---|
| | SO | EO | LG | Alfacs | Mor |
| SO | | *** | 0.017 | *** | *** |
| EO | | | *** | *** | *** |
| LG | | | | *** | 0.765 |
| Alfacs | | | | | *** |

| | k | | | | |
|---|---|---|---|---|---|
| | SO | EO | LG | Alfacs | Mor |
| SO | | *** | *** | 0.5 | 0.146 |
| EO | | | 0.016 | ** | *** |
| LG | | | | *** | *** |
| Alfacs | | | | | 0.244 |

498 *** p value < 0.001; * p value < 0.01; * p value < 0.05
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Table 3: Results of Tukey's honestly significant differences test among groups. From age 7 onwards, there were insufficient data from LG for comparison (only SO, EO and Alfacs could be compared), and from age 11 onwards there were insufficient data for Alfacs (only SO and EO could be compared).

| | 2 years | | | | 7 years | | | |
|---|---|---|---|---|---|---|---|---|
| | SO | EO | LG | Alfacs | SO | EO | LG | Alfacs |
| SO | | ** | * | 0.936 | | *** | — | *** |
| EO | | | *** | 0.161 | | | — | *** |
| LG | | | | 0.906 | | | | — |

| | 3 years | | | | 8 years | | | |
|---|---|---|---|---|---|---|---|---|
| SO | | *** | *** | 0.463 | | *** | — | *** |
| EO | | | *** | *** | | | — | *** |
| LG | | | | 0.085 | | | | — |

| | 4 years | | | | 9 years | | | |
|---|---|---|---|---|---|---|---|---|
| SO | | *** | *** | 0.072 | | *** | — | *** |
| EO | | | *** | *** | | | — | * |
| LG | | | | 0.398 | | | | — |

| | 5 years | | | | 10 years | | | |
|---|---|---|---|---|---|---|---|---|
| SO | | *** | *** | ** | | *** | — | 0.078 |
| EO | | | *** | *** | | | — | *** |
| LG | | | | 0.775 | | | | — |

| | 6 years | | | | 11 years° | | | |
|---|---|---|---|---|---|---|---|---|
| SO | | *** | *** | *** | | *** | — | — |
| EO | | | *** | *** | | | — | — |
| LG | | | | 0.977 | | | | — |

*** p value < 0.001; ** p value < 0.01; * p value < 0.05; — insufficient data for comparison.

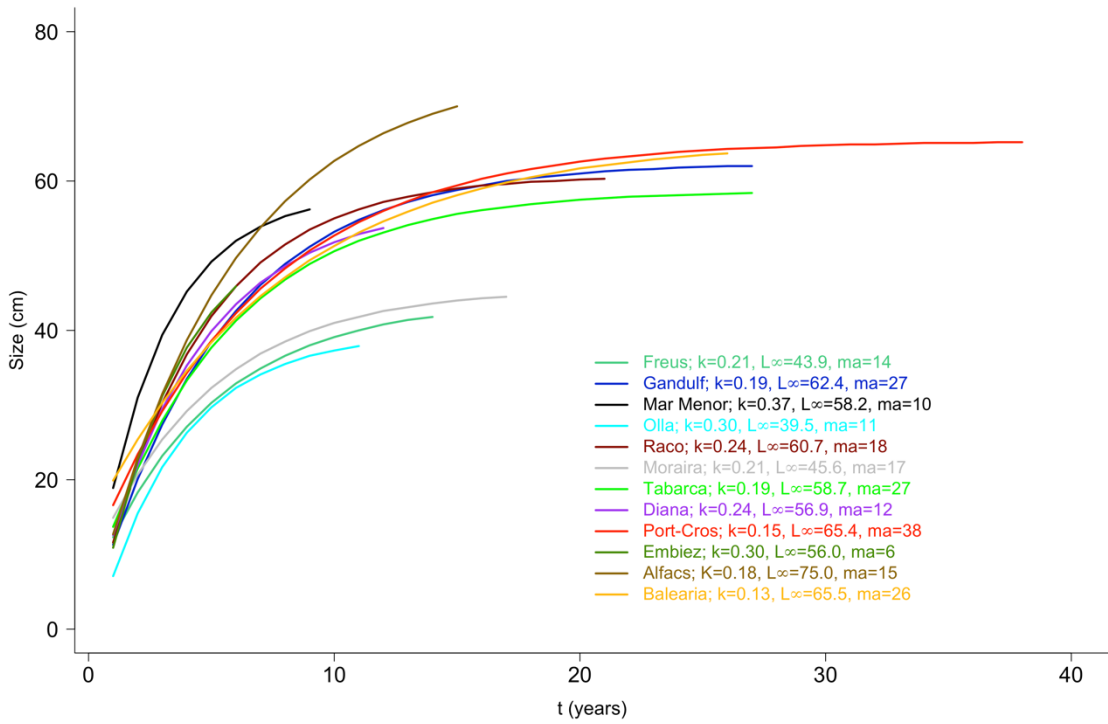°SO and EO showed significant differences from ages 11 to 14, the last age in EO for which there were sufficient data for comparison.
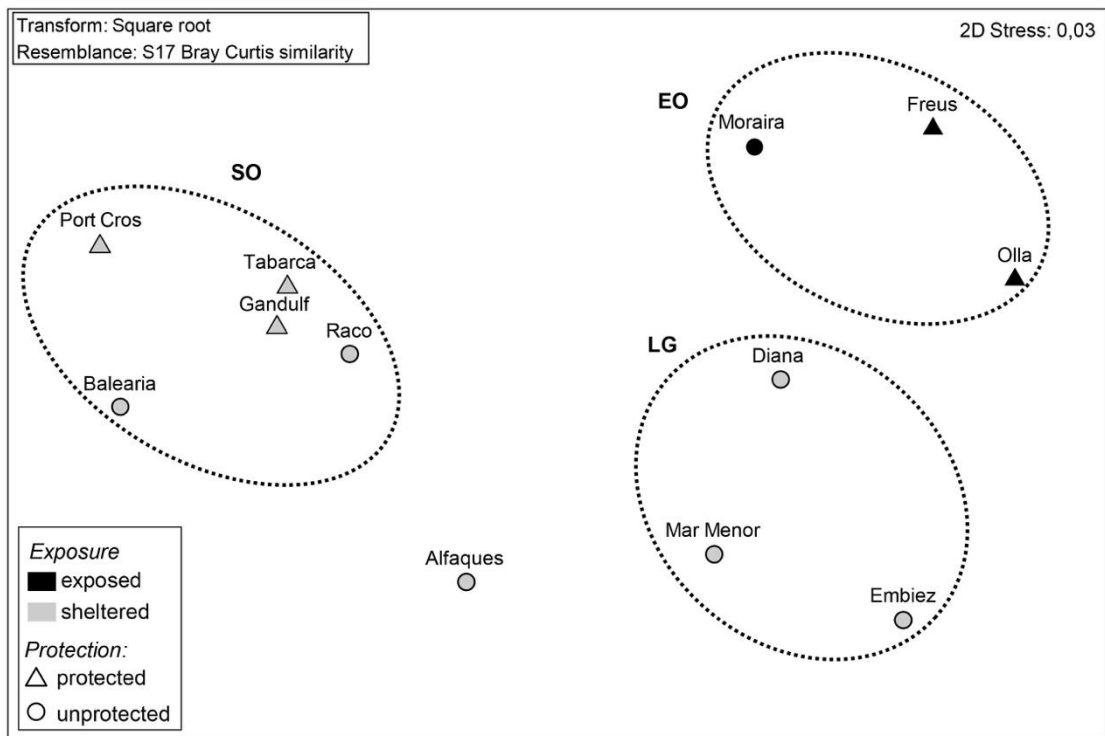
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522    Figure 1.
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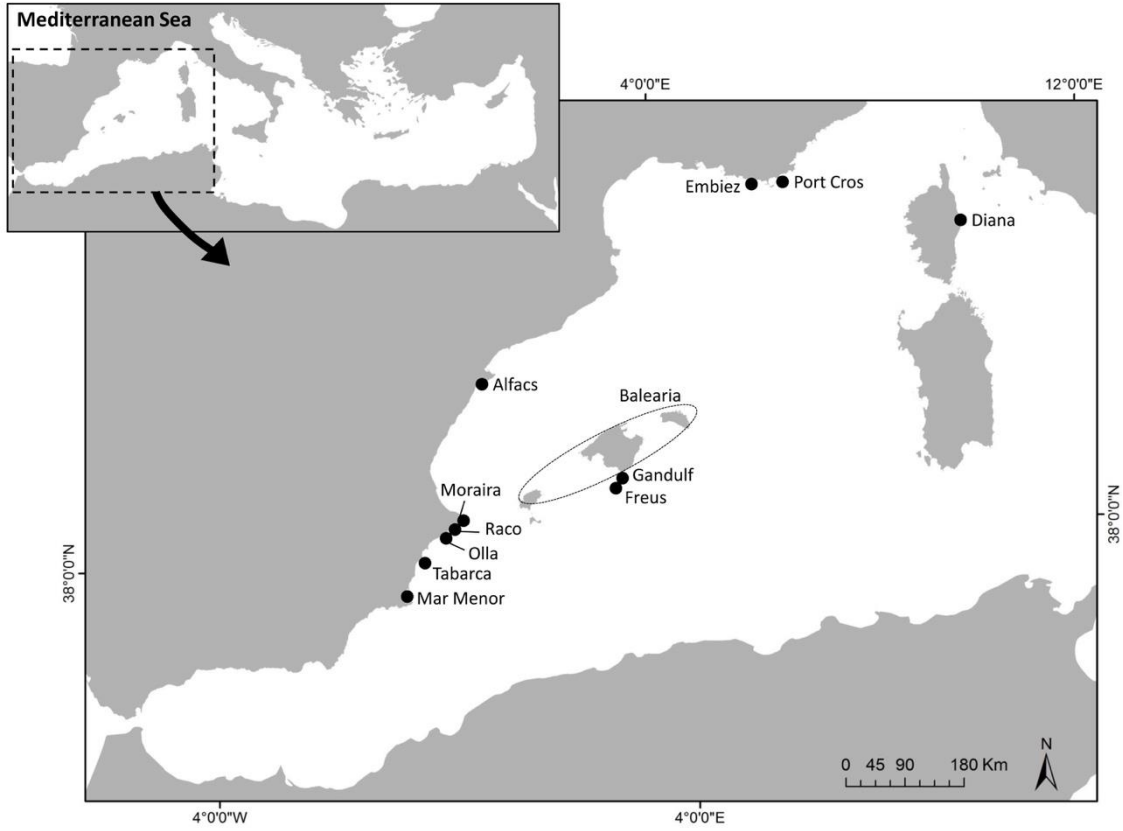
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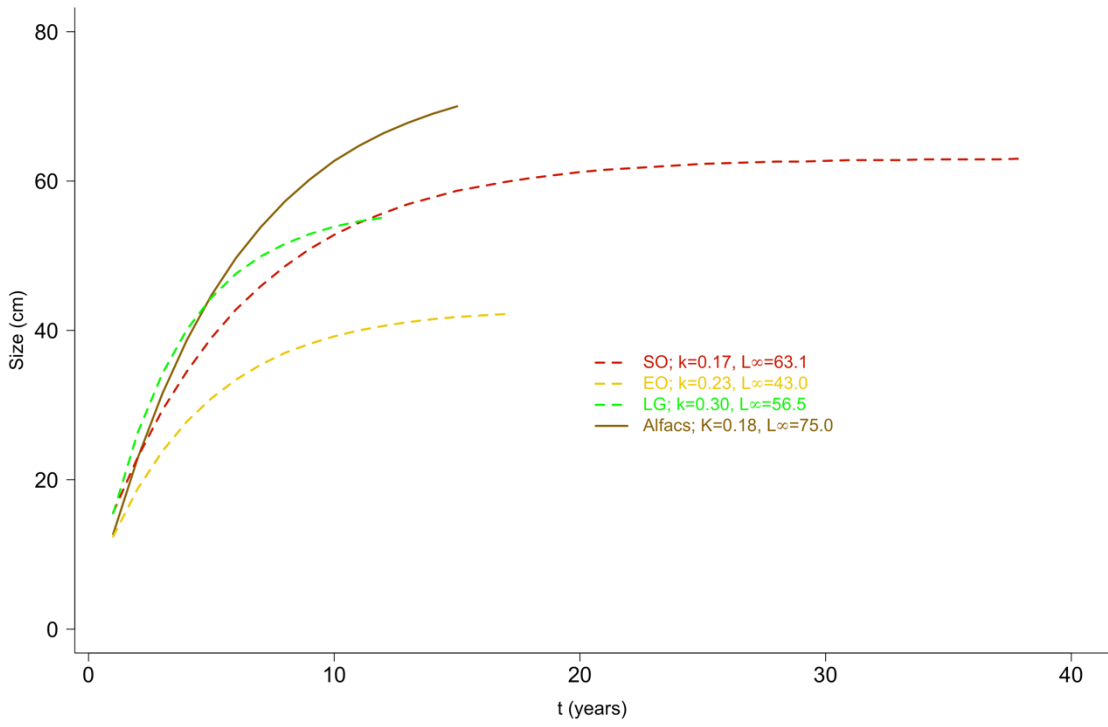526     Figure 2.

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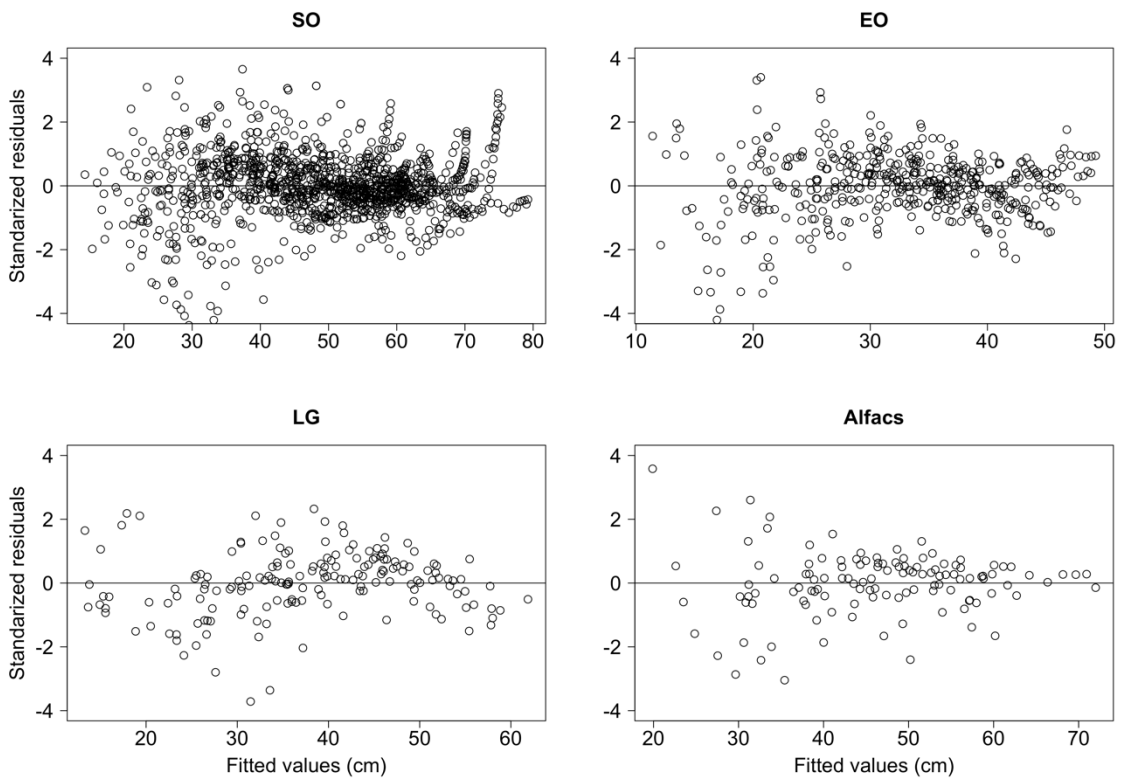529     Figure 3.

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531    Figure 4.

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535    Figure 5.

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537     **5. Bibliography**

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