Factors affecting the feeding patterns of the horned octopus (*Eledone cirrhosa*) in Atlantic Iberian waters

M. Regueira[1,2*], Á.Guerra[1], C.M. Fernández-Jardón[3],Á.F. González[1]

[1]Instituto de Investigaciones Marinas (IIM-CSIC), Eduardo Cabello 6, 36208 Vigo, Spain.

[2]Departamento de Biologia, Universidade de Aveiro. 3810–193 Aveiro, Portugal.

[3]Facultad de Ciencias Económicas y Empresariales, Departamento de Economía Aplicada, Universidad de Vigo, Campus de Vigo. 36310, Vigo, Spain.

*Corresponding author: regueira@iim.csic.es

Tel. (+34) 986 23 19 30

Fax. (+34) 986 29 27 62

RUNNING TITLE: Feeding patterns of *Eledone cirrhosa*

KEY WORDS: Cephalopods, *Eledone cirrhosa*, diet, feeding patterns, Atlantic Iberian waters, Multinomial Logistic Regression.

Abstract The present study combines morphological and molecular analysis of stomach contents (n=2,355) and Multinomial Logistic Regression (MLR) to understand the diet and feeding patterns of the horned octopus *Eledone cirrhosa* inhabiting Atlantic Iberian waters. Specimens were collected monthly from commercial bottom trawl fisheries between February 2009 and February 2011 in three fishing grounds (North Galicia, West Galicia and North Portugal), located between 40.6–43.6°N and 8.6–7.36°W. Based on stomach analysis, horned octopuses in the region consumed mainly crustaceans, followed by teleost fish, echinoderms, molluscs and polychaetes. Molecular analysis of 14 stomach contents confirmed the visual identification of prey items as well as cannibalistic events. Statistical tests found that sex and size did not significantly affect prey selection, while the maturity stage of octopuses significantly affected the probability of selecting a particular prey. Among external variables, season and fishing ground showed significant effects on the dietary patterns of *E. cirrhosa*. In addition to taking into account the main effects affecting feeding patterns according to the model, we discuss the ecological implications of other parameters suggested by MLR.

Introduction

Eledone cirrhosa (Lamarck, 1798) is a moderate–sized incirrate octopod inhabiting benthic grounds in the continental shelf and slope of the north–western Atlantic and throughout the Mediterranean Sea (Belcari et al., 2015). In Atlantic waters off the Iberian Peninsula, this species is mainly caught in trawling fisheries targeting other species (mainly hake, norway lobster and monkfish) usually operating between 200 and 800 m depth, although spawning females have occasionally been caught by pots in shallower areas (unpubl data). Annual catches of *E. cirrhosa* in Galician waters reached an average of 1,634 metric tonnes in the last ten years (www.pescadegalicia.com). The species is also harvested for human consumption on a large scale in the Mediterranean Sea, where catch statistics are reported in combination with the smaller catch of *E. moschata*. These species are caught primarily with bottom trawls and, to a lesser extent, with seines in the Mediterranean (Jereb et al., 2014).

As in other cephalopods, there are problems identifying the diet of *E. cirrhosa* because hard parts of their prey (such as crustacean integuments, cephalopod beaks and sucker rings, as well as fish otoliths and skeletons) that are usually necessary for identification are torn into small pieces and often rejected. It has also been observed that rapid digestion means that many specimens have little or no food in their stomachs (Boyle & Rodhouse, 2005). Another major limitation is that stomach contents represent the last feeding events with no indication of long–term dietary habits. Despite these problems, there is a considerable amount of information on the trophic relationships of cephalopods that has been collected using conventional visual analysis of the stomach contents of specimens from fisheries, laboratory studies and analyses of prey remains around middens in the case of some coastal octopods (Mather, 1991; Nixon, 1987).

Serological analysis of cephalopod diets has been used to identify prey species (Grisley & Boyle, 1985; 1988). Using this technique, Boyle et al. (1986) demonstrated that *E. cirrhrosa* from Scottish waters preys on several crustacean species. However, the method is too expensive to be used to identify all prey (Boyle & Rodhouse, 2005). Naturally occurring stable isotopes of nitrogen present in animal tissues differ among species and trophic levels and therefore provide a way of estimating species level in the trophic web (Cherel & Hobson, 2005). Lipid contents of the digestive gland of some cephalopod

species have also been used to identify major prey items in some cephalopod species (Jackson et al., 2007; Phillips et al., 2003). Nevertheless, lipid signatures can be misleading when tracing the cephalopod prey of their predators (Boyle & Rodhouse, 2005). Finally, DNA sequence analysis has proven to be an excellent technique for dietary cephalopod studies, even in paralarvae (Boyle & Rodhouse, 2005; Jackson et al., 2007; Roura et al., 2012).

Available dietary information concerning wild *E. cirrhosa* note that this species mainly preys on decapod crustaceans, mostly alpheids and brachyurans, although molluscs and the eggs of other cephalopods have also been reported as prey to a lesser extent in the Mediterranean (Auteri et al., 1988; Ezzeddine et al., 2012; Moriyasu, 1984; Sánchez, 1981). Cannibalistic behaviour has also been reported (Guerra, 1992; Moriyasu, 1981). Otherwise, feeding studies carried out on this species in the Atlantic have been mostly focused on the feeding and hunting behaviour of captive animals (Boyle & Knobloch, 1981; Grisley et al., 1999; Runham et al., 1997). In spite of this, no detailed studies on the diet of this species in the Atlantic exist, although studies with a more general approach support the predominance of crustaceans (Boyle, 1986).

As an important ecosystem component, cephalopods and their fluctuations influence the population dynamics of both higher predators and their own prey (Pierce et al., 2008). Advances in statistical modelling have facilitated increasingly sophisticated approaches in ecological surveys that study the effects of multiple explanatory variables and their interactions on a dependent variable (e.g. Multiple Linear Regression, Generalized Linear Models, Generalized Additive Models, Generalized Additive Mixed Models, Neural Networks and Bayesian models) (Pierce et al., 2008). These analyses are becoming more frequent for modelling processes in cephalopods with diverse objectives, including mainly fishery forecasting but also studying habitat requirements of a species and the influence of environmental variables on particular life history events or stages (Guerra et al., 2015; Pierce et al., 2008). They have also been used to evaluate foraging behaviour. Thus, Leite et al. (2009) used a Multinomial Logistic Regression (MLR) to assess relationships between individual (*Octopus insularis* size) and environmental (e.g. depth, substrate) variables and the occurrence of the three main foraging behaviours and also to assess the effects of the previously mentioned variables in addition to the swimming and moving behaviour on the four main body patterns. As far as we know, this is the only case in which this technique has been used in cephalopod dietary studies. MLR is useful to predict the probability of category membership on a multinomial dependent variable based on multiple independent variables (Starkweather & Moske, 2011).

The aim of this paper is to provide a comprehensive view of the role of *E. cirrhosa* in the marine trophic web through the identification of its diet composition in Atlantic Iberian waters, based on both visual and genetic identification of the gut contents from wild caught animals and testing of horned octopus feeding patterns using MLR.

Materials and Methods

A total of 2335 specimens of *E. cirrhosa* were obtained from commercial landings in Atlantic Iberian waters. Samples were acquired between February 2009 and February 2011 in Burela's port (northern Galician fishing ground, NG), Ribeira or Bueu ports, (west Galicia fishing grounds, WG), and Aveiro (western Portuguese fishing ground, WP) (Figure 1). All specimens were sexed, and body weight (BW)

was measured to the nearest g. The macroscopic maturity scale of Inejih (2000) was adapted to assign a specific maturity stage to each individual. For males, this scale was as follows: I: immature, II: maturing, III: pre–spawning, with some spermatophore in Needham's sac, and IV: mature, fully developed spermatophore, and for females: I: immature, II: maturing, III: pre–spawning, IV: mature and V: post–spawning. To determine if there were differences in diet between seasons, two categories were defined: "warm season" (April to September) and "cold season" (October to March). Four size classes were considered (S1: 0–250 g, S2: 251–500 g; S3: 501–750 g, and S4: >750 g) for Emptiness Index (EMI) comparisons.

Stomachs were preserved in 70% ethanol until further examination in the laboratory. Stomachs and their contents were weighted separately to the nearest 0.01 g. Stomach content was filtered through a 300 µm mesh to remove silt and organic detritus that would hinder identification of the remaining contents. Hard structures, namely otoliths, fish vertebrae and jaws, pedunculated eyes, traces of chelae and other hard pieces of the body of crustaceans, echinoderm ossicles, pieces of shells of molluscs or traces of cephalopod beaks and radula, were identified to the lowest possible taxon by means of available bibliography (Bouvier, 1940; González–Gurriarán & Mendez, 1986; Guerra, 1992; Perrier, 1954; Tuset et al., 2008; Watt et al., 1997; Zariquiey, 1968). All remains were counted, and minimum number of items was estimated for each stomach. In the case of no quantifiable material present, minimum number of items was recorded as one.

A subset of 21 samples of no identifiable tissues was randomly caught from the stomachs of 20 individuals. Tissue samples were labelled, fixed with 95% ethanol and stored for genetic analysis. Genomic DNA was extracted from each muscle tissue sub–sample by homogenisation and digestion using NucleoSpin® tissue extraction kit, following manufacturer's instructions. Absorbance ratio at 260/280 nm was used to assess the purity of the extracted DNA, with values from 1.8 to 2 representing highly purified DNA (Gallagher & Desjardins, 2006). Cytochrome c oxidase subunit I (COI) was amplified using HCO and LCO universal primers (Folmer et al., 1994). Cycling conditions were as follows: initial denaturation at 94ºC 1 min, followed by 39 cycles of denaturation at 94ºC for 15 seconds, annealing for 30 seconds at 48 Cº, extension at 72ºC for 45 seconds and final elongation for 7 minutes at 72 ºC. Reaction mix was composed by 1,5 µl $MgCl_2$, 2,50 Buffer 10x, 0,2 µl dNTP (10mM), 0,50 µL of each primer (10 µM), 0.13 µl Roche Applied Science Taq DNA polymerase (5 U/µl), 1 µl of DNA and distillate water until 25 µl. 2 µl of each PCR product were checked on 1.5% agarose gels. Those that present a clear band of expected size were clean using USB® ExoSAP–IT® PCR Product Cleanup following manufacturer protocol and sequenced by Sanger sequencing. Obtained DNA sequences were managed (aligned) with MEGA 6 software (Tamura et al., 2013) and compared to sequences in GenBank using BLAST algorithm. Genetically identified preys were added to the main database for further diet analyses.

In order to assess feeding habits, four indexes were calculated: (i) Index of Occurrence (%F), which correspond to the percentage of full stomachs containing a particular prey category; (ii) Percentage by Number (%N), the percentage of each category of prey compared to the total number of prey consumed; (iii) Percentage by weight (%W), total weight of each food category expressed as the percentage of the total weight of all stomach contents. When more than one type of prey was found in a single stomach, and

because it was impossible to discriminate one from each other, the total weight of the stomach content was divided by the total number of prey items (Castro & Guerra, 1990); and (iv) Emptiness Index (EMI), the number of empty stomachs compared with the total number of stomachs, expressed as a percentage. Comparison between EMI in each size class was carried out using a Chi square test.

The relative importance of each item group was estimated using two different indices: (i) Feeding Coefficient (Q= %N * %W)(Hureau, 1970); considering main prey for Q>200, secondary for 20<Q<200 and occasional for Q<20 and (ii) Index of Relative Importance (IRI = (%N + %W) * %F)(Pinkas et al., 1971). Pearson's correlation between both indexes was estimated.

MLR was used to analyse *E. cirrhosa* feeding patterns. The dependent variable was the prey item, categorized into the five main zoological groups consumed. Preliminary assessment of multicollinearity was performed using the condition index of the tetrachoric matrix (Belsley, 2004). A Likelihood Ratio Test (LRT) was used to evaluate the global impact of the independent variables on the dependent one, i.e., the effect of introducing the independent variable in the model. In LRT, the Chi–square parameter is the difference of Log likelihood–2 between the final and the reduced model, which is made by omitting an effect of the final model. The null hypothesis is that all the parameters of that effect are zero.

The Wald test was used to evaluate the impact of each category of independent variables on the dependent one. One category was used as a reference to avoid multicollinearity. The category Arthropoda was used as reference in the dependent variable.

The statistical software SPSS 23.0 was used.

Results

Diet description

Of the 2335 stomachs examined, only 618 contained prey items. In 120 of them, these contents were mainly composed of semi–digested fleshy material, which were not visually identifiable. In the remaining 498 stomachs, 64.1% had only one type of prey, 25.7% presented two types of prey, and 7.83% three different prey items. More than three types of preys were detected in 2.37% of the stomachs with prey items. The maximum number of prey items recorded in a single stomach was seven, belonging to a mature female of 243 g BW. The mean number and standard deviation of different prey by stomach were 1.91 and 1.07, respectively.

Empty stomachs represented 73.79% of the total. In overall, EMI did not show significant differences between sexes in any fishing ground (χ^2= 0.26, p>0.05). EMI exhibited monthly variability, ranging from 26.47% to 92.1%, with lower values during the cold season in the three fishing grounds. Regarding size classes, EMI values significantly decreased as BW increased (χ^2= 105.9, d.f. =3, p<0.05). Specifically, EMI corresponding to S1, S2, S3 and S4 size classes were 82.61%, 69.32%, 56.05% and 46.67%, respectively.

Five different prey species were successfully identified by molecular analysis in 14 stomach contents: the crustaceans *Polybius henslowii*, *Liocarcinus holsatus*, *Goneplax rhomboides* and *Munida rugosa*; and the cephalopod *E. cirrhosa*.

Calculated values of different indexes for each prey category are summarized in Table 1. The most important prey species by frequency of occurrence (F%) was the snapping shrimp *Alpheus glaber*, present

179 in a 20.88% of the examined stomachs and comprising 19.82% of the identified items. This prey was
180 follow by the bony fish *Callionymus sp.* (6.02%) and the crab *Goneplax rhomboids* (5.62%).
181 Across prey groups, F% of crustaceans was 88.15%. The F% for crustacean decapods was 53.6%.
182 Teleost or bony fish appeared in 19.48% of the examined stomachs, and the most preyed upon order was
183 Perciformes, with 8.43%F. Remains of molluscs appeared in 6.43% of the analysed stomachs. Among
184 them, two species of cephalopods were identified: *E. cirrhosa* (1%F) and *Alloteuthis subulata* (0.2% F).
185 *E. cirrhosa* remains were found in five specimens (2 from NG and 3 from WG fishing grounds), with
186 weights ranging from 159 to 673 g. Echinoderms were present in 7.23% of cases and, finally, the least
187 abundant prey group was polychaetes, with 2.81%F.
188 Dietary indexes calculated for each prey category are shown in Table 1.Q and IRI indexes showed a
189 strong linear correlation (r^2=0.99).
190

191 Feeding patterns

192 Type of prey was initially considered as dependent variable for MLR. As stated in Table 1, polychaetes
193 were an uncommon prey, which would generate singularities in the hessian matrix and, consequently, this
194 prey category was ruled out of the model. The condition index of the tetrachoric matrix was used as an
195 approach to assessing multicollinearity and was found to be 3.60—well below the limit of 10 suggested in
196 the literature (Belsley, 2004). Our sample fit the requirement of a minimum sample size of 10 cases per
197 independent variable (Schwab, 2002).

198 Likelihood ratio tests found significant differences between the final and null model (χ^2 = 70.77; 24
199 d.f.; p <0.001). The value of log likelihood–2 (deviation) indicates up to what point the model adjusts
200 well to the information (smaller, better adjustment). In our case, likelihood fell between 0 and 1 and, in
201 consequence, the log of a likelihood is less than or equal to zero. The Pseudo R–Square of McFadden
202 (0.062), Cox and Snell (0.093) and Nagelkerke (0.117) were treated as measures of effect size, similar to
203 how R^2 is treated in standard multiple regressions.
204 The global model test (Table 2) shows main effects, comparing likelihood ratios between reduced and
205 final models, and indicates which variables significantly affect dependent variables. According to this
206 test, the probability of preying on a particular zoological group is significantly affected by the
207 environmental variables "Season" and "Fishing ground", as well as the individual variable "Maturity
208 stage". On the contrary, sex and weight did not appear to influence the diet of *E. cirrhosa* in Atlantic
209 Iberian waters. Parameter estimations of the final model are shown in Table 3, which shows which
210 concrete categories of each variable affect relative probability of feeding. Moreover, the relative direction
211 and strength of each effect is given by the parameter β. The adjusted model indicates that *E. cirrhosa*
212 feeding patterns are significantly affected by several variables (Sig <0.05) with regard to the probability
213 of occurrence of certain taxonomic prey groups. Dietary composition according to significant variables
214 highlighted by the model is shown in Figure 2. The model predicted a higher probability of bony fish
215 consumption with respect to the reference group (crustaceans) depending on the fishing ground.
216 Specifically, the relative probability of fish consumption decreased southwards. During the cold season,
217 the probability of the occurrence of echinoderms with respect to crustaceans significantly increased.

There was a slight tendency, albeit significant, towards decreasing relative consumption of molluscs depending on the weight of the animal. Maturity stage significantly influenced the probability of mollusc consumption in relation to crustaceans, which decreased as the animal matured.

Discussion

DNA identification techniques can help identify decomposed species in the gut contents of cephalopods (Symondson, 2002). Nevertheless, at present, very few papers have been published on this subject (Deagle et al., 2005; Roura et al., 2012). Despite the limited number of successfully identified prey items by molecular techniques in the present paper, it is striking that the majority of prey identified by this technique were decapods, reinforcing the results obtained from visual analyses.

The main prey species of *E. cirrhosa* from Atlantic Iberian waters were crustaceans, which was similar to those found in captivity. In confined experiments carried out in the Zoological Laboratory (Aberdeen, Scotland), the species feeds on a wide variety of crustaceans, from lobsters to hermit crabs, including *Carcinus* (Boyle, 1983). Moreover, molluscs, when offered, were very rarely eaten in captivity (Boyle, 1983), which agrees with the relative scarcity (6.43%) of this kind of prey found in the present study. Crustaceans, including *Alpheus glaber* and *Goneplax rhomboides*, were the main prey item presents in the diet of *E. cirrhosa* from the Gulf of Lion, with a frequency of >50% in both sexes year round. In this area, the species also feeds upon fish, gastropods, cephalopods, polychaetes, and ophiuroids (Moriyasu, 1981), though at lower frequencies. The composition of the diet of *E. cirrhosa* described herein also shows clear similarities with its congeneric *E. moschata* from the Adriatic Sea, which also preferentially feeds on crustaceans and fish. Moreover, several factors have been shown to be involved in dietary changes in this species, such as the onset of sexual maturity and size (Sifner & Vrgoc, 2009). Results found in the present paper indicate that individual size did not affect feeding patterns, whereas maturity significantly affected prey selection.

Our results suggest that cannibalism is occasional in this species and, although it is unknown whether it takes place between animals of different sizes, observations in *O. vulgaris* (Hernández-Urcera et al., 2014) noted that prey/predator weight ratios range from 20% to 25% body weight. Moreover, our data indicate that cannibalistic episodes occurred in animals of various sizes and during winter and early spring, both periods with eventual abundance of juveniles (Regueira et al., 2014), so the presence of small animals could trigger this behaviour, as has been argued in the congeneric *E. moschata* (Sifner & Vrgoc, 2009).

The emptiness index (EMI) in this study showed a wide variability across months, which agrees with the results by Moriyasu (1981), who also found significantly higher EMI (53.43% in average) in the warm season than in the cold one (39.67%), as found in this study. EMI decreased with BW in our specimens. This result, together with the higher EMI values attained during the cold season when mean size of the population is larger and individuals are close to the reproductive season (Regueira et al., 2013), suggests that food intake in larger animals increases with maturation. This hypothesis seems to be supported by the fact that gonadal development mainly depends on the energy intake from food (Regueira et al., 2013) and also because one of the major effects given by the model was stage of maturity. Nevertheless, as no clear pattern was inferred from EMI monthly evolution, significantly higher values of

EMI reached by larger animals could indicate that larger individuals caught in net tows were less prone to regurgitate food than smaller ones. On the other hand, because the duration of throws of trawlers in this zone lasted approximately 6 hours, it could also be that the vacuity of stomachs might be due to the digestion of food. Nevertheless, given that *E. cirrhosa* needs 16 hours to free its digestive tract at a temperature of 18° C (Boucher-Rodoni, 1975) and the water temperature at depths where the throws are carried out is approximately 12°C, this possibility does not appear to be a likely explanation. In any case, it is difficult to conduct dietary studies in the field as there are many variables that can introduce serious biases. Thus, the genetically identified chaetognath *Sagitta enflatta* was discarded because it was considered a secondary prey species due to the Russian doll effect.

Although the full model was significantly better than the null model, Pseudo R-squared values indicated a poor fit. However, these types of metrics do not represent the amount of variance in the outcome variable accounted for by the predictor variables. Higher values indicate better fit, but they should be interpreted with caution. If a model has a very low likelihood, then the log of the likelihood will have a larger magnitude than the log of a more likely model, as occurred in the present study. Thus, a small ratio of log likelihoods indicates that the full model is a far better fit than the intercept model. As with most Chi-square based tests however, it is prone to inflation as sample size increases. Here, we see the model fit is significant ($p < 0.001$), which indicates that our full model predicts significantly better, or more accurately, than the null model. In other words, when p-value is less than the established cut-off (generally 0.05) a good fit is obtained (Starkweather & Moske, 2011).

A possible interpretation of the low Pseudo R-square values obtained would be that the diet of *E. cirrhosa* is not ruled strictly by the considered variables or because of high data dispersion. This fact can be easily associated with opportunist feeding behaviour as described for some octopod species (e.g. Leite et al., 2009). Nevertheless, previous studies on the abundance of crustaceans in the Galician shelf (González-Gurriarán & Olaso, 1987) have indicated that the most frequent items found in the stomachs of *E. cirrhosa* (*A. glaber* and *G. rhomboides*) actually show low abundances in the wild, with a merely occasional presence in the study area, while other species such as *Munida* spp. and *P. henslowii* exhibit patchy distribution, being locally abundant. However, relative importance indexes obtained by *P. henslowii* were notable while *Munida* spp. were present in the diet at very low frequencies. Although diet composition is, logically, a compromise between food preference and availability, as well as between the energy obtained and the vulnerability to be detected by a predator during foraging activity, the abovementioned facts suggest that despite the wide variety of prey available, *E. cirrhosa* is not an entirely opportunistic hunter but rather a selective generalist, as noted by Anderson for *O. vulgaris* in the Caribbean Sea.

Over the short life-span of cephalopods, feeding rapidly shifts from small to large prey, so an ontogenetic shift in prey selection does exist. A common pattern is for juveniles to prey on crustaceans and then switch to fish and other prey such as cephalopods as they grow larger (e.g. Castro & Guerra, 1990; Nixon, 1987; Wangvoralak et al., 2011). With regard to the main effects found by MLR (Table 2), BW did not have a significant effect on *E. cirrhosa* prey selection. However, parameters from the full model (Table 3) showed that BW had a small but significant effect on mollusc consumption in relation to crustaceans: the use of mollusc prey diminished with regard to crustaceans as the animal grew larger.

298 Because sexual maturity is positively and intimately related to size (BW), it is surprising that the model
299 did not detect this relationship, which might owe to data masking.
300 Differences in the diet of *E. cirrhosa* between different geographic regions and seasons, as found in
301 the present paper, had not been examined until now but these are common patterns for many cephalopods
302 species (Hastie et al., 2009; Hatanaka, 1979). These dietary differences between regions and seasons may
303 have been the result of the types of habitat that dominate these regions and the abundance and availability
304 of the different prey types within these habitats. Similarly, Fariña (1996) noted a significant decrease of
305 fish biomass and a simultaneous rise of crustaceans with depth, which, in addition to the aggregated
306 distribution and reproductive migration to shallow waters described in *E. cirrhosa* in Atlantic Iberian
307 waters (Regueira et al., 2014), could trigger an ontogenetic dietary change depending on maturation stage,
308 as indicated by our model.
309 MLR is a powerful and useful tool for this type of study. Leite et al. (2009) succeeded in using this
310 type of statistical analysis to study the influence of several factors on the feeding behaviour of *O.*
311 *insularis*. Nevertheless, judging by their results, they limited themselves to presenting the first part of the
312 regression, which measures the main effects (the impact of the independent variables on the dependent
313 variable in a global view) without indicating which category it specifically affects. In our case, however,
314 we attempted to bring biological sense to the **values of β (Table 3),** **which indicate the relative (to the**
315 reference group) direction and intensity of the effect of every independent variable.

316 Acknowledgments

317 **This research was supported by the project "Impacto do ambiente sobre o polbo** *Eledone cirrhosa* **no**
318 **sistema de afloramento costeiro das augas de Galicia" (INCITE08PXIB402074PR), funded by Xunta de**
319 Galicia. Marcos Regueira was supported by a FCT grant (SFRH / BD / 51038 / 2010). We thank to Mª.T.
320 Fernández and M. E. Garci for their help in the dissection of specimens and samples obtaining and also to
321 M. Cueto for her help in genetic extraction.

322 References

323 Auteri, R., P. Manini & C. Volpi, 1988. Regime alimentare di *Eledone cirrhosa* (Lamarck, 1798) e *Sepia*
324 *orbignyana* (Férrussac in Orbigny, 1826) (Mollusca, Cephalopoda) nel Tirreno Settentrionale.
325 Quaderni del Museo di Storia Naturale di Livorno 9: 67–73.
326 Belcari, P., P. Sartor, P. Jereb, E. Lefkaditou, G. J. Pierce, U. Piatkowski, T. Borges & A. L. Allcock,
327 2015. *Eledone cirrhosa*. In: Jereb P., A.L. Allcock, E. Lefkaditou, U. Piatkowski, L. Hastie & G.
328 J. Pierce (eds), Cephalopod biology and fisheries in Europe: II. Species Accounts, ICES
329 Cooperative Report 325: 30–41.
330 Belsley, D. A., 2004. Conditioning Diagnostics. In: John Wiley & Sons I (ed) Encyclopedia of Statistical
331 Sciences doi:10.1002/0471667196.ess0275.pub2.
332 Bouvier, E. L., 1940. Faune de France. Décapodes marcheurs. Féderation Française des Sociétés de
333 Sciences Naturelles, vol. 37. Paris.
334 Boucher-Rodoni, R., 1975. Vitesse de digestion chez les céphalopodes *Eledone cirrhosa* (Lamarck) et
335 *Illex illecebrosus* (Lesueur). Cahiers de Biologie Marine 16: 159–175.
336 Boyle, P & P. Rodhouse, 2005. Cephalopods: Ecology and Fisheries. Blackwell Science Ltd. Oxford UK.

337 Boyle, P. R., 1983. *Eledone cirrhosa*. In: Boyle PR (ed) Cephalopod life cycles, vol 1: Species Accounts.
338 Academic Press, London.
339 Boyle, P. R., 1986. A descriptive ecology of *Eledone cirrhosa* (Mollusca: Cephalopoda) in Scottish
340 waters. Journal of the Marine Biological Association of the UK 66: 855–865.
341 Boyle, P. R., M. S. Grisley & G. Robertson, 1986. Crustacea in the diet of *Eledone cirrhosa* (Mollusca:
342 Cephalopoda) determined by serological methods. Journal of the Marine Biological Association
343 of the UK 66: 867–879.
344 Boyle, P. R. & D. Knobloch, 1981. Hole boring of crustacean prey by the octopus *Eledone cirrhosa*
345 (Mollusca: Cephalopoda). Journal of Zoology 193: 1–10.
346 Castro, B. G. & A. Guerra, 1990. The diet of *Sepia officinalis* (Linnaeus,1758) and *Sepia elegans*
347 (Cephalopoda, Sepioidea) from the Ria de Vigo (NW Spain). Scientia Marina 54: 375–388.
348 Cherel, Y. & K. A. Hobson, 2005. Stable isotopes, beaks and predators: a new tool to study the trophic
349 ecology of cephalopods, including giant and colossal squids. Proceedings of the Royal Society
350 B: Biological Sciences 272: 1601–1607.
351 Deagle, B.E., S. N. Jarman, D. Pemberton & N. J. Gales, 2005. Genetic Screening for Prey in the Gut
352 Contents from a Giant Squid (*Architeuthis sp.*). Journal of Heredity 96: 1–7.
353 Ezzeddine, S., M. Rjeibi & B. Chemmam, 2012. Regime alimentaire d´*Eledone cirrhosa* (Cephalopoda,
354 Octopoda) des côtesnord et est Tunisiennes. Bulletin de l'Institut National des Sciences et
355 Technologies de la Mer de Salammbô 39: 25–33.
356 Fariña, A. C., 1996. Megafauna de la plataforma continental y talud superior de Galicia. Biología de la
357 cigala, *Nephrops norvegicus*. Universidad de La Coruña.
358 Folmer, O., M. Black, W. Hoeh, R. Lutz & R. Vrijenhoek, 1994. DNA primers for amplification of
359 mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular
360 Marine Biology and Biotechnology 3: 294–299.
361 Gallagher, S. R. & P. R. Desjardins, 2006. Quantitation of DNA and RNA with absorption and
362 fluorescence spectroscopy. Current Protocols in Molecular Biology 76: 3D:A.3D.1–A.3D.21.
363 Grisley, M. S. & P. R. Boyle, 1985. A new application of serological techniques to gut content analysis.
364 Journal of Experimental Marine Biology and Ecology 90: 1–9.
365 Grisley, M. S. & P. R. Boyle, 1988. Recognition of food in Octopus digestive tract. Journal of
366 Experimental Marine Biology and Ecology 118: 7–32.
367 Grisley, M. S., P. R. Boyle, G. J. Pierce & L. N. Key, 1999. Factors affecting prey handling in lesser
368 octopus *Eledone cirrhosa* feeding on crabs *Carcinus maenas*. Journal of the Marine Biological
369 Association of the UK 79: 1085–1090.
370 González–Gurriarán, E. & M. Méndez, 1986. Crustáceos decápodos das costas de Galicia. I. Brachyura.
371 Cuadernos da Área de Ciencias Biolóxicas, Seminario de Estudos Galegos, Vol. 2. O Castro–
372 Sada, A Coruña.
373 Guerra, A., 1992. Mollusca, Cephalopoda. In: *Fauna Ibérica*. Vol. 1 Ramos, M. A. *et al* (Eds.). Museo
374 Nacional de Ciencias Naturales. CSIC. Madrid.
375 Guerra, A., J. Hernández–Urcera, M. E. Garci, M. Sestelo, M. Regueira, A. F. González, M. Cabanellas–
376 Reboredo, M. Calvo–Manazza & B. Morales–Nin, 2015. Spawning habitat selection by *Octopus*

377 *vulgaris*: New information for management of this resource within a National Park. Fisheries
378 Research 167: 313–322.
379 Hastie, L. C., G. J., Pierce, J. Wang, I. Bruno, A. Moreno, U. Piatkowski & J. P. Robin, 2009.
380 Cephalopods in the North–Eastern Atlantic: Species, Biogeography, Ecology, Exploitation and
381 Conservation. Oceanography and Marine Biology, An Annual Review 47: 111–190.
382 Hatanaka, H., 1979. Studies on the fisheries biology of common octopus off the northwest coast of
383 Africa. Bulletin of the Far Seas Fisheries Research Laboratory 17: 13–124.
384 Hernández–Urcera J, M. E. Garci, A. Roura, A. F. González, M. Cabanellas–Reboredo, B. Morales–Nin &
385 A. Guerra, 2014. Cannibalistic Behavior of *Octopus vulgaris* in the wild. Journal of Comparative
386 Psychology 128: 427–430.
387 Hureau, J. C., 1970. Biologie compareé de quelques poissons antarctiques (Nototheniidae). Bulletin de
388 l'Institut océanographique de Monaco 68: 1–50.
389 Jackson, G. D., P. Bustamante, Y. Cherel, E. A. Fulton, E. P. M. Grist, C. H. Jackson, P. D. Nichols, H.
390 Pethybridge, K. Phillips, R. D. Ward & J. C. Xavier (2007) Applying new tools to cephalopod
391 trophic dynamics and ecology: perspectives from the Southern Ocean. Reviews in Fish Biology
392 and Fisheries 17: 79–99.
393 Jereb, P., C. F. E. Roper, M. D. Norman & J. K. Finn, 2014. Cephalopods of the world. An annotated and
394 illustrated catalogue of cephalopod species known to date. Octopods and Vampire Squids. FAO
395 Species Catalogue for Fishery Purposes. No. 4, Vol. 3. Rome.
396 Leite, T. S., M. Haimovici & J. Mather, 2009. *Octopus insularis* (Octopodidae), evidences of a
397 specialized predator and a time–minimizing hunter. Marine Biology 156: 2355–2367.
398 Mather, J. A., 1991. Foraging, feeding and prey remains in middens of juvenile *Octopus vulgaris*
399 (Mollusca: Cephalopoda). Journal of Zoology 224: 27–39.
400 Moriyasu, M., 1981. Biologie des peches de céphalopodes benthiques. Applica–tion aux eledones,
401 *Eledone cirrhosa* (Lam. 1798) du golfe du Lion. These Doctorat, Université des Sciences et
402 Techniques du Langue–doc, Montpellier.
403 Moriyasu, M., 1984. Egg clusters observed in the stomach contents of spawning females of *Eledone*
404 *cirrhosa* (Lamarck) (Cephalopoda: Octopoda) from the North–western Mediterranean Sea.
405 Venus; the Japanese journal of malacology 43: 189–192.
406 Nixon, M., 1987. Cephalopod diets. In: Cephalopod life cycles vol II. Academic press, pp 201–219
407 Perrier, R., 1954. La faune de la France illustrée. Arachnides et Crustacés. ed. Delagrave, Paris.
408 Phillips, K. L., G. D. Jackson & P. D. Nichols, 2003. Temporal variations in the diet of the squid
409 *Moroteuthis ingens* at Macquarie Island: stomach content and fatty acid analysis. Marine
410 Ecology Progress Series 256: 135–149.
411 Pierce, G. J., V. D. Valavanis, A. Guerra, P. Jereb, L. Orsi–Relini, J. M. Bellido, I. Katara, U. Piatkowski,
412 J. Pereira, E. Balguerias, I. Sobrino, E. Lefkaditou, J. Wang, M. Santurtun, P. R. Boyle, L. C.
413 Hastie, C. D. MacLeod, J. M. Smith, M. Viana, A. F. González & A. F. Zuur, 2008. A review of
414 cephalopod–environment interactions in European Seas. Hydrobiologia 612: 49–70.
415 Pinkas, L., M. S. Olipham & I. L. K. Iversor, 1971. Food habits of albacore, bluefin tuna and bonito in
416 California waters. Fisheries Bulletin 152: 1–105.

417 Regueira, M., A. F. González & A. Guerra, 2014. Habitat selection and population spreading of the
418 horned octopus *Eledone cirrhosa* (Lamarck, 1798) in Galician waters (NW Atlantic). Fisheries
419 Research 152: 66–73. doi:http://dx.doi.org/10.1016/j.fishres.2013.09.003.
420 Regueira, M., A. F. González, A. Guerra & A. Soares, 2013. Reproductive traits of horned octopus
421 *Eledone cirrhosa* in Atlantic Iberian waters. Journal of the Marine Biological Association of the
422 UK 93: 1641–1652. doi:10.1017/S0025315413000118.
423 Roura, A, A. F. González, K. Redd & A. Guerra, 2012. Molecular prey identification in wild *Octopus*
424 *vulgaris* paralarvae. Marine Biology 159: 1335–1345. doi:10.1007/s00227-012–1914–9.
425 Runham, N. W., C. J. Bailey, M. Carr, C. A. Evans & S. Malham, 1997. Hole drilling in crab and
426 gastropod shells by *Eledone cirrhosa* (Lamarck, 1798). Scientia Marina 61: 67–76.
427 Sánchez, P., 1981. Regime alimentare d'*Eledone cirrhosa* (Lamarck, 1798) (Mollusca, Cephalopoda)
428 dans la Mer Catalane. Rapport Commission International Mer Mediterranee 27: 209–212.
429 Schwab, J. A., 2002. Multinomial logistic regression: Basic relationships and complete problems.
430 http://www.utexas.edu/courses/schwab/sw388r7/SolvingProblems/.
431 Sifner, S. K. & N. Vrgoc, 2009. Diet and feeding of the musky octopus, *Eledone moschata*, in the
432 northern Adriatic sea. Journal of the Marine Biological Association of the UK 89: 413–419.
433 Starkweather, J. & A. K. Moske, 2011. Multinomial Logistic Regression
434 http://www.unt.edu/rss/class/Jon/Benchmarks/MLR_JDS_Aug2011.pdf.
435 Symondson, W. O. C., 2002. Molecular identification of prey in predator diets. Molecular Ecology 11:
436 627–641.
437 Tamura, K., G. Stecher, D. Peterson, A. Filipski & S. Kumar, 2013. MEGA6: Molecular Evolutionary
438 Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.
439 Tuset, V. M., A. Lombarte & C. A. Assis, 2008. Otolith atlas for the western Mediterranean, north and
440 central eastern Atlantic. Scientia Marina. 72: 7–198.
441 Wangvoralak, S., L. C. Hastie & G. J. Pierce, 2011. Temporal and ontogenetic variation in the diet of
442 squid (*Loligo forbesii* Streenstrup) in Scottish waters. Hydrobiologia 670: 223–240.
443 Watt, J., Pierce, G. J. & Boyle, P. R., 1997. Guide to the identification of north sea fish using premaxillae
444 and vertebrae. ICES Cooperative Research Report. 220, 231 pp.
445 Zariquiey A., R., 1968. Crustáceos decápodos Ibéricos. Investigacion Pesquera. Vol. 32. Barcelona.
446

447 Figure Captions

448 Fig. 1 Map showing the location of the fishing ports where samples for this study were obtained

449 Fig. 2 Diet composition of Eledone cirrhosa according to the three significant variables highlighted by Multinomial
450 Logistic Regresion (MLR) model: Maturity stage (a), Fishing ground (b) and Season (c)

451

452

Figure 1

Click here to download Figure Figure 1.docx

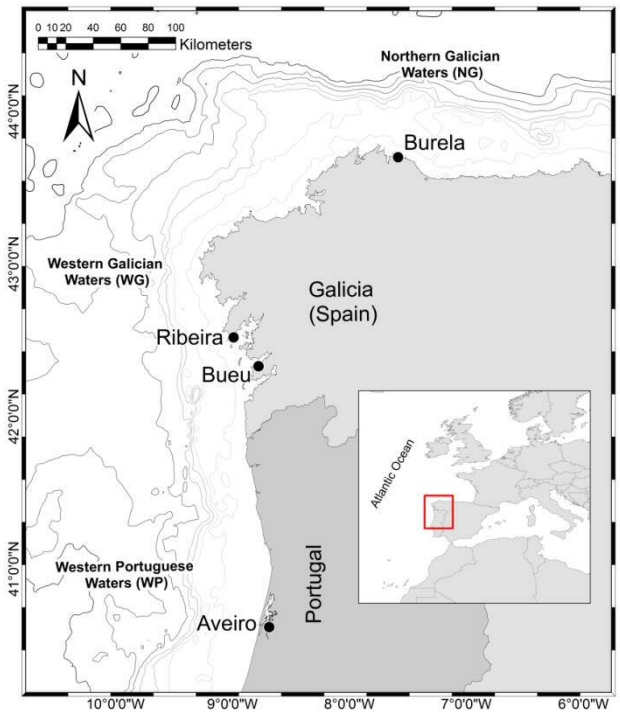

Figure 2

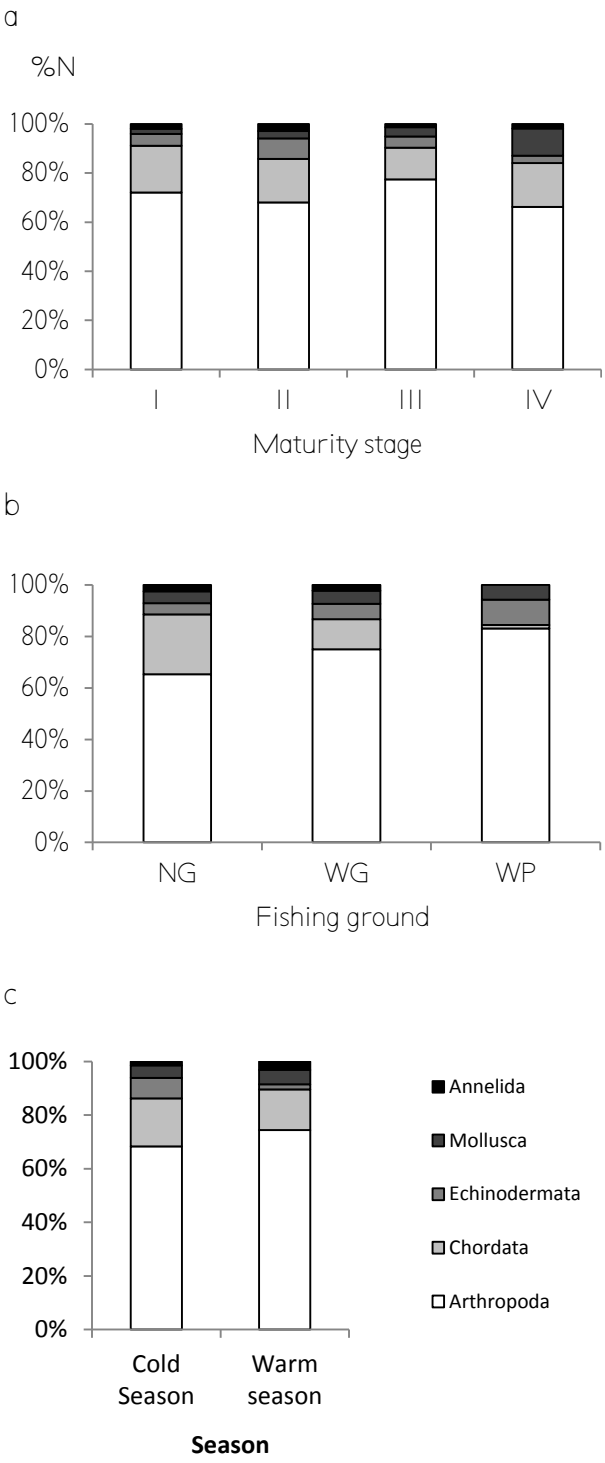

Table 1. Percentage of occurrence (%F), percentage by number (%N), weight percentage (%W), and index of relative importance (IRI) and feeding coefficient (Q) of prey in the diet of *Eledone cirrhosa*.

	%F	%N	%W	IRI	Q
POLYCHAETA	2.81	2.09	0.66	7.72	1.38
CRUSTACEANS	88.15	70.64	71.46	12526.81	5048.19
Malacostraca	54.62	45.01	43.16	4815.66	1942.59
Amphipoda	0.80	0.60	0.24	0.67	0.15
Decapoda	53.61	44.26	42.69	4661.83	1889.50
Anomura	6.43	4.92	3.45	53.75	16.95
Family: Galatheidae					
Galathea sp.	4.02	3.13	2.06	20.84	6.45
Galathea squamifera	0.20	0.15	0.08	0.05	0.01
Family: Munididae					
Munida rugosa	0.20	0.15	0.35	0.10	0.05
Munida sp.	0.20	0.15	0.06	0.04	0.01
Family: Paguridae					
Anapagurus laevis	0.40	0.30	0.12	0.17	0.04
UnndentifiedAnomura	1.41	1.04	0.78	2.56	0.81
Brachyura	8.23	6.11	6.34	102.52	38.75
Family: Atelecyclidae					
Atelecyclus undecimdentatus	0.20	0.15	0.06	0.04	0.01
Family: Goneplacidae					
Goneplax rhomboides	5.62	4.17	2.96	40.09	12.34
Family: Homolidae					
Paromola cuvieri	0.40	0.30	0.34	0.25	0.10
Family: Portunidae					
Liocarcinus holsatus	0.20	0.15	0.10	0.05	0.01
Polybius henslowii	1.61	1.19	2.84	6.48	3.39
Unidentified Portunidae	0.20	0.15	0.05	0.04	0.01
Caridea					
Family: Alpheidae					
Alpheus glaber	20.88	19.82	11.44	652.81	226.72
Unidentified Decapoda	27.11	20.12	21.46	1127.17	431.78
Unidentified Crustacea	36.14	27.57	23.97	1863.03	660.96
PISCES	19.68	16.84	17.73	680.23	298.52
Chondrichthyes	0.20	0.15	0.13	0.06	0.02
Osteichthyes	19.48	16.69	17.60	667.95	293.79
Argentiniformes					
Argentina sp.	0.20	0.15	0.11	0.05	0.02
Clupeiformes	0.40	0.30	0.35	0.26	0.10
Perciformes	8.43	8.49	3.89	104.42	33.02
Callionymidae	6.02	6.56	2.28	53.25	14.97
Callionymus maculatus	0.20	0.15	0.02	0.03	0.00
Callionymus sp.	5.82	6.41	2.26	50.50	14.50
Gobiidae	4.02	3.13	1.60	19.01	5.02
Pleuronectiformes					
Arnoglosus sp.	0.20	0.15	0.08	0.05	0.01
Scorpaeniformes					
Triglalyra	0.20	0.15	0.12	0.05	0.02
Unidentified Osteichthyes	11.04	8.20	13.06	234.72	107.02
ECHINODERMS	7.23	5.51	2.53	58.15	13.95
Ophiuridae	2.81	2.24	1.08	9.31	2.41
Unidentified Echinodermata	4.42	3.28	1.45	20.90	4.76
MOLLUSCS	6.43	4.92	7.62	80.57	37.48
Cephalopoda	4.42	3.28	5.98	40.92	19.62
Octopoda					
Eledone cirrhosa	1.00	0.75	0.52	1.27	0.39
Teuthida					
Alloteuthis subulata	0.20	0.15	0.14	0.06	0.02
UnidentifiedCephalopoda	3.21	2.38	5.32	24.74	12.68
Gastropoda	0.40	0.45	0.13	0.23	0.06
Unidentified Mollusca	1.81	1.34	1.51	5.15	2.02

Table 2. Likelihood Ratio Test (LRT) between the final and the reduced model. ([a]) This reduced model is equivalent to the final model, because omitting the effect does not increase the degrees of freedom; (*) indicates significant effect over probability of *Eledone cirrhosa* prey consumption.

Effect	Fitting model criteria	Likelihood ratio contrast		
	Log likelihood -2 of the reduced model	Chi-square	d.f.	Sig.
Intercept	869.967[a]	0.000	0	.
BW	875.605	5.639	3	0.131
Season	881.369	11.402	3	0.010 *
Fishing ground	898.391	28.424	6	0.000 *
Maturity	895.831	25.864	9	0.002 *
Sex	873.145	3.178	3	0.365

Table 3. *Eledone cirrhosa* feeding patterns. Full model parameters estimation for Multinomial Logistic Regression (MLR); ([a]) Reference category was Arthropod; ([b]) This parameter is 0 because this category is redundant; (*) Indicates signification (Sig<0.05).

Prey item group[a]		β	Standard error	Wald	d.f.	Sig.	Exp(β)	95% confidence interval for Exp(B)	
								Lower limit	Upper limit
Fish	Intercept	-0.948	0.409	5,377	1	0.02 *			
	Body weight	0	0.001	0.289	1	0.591	1.000	0.998	1.001
	Season: Cold	0.265	0.241	1.206	1	0.272	1.303	0.812	2.092
	Season: Warm	0[b]	.	.	0	.	.	.	.
	Fishing ground: WG	-0.679	0.237	8,216	1	0.004 *	0.507	0.319	0.807
	Fishing ground: WP	-2.967	1.029	8.316	1	0.004 *	0.051	0.007	0.387
	Fishing ground: NG	0[b]	.	.	0	.	.	.	.
	Maturity: I	-0.227	0.352	0.418	1	0.518	0.797	0.4	1,587
	Maturity: II	-0.144	0.298	0.232	1	0.63	0.866	0.483	1,554
	Maturity: III	-0.382	0.339	1,270	1	0.26	0.683	0.351	1,326
	Maturity IV	0[b]	.	.	0	.	.	.	.
	Sex: Female	-0.128	0.26	0.242	1	0.623	0.88	0.528	1,465
	Sex: Male	0[b]	.	.	0	.	.	.	.
Echinoderm	Intercept	-3.406	0.822	17.171	1	0			
	Body weight	0	0.001	0.011	1	0.915	1.000	0.997	1.003
	Season: Cold	1.261	0.518	5.918	1	0.015 *	3.529	1.278	9.746
	Season: Warm	0[b]	.	.	0	.	.	.	.
	Fishing ground: WG	0.277	0.393	0.497	1	0.481	1.319	0.611	2.850
	Fishing ground: WP	0.78	0.596	1,713	1	0.191	2.181	0.678	7.013
	Fishing ground: NG	0[b]	.	.	0	.	.	.	.
	Maturity: I	-0.242	0.695	0.121	1	0.728	0.785	0.201	3.067
	Maturity: II	0.242	0.569	0.182	1	0.67	1.274	0.418	3.884
	Maturity: III	-0.241	0.627	0.147	1	0.701	0.786	0.23	2.685
	Maturity IV	0[b]	.	.	0	.	.	.	.
	Sex: Female	-0.625	0.438	2,.036	1	0.154	0.535	0.227	1.263
	Sex: Male	0[b]	.	.	0	.	.	.	.
Mollusc	Intercept	-1.101	0.657	2.806	1	0.094			
	Body weight	-0.003	0.001	5.042	1	0.025 *	0.997	0.994	1.000
	Season: Cold	0.895	0.456	3.847	1	0.05	2.447	1.001	5.986
	Season: Warm	0[b]	.	.	0	.	.	.	.
	Fishing ground: WG	-0.055	0.404	0.019	1	0.891	0.946	0.429	2.087
	Fishing ground: WP	-0.56	0.69	0.658	1	0.417	0.571	0.148	2.210
	Fishing ground: NG	0[b]	.	.	0	.	.	.	.
	Maturity: I	-2.726	0.738	13.626	1	0 *	0.065	0.015	0.278
	Maturity: II	-2.008	0.541	13.779	1	0 *	0.134	0.047	0.388
	Maturity: III	-1.576	0.531	8.796	1	0.003 *	0.207	0.073	0.586
	Maturity IV	0[b]	.	.	0	.	.	.	.
	Sex:Female	0.449	0.506	0.786	1	0.375	1.567	0.581	4.227
	Sex: Male	0[b]	.	.	0	.	.	.	.