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Decision tree analysis for the determination of relevant variables and quantifiable reference points to establish maturity stages in *Enteroctopus megalocyathus* and *Illex argentinus*

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Determining the maturity condition of a large number of individuals is crucial for stock assessment and management of cephalopod populations, but this task is difficult to conduct in practice. We propose a novel approach for maturity stage classification using observer-independent criteria. Relevant morphological variables for classification are determined via decision tree (DT) analysis. Using *Illex argentinus* and *Enteroctopus megalocyathus* as case studies, individuals were sexed and assigned to a maturity stage defined by specific macroscopic maturity scales. Also, for each individual, the weight of the gonad, accessory glands/ducts, mantle length, and total weight were recorded and maturity indices were calculated (Hayashi index and gonadosomatic index). Two different DT models were fitted: one considering all maturity stages and the other considering only intermediate maturity stages since these are the most difficult to determine in practice. For the classification of *I. argentinus* among all stages, the weights of the nidamental gland and oviducts were the most relevant variables for females (misclassification 23%), while spermatophoric complex and testis weights were the key variables for males (misclassification 23%). For classification of intermediate stages only, the nidamental gland and spermatophoric complex weights were the most relevant variables to classify females (misclassification 19%) and males (misclassification 21%), respectively. For *E. megalocyathus*, the oviducts and ovary weights of females and the terminal organ weight of males were the most relevant variables for classification among all maturity stages (misclassification 16% and 18%, respectively). For intermediate maturity stages, the same variables were most important and misclassification improved to 13% for both sexes. Gonadosomatic and Hayashi's indices were not relevant in either model. DTs based on measurements of cephalopod reproductive systems revealed a simple classification system for maturity stages using only a few variables that are easy to measure in the field and are independent of observer training.

Keywords: cephalopods, decision tree, fisheries assessment, maturity scales, octopus, sexual maturation, squid.

Introduction

Fishery and ecological studies on cephalopods require accurate maturity assessment of large numbers of individuals to estimate the timing and location of breeding aggregations, the size at maturity, and the sex ratio. Maturity scales are developed based on several aspects of reproductive system development that are based on microscopic changes in gonad condition (histological analysis to evaluate the gametogenesis process), reproductive indices (dividing gonad weight by some other body measure) or macroscopic maturity stages (division of the maturity process in recognizable stages using morphochromatic features of the reproductive system), or a combination of these methods (Mangold, 1987; Sauer and Lipiński, 1990; Lipiński and Underhill, 1995; Boyle and Rodhouse, 2005). Each approach provides a guide for the assessment of maturity stages of individuals, thus allowing for the documentation of chronology of reproductive activity of populations. However, important limitations arise when trying to apply them.

Histological analysis has been considered an important approach to understand the maturity process, since it focuses mainly on gametogenesis accuracy, but it is time-consuming and expensive (Durward *et al.*, 1979; Lipiński and Underhill, 1995; Ortiz, 2013). For reproductive indices, the most frequently and widely used are: gonadosomatic index (GSI), in squids, and Hayashi's index (HI, Hayashi, 1970), or Guerra's index (GI, Guerra, 1975), in octopuses. However, although they are simple to calculate, there are several problems with these indices. First, dividing gonad weight by some measure of body size (e.g. GSI) does not necessarily make the index independent of this measure (Orange, 1961; Gonor, 1972). Thus, problems may arise when individuals of different sizes are compared (De Vlamming *et al.*, 1982). In addition, ratios in general may present difficulties for meeting the statistical assumptions required for several parametric tests (e.g. normality, homoscedasticity, independence). This is particularly pronounced in situations when the denominator of the ratio is highly variable (Bolger and Connolly, 1989), as is true for body weight in cephalopods (Boyle and Rodhouse, 2005). On the other hand, macroscopic maturity scales represent a relatively rapid manner for assessing the maturity status of individuals. This method is frequently used in fishery management and ecological studies, since it allows researchers to make fast and gross determinations of maturity condition in the field (Boyle and Rodhouse, 2005; Crespi-Abril *et al.*, 2008; Perez *et al.*, 2009; Ortiz *et al*, 2011). However, macroscopic analysis alone may lead to misclassification of maturity state of animals and thus, validation with histological observations is recommended (Sauer and Lipiński, 1990; Gonçalves *et al.*, 2002; Díaz-Uribe *et al.*, 2006; Ortiz, 2013). In addition, identifying discrete maturity stages based on macroscopic reference points is often difficult since maturity of an individual changes continuously (Mangold, 1987; Sauer and Lipiński, 1990) and also it could contain a subjective judgement (Gerritsen and McGrath, 2006). However, it is possible to assess maturity accurately using macroscopic criteria when observers are trained to identify the differences between stages. One limitation is that adequate training requires substantial effort since each observer must look at a large number of individuals to be able to recognize the intrinsic variability among individuals and to assign each individual into a maturity stage of the scale. Therefore, it is crucial to establish low-effort classification criteria for grouping each individual accurately into a particular maturity stage that is based on relevant characteristics that can be easily and objectively obtained (e.g. weight, length). This is particularly relevant when a trade-off between cost and efficiency of the staging process become important, like in fisheries assessment and management.

Methods used in neural networks, particularly in pattern recognition, have proven to be useful in determining decision criteria for classification of observations (Ripley, 1996; Basu *et al.*, 2010). One key point in neural networks is classification which is, in simplified terms, the assignment of a label to a given input value. Several algorithms have been developed to classify data and they are generally categorized into supervised and unsupervised, according to the type of procedure used to generate the outcome (Ripley, 1996). Unsupervised procedures attempt to find inherent patterns in unlabeled data (e.g. hierarchical clustering, principal component analysis). Supervised procedures, on the other hand, generate a function that maps inputs to desired outputs (labels or categories) that have been properly determined by experts [e.g. linear discriminant analysis, logistic regression, decision trees (DT)]. Since inputs are previously labelled, the classification error of the function can be estimated and minimized (Bishop, 1995; Ripley, 1996). Then the function can be used to determine the correct output value for new data instances (generalization). The supervised procedure most frequently used in ecological studies to classify datasets is linear discriminant analysis (Macy, 1982; James and McCulloch, 1990; Perez *et al.*, 2009). However, this method has important statistical assumptions that usually are not met, since it is based on a family of models with a small number of parameters (Williams, 1983). In particular, discriminant analysis assumes multivariate normality and it is not well known how robust these methods are to the violation of those assumptions. DT are more appropriate to classify most datasets since this method is not based on parametric models.

The squid *Illex argentinus* and the octopus *Enteroctopus megalocyathus* are two commercially important species of cephalopod occurring in the coastal waters of Northern Patagonia (Ré, 1998; Ortiz *et al.*, 2006; Crespi-Abril and Barón, 2012; Crespi-Abril *et al.*, 2013, 2014). *Illex argentinus* is an ommastrephid squid that occurs from southern Brazil (24°S) to the Malvinas Islands (50°S).The population structuring comprises at least five spawning groups (spring-spawning group, summer-spawning group, Bonaerensis-Northpatagonic, South Patagonic, South Brazilian) along the continental shelf and slope, based on the spatio-temporal location of reproductive activity (Haimovici *et al.*, 1998; Crespi-Abril and Barón, 2012). This species has sustained the most important fishery of cephalopods based on landings over the last 10 years (FAO, 2012). *Enteroctopus megalocyathus* is an incirrate octopus that is distributed from San Matias Gulf to Beagle Channel, including the Malvinas Islands and Burwood Bank in the Atlantic Ocean and from 42°S to 54°S in the Pacific Ocean (Ré, 1998). Along the northern Patagonian Atlantic coast, the most important spawning activity occurs in summer, while a small fraction of the population spawns in winter. This octopus is captured by artisanal fishers in coastal areas and represents an important resource for regional economies of both the Argentinean and Chilean coasts (Chong Lay-Son *et al.*, 2001; Ortiz *et al.*, 2011).

In this paper, we applied DT analysis of quantitative morphological variables and indices to determine relevant quantitative criteria that characterize the maturity condition of individuals of *I. argentinus* and *E. megalocyathus*. This approach is not aimed to replace histological analysis and macroscopic maturity scales in reproductive studies but to provide a simple classification key for monitoring and management goals. Thus, based on previously published macroscopic maturity scales and our newly reported results, we suggest a new practical key to easily identify the maturity condition of individuals by relevant measurements of the reproductive system.

Material and methods

Data collection

A total of 4551 individuals of *I. argentinus* (Table 1) comprising two different spawning groups were analysed: spring-spawning group (1146 females and 1555 males) and summer-spawning group (742 females and 1108 males). Samples were obtained monthly from June 2005 to November 2007 on 25 m-long bottom trawlers operating in San Matías Gulf (SMG, Figure 1), from hauls conducted with 120 mm mesh-size nets. The specimens were stored in sealed plastic bags and preserved in ice-chillers until examination in the laboratory. Dorsal mantle length (DML) and total weight (TW) of

Table 1. Mean values and standard deviation (in parentheses) of the variables and indices in each maturity stage and sex for both species.

Species	Maturity stage	Females								Males							
		N	DML	TW	OvW	OdW	NiW	HI	MI	N	DML	TW	TeW	SCoW	TOW	HI	MI
Enteroctopus megalocyathus	I	12	8.2 (13)	240 (88)	0.29 (0.1)	0.1 (0.1)	–	0.23 (0.1)	0.16 (0.1)	14	7.1 (0.95)	175.7 (88)	0.09 (0.05)	0.1 (0.08)	0.02 (0.01)	0.51 (0.13)	0.06 (0.06)
	II	338	10.8 (17)	644 (252)	1.44 (1.2)	0.9 (0.8)	–	0.35 (0.1)	0.33 (0.2)	235	10.54 (1.68)	586.6 (241.2)	3.61 (4.8)	0.9 (0.94)	0.15 (0.12)	0.41 (0.18)	0.22 (0.17)
	III	327	13.1 (18)	1096 (331)	6.63 (5.6)	3.95 (2.4)	–	0.38 (0.1)	0.91 (0.5)	236	12.81 (1.59)	921.6 (259)	16.2 (10.7)	4.7 (2.88)	0.54 (0.33)	0.30 (0.1)	0.72 (0.39)
	IV	55	16.3 (28)	1639 (559)	62.8 (37.5)	16.4 (7.2)	–	0.22 (0.1)	4.93 (2.3)	219	15.29 (2.08)	1404.7 (434.2)	48.9 (21.1)	21.7 (15.5)	2.32 (1.87)	0.37 (0.16)	2.4 (1.57)
	V	11	14.3 (9.2)	1042 (434)	46.7 (50.6)	12.03 (6.5)	–	0.29 (0.2)	5.72 (4.6)	–	–	–	–	–	–	–	–
Illex argentinus	I	331	15.5 (1.9)	70.8 (33)	0.22 (0.4)	0.13 (0.1)	0.1 (0.1)	0.56 (0.2)	0.68 (0.8)	240	14.2 (1.6)	55.8 (19.3)	0.17 (0.25)	0.1 (0.4)	–	0.49 (0.17)	0.63 (1.2)
	II	338	19.6 (3.3)	167 (105)	0.99 (1.2)	0.42 (0.4)	0.73 (0.9)	0.55 (0.1)	1.28 (1.2)	271	15.9 (1.9)	83.9 (44.7)	1.3 (1.3)	0.4 (0.5)	–	0.29 (0.14)	2.24 (2.2)
	III	323	22.8 (3.4)	270 (137)	5.92 (5.6)	1.21 (1.1)	6.23 (5.2)	0.57 (0.1)	6.29 (5.7)	206	17.9 (2.4)	137.6 (72.8)	4.3 (2.4)	1.5 (1.7)	–	0.24 (0.1)	4.44 (1.9)
	IV	421	22.9 (3.5)	279 (164)	15.15 (11)	4.27 (3.5)	18.3 (9.8)	0.61 (0.1)	14.9 (5.6)	471	18.9 (3.1)	184.2 (102.1)	5.4 (3.1)	3.1 (2.4)	–	0.37 (0.13)	4.8 (1.3)
	V	475	24.8 (4.6)	392 (268)	24.6 (19)	18.5 (17)	36.1 (21)	0.7 (0.1)	21.1 (5.6)	1475	19.8 (3.5)	239.8 (149.1)	6.5 (4.1)	6.3 (4.5)	–	0.49 (0.11)	5.57 (1.3)

DML, dorsal mantle length; TW, total weight; OvW, ovary weight; OdW, oviductal weight; NiW, nidamental weight; TeW, testis weight; SCoW, spermatophoric complex weight; TOW, terminal organ weight; HI, Hayashi index; MI, maturity index; *n*, number of individuals. Lengths are expressed in centimetres and weights in grammes. The symbol "–" means no available data.

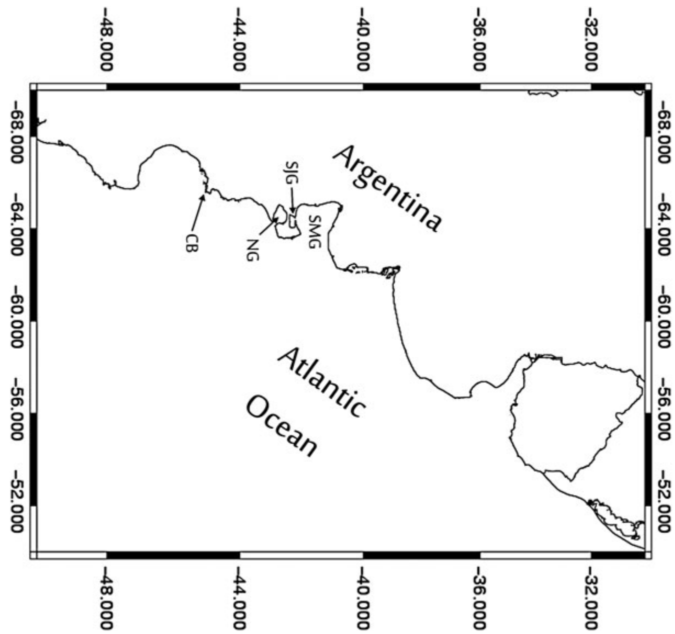

Figure 1. Location of the sampling sites on the Argentinean coast for *I. argentinus* (SMG, San Matias Gulf) and *E. megalocyathus* (SJG, San Jose Gulf; NG, Nuevo Gulf; CB, Camarones Bay).

each individual were measured and specimens were carefully examined by one of the authors (i.e. a highly trained observer) to determine, with a high level of certainty, the maturity condition of squids, and minimized misclassification. The scale used for the assessment of maturity was the one proposed by Nigmatullin (1989) which was modified and histologically corroborated by Brunetti (1990). This scale categorizes the process of maturation in seven stages for males and eight for females: stages I, II, and III correspond to immature individuals with progressive formation of gonads, stage IV consists of specimens in physiological maturation, stages V and VI comprise animals at functional maturity (capable to mate), and stages VII and VIII include fully spawned individuals in males and females, respectively. Measurements of reproductive organs were also recorded: weights of the nidamental glands (NiW), ovary (OvW), and oviducts (OdW) in females and weights of the testes (TeW) and spermatophoric complex (SCoW) in males.

A total of 1447 *E. megalocyathus* individuals (704 males and 743 females, Table 1) were obtained monthly by diving surveys (up to 25 m depth) on Nuevo Gulf (NG, Figure 1) and San José Gulf (SJG, Figure 1) and by artisanal fisheries in the intertidal area of Camarones Bay (CB, Figure 1) from June 2004 to March 2007. Octopuses were extracted from holes or crevices present in rocky outcrops or abrasion limestone-platforms using iron hooks. In the laboratory, DML and total TW of each individual were recorded and maturity condition was determined, via careful examination of individuals by one of the authors, following the scale proposed by Ortiz *et al.* (2011) and validated by Ortiz (2013). This scale categorizes the process of maturation into four stages for males and six stages for females. In males, stages I and II correspond to immature individuals with progressive formation of gonads, stage III comprises specimens in physiological maturation, and stage IV comprises animals in functional maturation (capable to mate) or fully spent individuals. In females, stages I and II correspond to immature individuals, stages III and IV comprises specimens in physiological maturation, and stages V and VI comprise animals in functional maturation or fully spent individuals. Additionally, testis weight (TeW), spermatophoric complex weight (SCoW), and terminal organ weight (TOW) were recorded for males; and ovary weight (OvW) and oviducts weight (OdW) were recorded for females. Hayashi index (HI) and Maturity index (MI) were calculated as:

(i) $HI = SCoW/(SCoW + TeW + TOW)$ for *E. megalocyathus* males and

(ii) $HI = SCoW/(SCoW + TeW)$ for *I. argentinus* males and

(iii) $HI = OdW/(OdW + OvW)$ for females of both species,

(iv) $MI = (SCoW + TeW + TOW)/(TW - SCoW - TeW - TOW)$ for *E. megalocyathus* males, and

(v) $MI = (SCoW + TeW)/(TW - SCoW - TeW)$ for *I. argentinus* males, and

(vi) $MI = (OdW + OvW)/(TW - OdW - OvW)$ for *E. megalocyathus* females, and

(vii) $MI = (OdW + OvW + NiW)/(TW - OdW - OvW - NiW)$ for *I. argentinus* females

DT analysis

To determine the relevant variables that minimize misclassification of individuals into each maturity stage, a DT analysis was conducted. For building each tree, all the above-mentioned variables and the two estimated indices (HI and MI) were used (Table 1) and the macroscopic maturity condition of individuals was used as an independent category to supervise the classification process. Two different tree classification analyses were conducted for males and females of each species using all the variables and indices. The first analysis (M1) was conducted considering all the categories of the maturity scales. In the particular case of *I. argentinus*, the spawning group (SG) was used as a categorical variable in the model. Considering that intermediate stages of the macroscopic scales are the most difficult maturity conditions to determine in practice (since they are usually defined by the relative size and colour of the different parts of the reproductive organs (Gerritsen and McGrath, 2006; Ortiz *et al*, 2011), a second analysis (M2) was conducted including only the intermediate categories of the maturity scales (stages II, III,

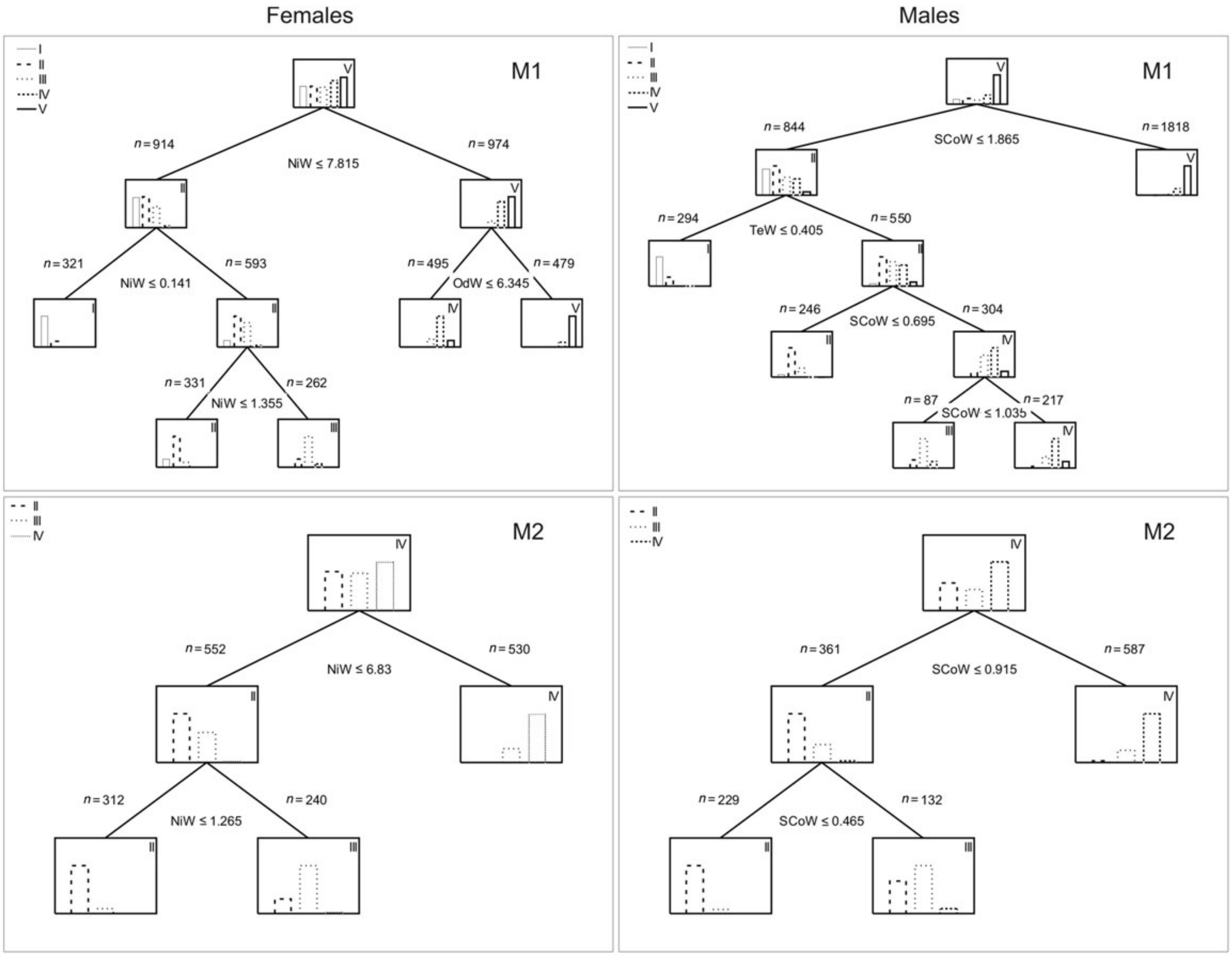

Figure 2. DT diagrams for males and females of *I. argentinus*. M1: model that considers all maturity stages and M2: model that considers only intermediate maturity stages. NiW, nidamental gland weight; OdW, oviduct weight; TeW, testis weight; SCoW, spermatophoric complex weight.

and IV, except for males of *E. megalocyathus* in which case only stages II and III were considered).

The result of DT is a tree-like plot composed of a root node, a set of internal nodes, and a set of terminal nodes (for illustration, see Figure 2). DT construction involves the recursive partitioning of the dataset (learning data) into increasingly homogeneous subsets based on tests applied to one or more of the feature values. In each node, the split that maximizes the reduction in impurity of the subsequent data subsets is chosen. Splitting continues until the terminal nodes are reached and they are labelled by majority voting. DT estimates the misclassification rate as the ratio between the number of correctly assigned cases and the total number of cases considered. It must be kept in mind that DT analysis attempts to divide the learning data into subsets that contain only a single class (Ripley, 1996). Thus, the result of this procedure is often a very large and complex tree. Usually, building a DT until all terminal nodes contain data for a single class may overfit to the intrinsic variability (noise) of the learning dataset. Therefore, the classification tree can lead to poor performance on unseen cases (test dataset; Breiman *et al.*, 1984). In this sense, the original tree can be pruned, retrospectively removing some parts to produce a less complex, more comprehensible, and general tree. To determine the optimal size of the tree, it is necessary to grow the model recursively and to estimate the misclassification rate of both learning and test datasets each time. The optimal model represents the trade-off between complexity (number of nodes) and the misclassification rate (best fit) of the learning and test data (Breiman *et al.*, 1984).

Owing to the lack of an independent dataset, we used the cross-validation technique to obtain the optimal tree model (Breiman *et al.*, 1984). The complete dataset was randomly split into ten subsets, stratified by the maturity stage of individuals. This assured that a similar distribution of maturity stages was present in each of the ten subsets. To perform the model-building procedure, one of these subsets was reserved for use as an independent dataset (test dataset) to determine the misclassification error, while the other nine subsets were combined for use as the learning dataset in the model-building procedure. The entire model-building procedure was repeated changing the subset of the data reserved for use as the test dataset each time, resulting in ten different models. The average performance of these ten models is an unbiased estimator of the performance of the original model (produced through the recursive learning using all data in ten learning combinations of nine subsets) on a future dataset (Breiman *et al.*, 1984).

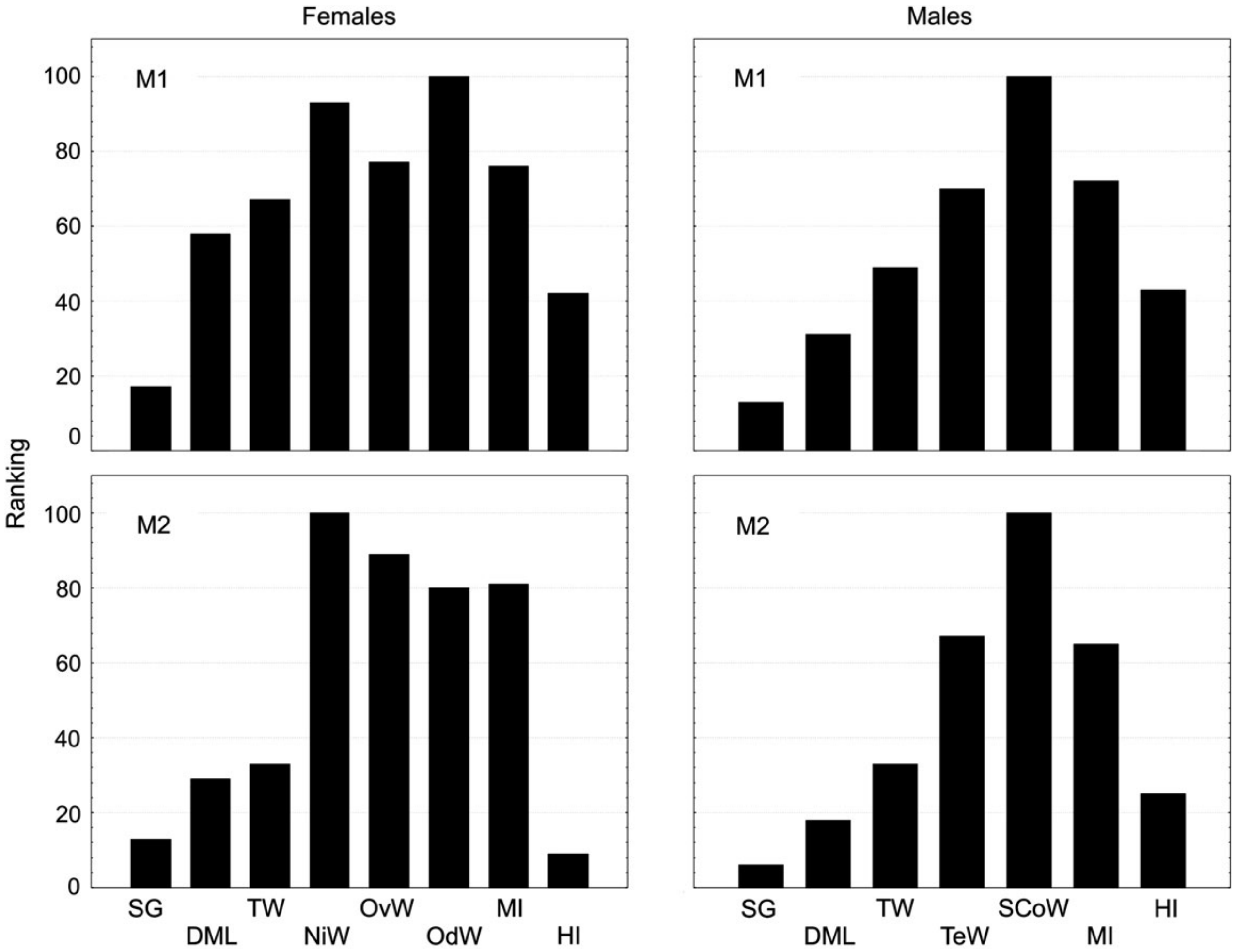

Figure 3. Ranking of importance of variables and indices (predictors) for males and females of *I. argentinus*. M1: model that considers all maturity stages and M2: model that considers only intermediate maturity stages. SG, spawning group; DML, dorsal mantle length; TW, total weight; OvW, ovary weight; OdW, oviductal weight; NiW, nidamental weight; TeW, testis weight; SCoW, spermatophoric complex weight; HI, Hayashi index; MI, maturity index.

In the present study, the ranking of importance of each predictor was estimated for each DT analysis conducted. To calculate a variable importance score, DT analysis looks at the improvement measure, called "Gini Index" (Breiman *et al.*, 1984), attributable to each variable in its role as either a primary splitter (criteria used of certain variable to create a node in DT) or a surrogate splitter (criteria which maximizes the probability of making the same decision at the node as the primary splitter; Breiman *et al.*, 1984). The values of all these improvements are summed over each node, and are then scaled relative to the best performing variable. The variable with the highest sum of improvements is scored 100, and all other variables will have lower scores ranging downwards towards zero (Breiman *et al.*, 1984).

Results

DTs and predictors importance

The classification tree obtained from M1 of *I. argentinus* females comprised four splits and five terminal nodes and was constructed using the variables NiW and OdW (Figure 2). Ranking of importance of predictors revealed NiW and OdW as the most important (Figure 3), while spawning group and HI were less important (Figure 3). The tree obtained of M2 was simpler, comprised two splits and three terminal nodes and was constructed using only NiW (Figure 2). The most important predictors were NiW and OvW, while the least important predictors were spawning group and HI (Figure 3).

For *I. argentinus* males, the tree obtained from M1 was made up of four splits and five terminal nodes and the variables used were TeW and SCoW (Figure 2). The tree obtained from M2 was simpler, comprised two splits and three terminal nodes, and was constructed using only SCoW (Figure 2). In both tree analyses, the most important predictor was SCoW followed by TeW and MI, while spawning group, DML and HI were the least important predictors (Figure 3).

For *E. megalocyathus*, the classification trees obtained from M1 and M2 for females are made up of two splits and three terminal nodes and were constructed using the variables OdW and MI (Figure 4). In both cases, the most important predictors were OdW and OvW followed by MI (Figure 5), while the least important was HI (Figure 5). For males, the tree obtained from M1 comprises two splits and three terminal nodes and the variables used were TOW and SCoW (Figure 4). The tree obtained from M2 was simpler, made up of one split and two terminal nodes and was constructed using only TOW (Figure 4). In both cases, the most important predictor was TOW followed by SCoW and TeW, while the least important was HI (Figure 5).

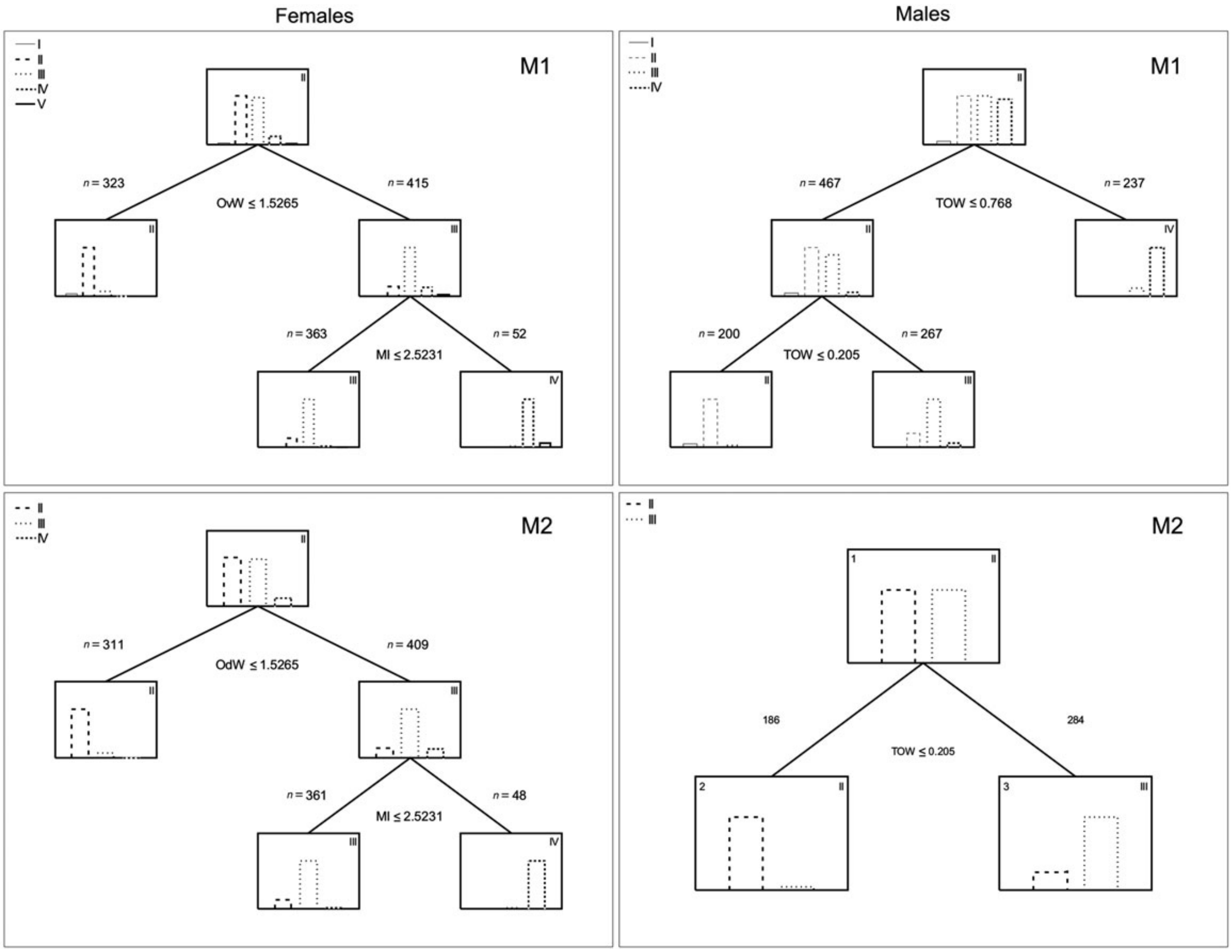

Figure 4. DT diagrams for males and females of *E. megalocyathus*. M1: model that considers all maturity stages and M2: model that considers only intermediate maturity stages. OdW, oviduct weight; MI, maturity index; TOW, terminal organ weight.

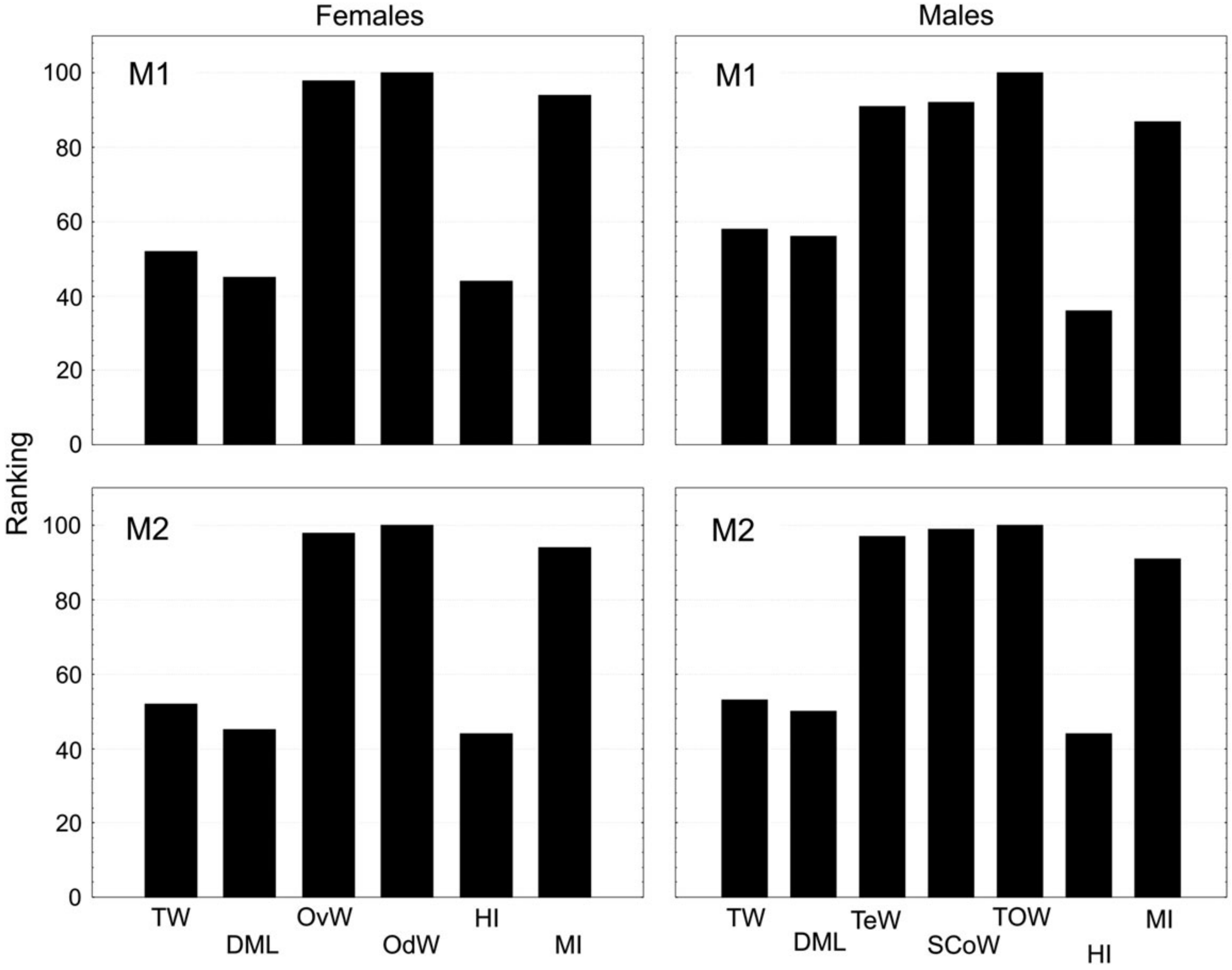

Figure 5. Ranking of importance of measurements and indices (predictors) for males and females of *E. megalocyathus*. M1: model that considers all maturity stages and M2: model that considers only intermediate maturity stages. DML, dorsal mantle length; TW, total weight; OvW, ovary weight; OdW, oviductal weight; TeW, testis weight; SCoW, spermatophoric complex weight; TOW, terminal organ weight; HI, Hayashi index; MI, maturity index.

Overall classification accuracies and individual class accuracies

The accuracies of tree models were better when only intermediate maturity stages were classified (Table 2). For *I. argentinus*, misclassification rates of the learning dataset of M1 were equal for males and females, but misclassification rate of the test dataset was lower in males than in females (Table 2). When maturity stages I and V were not considered (M2), misclassification rates of both learning and test datasets decreased for both sexes (Table 2). For *E. megalocyathus*, misclassification rates were lower than in *I. argentinus*.

In addition to considering overall classification accuracies, we examined the classification performance of each model with respect to each maturity stage. For *I. argentinus*, a consistent pattern of relative classification accuracies for each of the classes or maturity stages was observed in each model. The highest and lowest maturity stages had the best classification accuracies (Table 3). Maturity stage III had the lowest classification accuracy for both sexes, except M1 for males for which stage IV had the lowest accuracy (Table 3). When the extreme maturity stages (stages I and V in both sexes) were not considered in the analysis (M2), classification accuracies for each class increased markedly compared with the model that considered all maturity stages (M1; Table 3).

Table 2. Misclassification rates of learning and test (in parentheses) datasets for males and females of *I. argentinus* and *E. megalocyathus*.

	Enteroctopus megalocyathus		***Illex argentinus***	
Models	**Males**	**Females**	**Males**	**Females**
M1	18 (20)	16 (19)	23 (24)	23 (26)
M2	13 (15)	13 (17)	21 (22)	19 (21)

For *E. megalocyathus*, the maturity stage I had the lowest classification accuracy in the model that considered all maturity stages (M1; Table 3). When extreme values of maturity stages (stages I and IV for males and stages I and V for females) were not considered in the analysis (M2) classification accuracies of each class increased markedly compared with M1 (Table 3). Maturity stage III had the highest classification accuracy of all cases, except for males in M1 (Table 3).

Table 3. Average misclassification (expressed in percentage) of each maturity condition for both sexes of *I. argentinus* and *E. megalocyathus* derived from the tenfold cross-validation analysis.

		Males		Females	
	Macroscopic maturity stages	**Model I**	**Model II**	**Model I**	**Model II**
Illex argentinus	I	6.7	–	18.4	–
	II	35.4	16.2	29.9	16.3
	III	71.8	61.1	39.6	35.2
	IV	80.7	1.7	20.9	1.9
	V	1.9	–	14.5	–
Enteroctopus megalocyathus	I	71.4	–	100	–
	II	21.3	19.6	16.8	16.8
	III	18.6	4.2	9.2	9.2
	IV	7.8	–	14.5	14.5
	V	–	–	54.5	–

M1, DT model including all maturity stages; M2, DT model excluding extreme classes of maturity conditions. Maturity stage V is not defined for males of *E. megalocyathus.*

Discussion

Determining the maturity condition of cephalopods is crucial for population assessment and management, but this task is often difficult to conduct in practice. The present study provides simple criteria for classifying individuals into maturity stage based on gravimetric variables. DT analysis has been used successfully to determine decision criteria for classification of large datasets in ecological studies (De'ath and Fabricius, 2000; Vayssières *et al.,* 2000; De'ath, 2002), but has not been used frequently in cephalopod studies or in reproductive studies to date. This method provides practical advantages when it is applied in cephalopod studies. Outcomes of such analysis are simple to interpret and allow for the easy implementation of results in assessment and management of fishery resources. The most relevant variables as revealed by DT analysis are selected among all variables considered for their efficacy in reducing classification errors and variables making little or no contribution to classification success are not used. Moreover, this method allows for the management of missing values in predictor variables by developing splitting rules based on alternative measurements (derived from the ranking predictor analysis) that exhibit strong concordance with the primary splitting variable at any given point on the tree. Another important property of DT analysis is that the method is robust to outliers since they rarely define split points that correctly classify a significant number of cases. This is particularly important in cephalopods since the intrinsic plasticity of individuals (Moltschaniwskyj, 2004) can lead to the existence of extreme observations that may cause biases in most statistical analyses. Lastly, DT analysis provides a known classification error which is useful for assessment and management of cephalopod populations. This error can be taken into account either into assessment models that need estimations of spawning biomass such as stock–recruitment models or in the determination of reproductive seasons or areas based on the observation of the proportion of mature individuals to establish management strategies such as closures. While this approach cannot replace histological analysis and macroscopic maturity scales in reproductive studies, it can provide a classification key based on robust and simple criteria for assessing the maturity condition of a large number of individuals with a relatively low effort and a low observer training.

In most of the shelf species of cuttlefish, squid, and octopus, the enlargement of the ovary and of oviductal and nidamental glands in females and the enlargement of spermatophoric complex and the testis in males marks the beginning of sexual maturation (Boyle and Rodhouse, 2005). Thus, it is expected that the most relevant variables for classifying individuals into the maturity stages would be restricted to the measurements of different parts of the reproductive system. For *I. argentinus*, when all maturity stages were considered, two variables were enough to classify individuals into the maturity stages with an overall misclassification percentage lower than 25%. In females, the most relevant variables were the weight of oviducts and nidamental glands, while in males, the most relevant variables were testis and spermatophoric weights. When only intermediate maturity stages were considered, only one variable was enough to classify individuals and the percentage of misclassification was reduced to <21%. In this case, the nidamental gland weight and the spermatophoric complex weight were the most relevant variables in females and males, respectively. These results agree with the changes in the reproductive system of *I. argentinus* described in the macroscopic maturity scales (Brunetti, 1990). It has been pointed out that the nidamental gland changes markedly through maturation (reaching up to 7% of body weight, Boyle and Rodhouse, 2005) and the development of this gland is highly related to ovary development (Schuldt, 1979; Brunetti, 1990). Therefore, weight variation of this structure is an excellent indicator of the maturity stage of *I. argentinus* females. For males, the spermatophoric complex is a rather complex structure because of the number of accessory glands involved in spermatophore formation. Several changes occur in this structure throughout the maturity process. Accessory glands are barely visible in maturity stage I, and they gradually differentiate and increase in size as maturation progresses reaching a large structure with fully developed accessory glands and an enlarged Needham sac filled with spermatophores (Brunetti, 1990). Between stages III and IV, when the Needham sac begins to store spermatophores, the weight of the spermatophoric complex varies notably leading to high misclassification error (Table 3). This intrinsic variability has already been pointed out (Brunetti, 1990). In the present study, maturity stages VI and VII of males and stage VIII of females were not represented in samples. These stages are particularly hard to obtain due to the difficulties in catching them. However, individuals in such an advanced maturity condition are simple to recognize due to notable deterioration of the body and the reproductive system (Brunetti, 1990). It is worth drawing attention to the fact that spawning group was not important for classifying individuals into maturity stages, which

Table 4. Macroscopic maturity stages, and developmental phases of *E. megalocyathus* (from Ortiz *et al.*, 2011; Ortiz, 2013) and proposed criteria resulting from DT analysis.

	Maturity stage	**Macroscopic characteristics**	**Developmental phases of the reproductive system**	**Proposed criteria**
Males of *E. megalocyathus*	I Virginal	Difficult to determine sex and recognize the terminal organ to the eye naked. Hard to differentiate genital bag from the rest of the internal organs.		Same criteria used in the macroscopic characteristics
	II Immature	White testicle bigger than spermatophoric complex. Terminal organ easy to recognize. Well-defined reproductive system. Genital bag of smaller-size appearance than the rest of visceral mass	Physiologically and functionally immature	Terminal organ weight lower than 0.205 g
	III Advanced maturity	Large, yellowish testicle, of bigger size appearance than spermatophoric complex. Ducts of the spermatophoric complex slightly swollen with shreds of spermatophores in formation inside. Eventually, small fragments of an outer tunic without inner content can be found in the spermatophoric sac. The reproductive system has a similar size than the rest of the viscera	Physiologically mature and functionally immature	Terminal organ weight higher than 0.205 g and lower than 0.768 g
	IV Mature-spawning	Turgescent spermatophoric complex of a size appearance similar or bigger than testicle with wide duct lumen. Mature spermatophores stored in the spermatophoric sac and/or the terminal organ. Maximum-sized reproductive system (often bigger than the rest of visceral mass)	Physiologically and functionally mature	Same criteria used in the macroscopic characteristics
Females of *E. megalocyathus*	I Virginal	Difficult to determine sex to the eye naked. Hard to differentiate reproductive system from the rest of the internal organs		Same criteria used in the macroscopic characteristics
	II Immature	Smaller, white ovary filled with liquid. The reproductive system differentiates itself from the rest of the internal organs, but its size is still small compared with them. Distal oviducts narrow with respect to oviducal glands. White oviducal glands. Proximal oviducts are distended and easily visualized		Ovary weight lower than 1.5 g
	III Juvenile	Medium-sized ovary and from white to ivory in colour. Ivory oviducal glands. Distal oviducts slightly swollen. Proximal oviducts not easily visualized. A few, small and longitudinally striped oocytes become evident through the ovary wall. Slightly distinguishable rings in the proximal oviducts	Functionally and physiologically immature	Ovary weight higher than 1.5 g and lower than 20.5 g
	IV Advance maturity	Maximum-sized, ivory ovary of a firm consistence. Reproductive system of similar size to the rest of the visceral mass. Oviducal glands with two distinct bands: one narrow and ivory band and one broad and dark band. Distal oviducts very swollen, widened and filled with liquid. Medium-sized oocytes, with longitudinally striped appearance recognizable		Ovary weight higher than 20.5 g

Continued

Table 4. *Continued*

Maturity stage	Macroscopic characteristics	Developmental phases of the reproductive system	Proposed criteria
	through ovary wall. Ring-like structures in the proximal oviducts clearly distinguishable		
V Mature-spawning	Pink ovary lacking in firmness. Oviducal glands with ivory and dark bands. Unexpanded distal oviducts. Mature (smooth) oocytes in the proximal oviducts and free in the ovary lumen	Functionally and physiologically mature	Same criteria used in the macroscopic characteristics
VI Spent	Pink and flaccid ovary, with a few or no eggs inside. Post-ovulatory follicles distinguishable through the ovary wall. Thick and unexpanded distal oviducts. Widened proximal oviducts	Spent	Same criteria used in the macroscopic characteristics

indicates that this model can be used for *I. argentinus* in general, thus eliminating the need for the determination of spawning group for each individual.

For *E. megalocyathus*, the most relevant variables for classifying individuals in the maturity stages were also associated with measurements of different parts of the reproductive system, except in females where the maturity index was also important for separating stages III and IV in M1 and M2. However, according to the ranking of importance of predictors (Figure 5), MI in females can be replaced by ovary weight (20.5 g is the criteria for OvW) without modifying the tree structure (to separate between maturity stages III and IV in both models) with a misclassification percentage of 18%. In fact, the ovary only shows noticeable macroscopic differences (mainly in weight) between stages III and IV. From stages III to IV, ovary weight increases tenfold (Table 1) due to oocyte growth produced by vitellogenesis (Ortiz, 2013). Distal oviducts change macroscopically throughout maturation from nearly recognizable at stage I to slightly swollen at stage III. This could explain the incorporation of OdW as a classification variable among stages I, II, and III. It should be note that stage VI of females was not considered in the sample. However, as in *I. argentinus*, spent females are easy to recognize due to notable deterioration of the reproductive system (Ortiz, 2013). For males, terminal organ weight was a key variable for classification among all stages. The terminal organ in *E. megalocyathus* is involved in spermatophore storage and transfer and is a conspicuous sexual character that is easy to identify during dissections. The relative importance of the size of this organ in this species has been pointed out by Ré (1980) who noted that the penis is short, but diverticulum is extremely long leading to a large terminal organ that reaches up to 99% of the mantle length in mature animals. Therefore, the relevant variables for categorizing males that were selected by the tree analysis also involve the weights of structures that undergo noticeable change during the maturation process.

The HI is frequently used to determine maturity scales in octopus since each maturity stage is characterized by a range of HI (Guerra, 1975; Pujals, 1986; Grubert and Wadley, 2000; Otero *et al*, 2007). However, in practice, some problems arise. In our study, we observed an important overlap in HI index among maturity stages of *E. megalocyathus*, particularly at extremes of the scales. In immature animals, the reproductive system is underdeveloped and hardly recognizable by the naked eye. At this stage, the weight of the gonad and accessory glands is similar leading to HI values near 0.5 (Table 1). As maturation progresses, the weight of the gonad and accessory glands increases differentially leading to a high variability in HI values and low importance as a predictor in DT analysis. Therefore, based on our results, HI would not be an appropriate indicator of maturity stage of *E. megalocyathus*.

In both sexes of these species, a notable reduction in the misclassification percentage was observed when considering only intermediate stages ($<15\%$) compared with the case when all the maturity stages were considered ($<20\%$). Thus, eliminating easily recognizable stages such as virginal or fully mature leads to a simpler decision model with a better classification percentage since a smaller number of cases are classified into fewer maturity classes. In this sense, a simple guide that combines macroscopic criteria (for determining extreme stages of maturity scales) and DT criteria (for determining intermediate maturity stages) can be developed for assigning maturity stages of individuals.

Based on the results obtained in the present study, we propose new criteria for classifying individuals of *E. megalocyathus* and

Table 5. Macroscopic maturity stages, and developmental phases of *I. argentinus* (from Brunetti, 1990; Arkhipkin, 1992; Laptikhovsky and Nigmatullin, 1993) and proposed criteria resulting from DT analysis.

	Maturity stages	**Macroscopic characteristics**	**Developmental phases of the reproductive system**	**Proposed criteria**
Males of *I. argentinus*	I Virginal	The prostatic gland and the testis are transparent and easily recognizable	Immature	Testis weight lower than 0.4 g
	II Immature	The prostatic gland is transparent and Needham sac is evident in the posterior parte. The testis is transparent and its size reaches the prostatic gland		Testis weight higher than 0.4 g and spermatophoric complex weight lower than 0.7 g
	III Incipient maturity	The prostatic gland is whitish. The testis is triangular and of white colour with transparent borders. The anterior part of the testis is trilobulated and is thicker. The Needham sac starts to increase on its anterior part and small white filaments are present inside. The deferent conduct is evident by its white colour	Physiological maturation	Spermatophoric complex weight higher than 0.7 g and lower than 1 g
	IV Advance maturity	The testis is thick and white with 3–4 anterior lobules. The Needham sac is completely developed with several spermatophores in formation inside. The deferent conduct is thick and white with marked constrictions along it. The hectocotilization reaches 35–45%	Physiological maturity	Spermatophoric complex weight higher than 1 g and lower than 1.8 g
	V Mature	The testis is white and thinner with flaccid borders. The Needham sac is fully developed and spermatophores are completely developed. The hectocotilization reaches more than 45%	Functional maturation	Spermatophoric complex weight higher than 1.8 g
	VI Mature (copulating)	The testis is softer and whitish/greyish. The Needham sac is enlarge and part of its content is missing. Loosen spermatophores can be seen in the paleal cavity. The hectocotilization reaches more than 50%	Functional maturity	Same criteria used in the macroscopic characteristics
	VII Spent	The testis is whitish/greyish and its volume is significantly smaller. The Needham sac is almost empty with few spermatophores. The muscle of the mantle is notably deteriorated		Same criteria used in the macroscopic characteristics
Females of *I. argentinus*	I Virginal	Nidamental glands are thin and transparent. The ovary is transparent and is only visible the muscular axis	Immature	Nidamental gland weight lower than 0.1 g
	II Immature	Nidamental glands are thin and transparent. On its posterior region, the oviductal glands are visible, but are small and transparent. Oviducts are visible on the anterior region. The ovary is transparent with granules on the muscular axis		Nidamental gland weight higher than 0.1 g and lower than 1.4 g
	III Incipient maturity	Nidamental glands are enlarged, white and with transparent borders. Oviducts are plane and transparent through their length. Oviductal glands are white. The ovary is whitish and enlarged on its anterior region with more granules on the muscular axis	Physiological maturation	Nidamental gland weight higher than 1.4 g and lower than 7.8 g
	IV Advance maturity	Nidamental glands are white, wider, and thicker with a visible groove on the anterior region. Oviducts are plane with internal folds and without oocytes in them. The ovary is large and yellowish with large oocytes on the surface	Physiological maturity	Nidamental gland weight higher than 7.8 g and oviductal weight lower than 6.3 g
	V Mature	Nidamental glands are white and thick covering all the digestive gland. Oviducts are green and filled with oocytes. The ovary is large and yellowish	Functional maturation	Nidamental gland weight higher than 7.8 g and oviductal weight higher than 6.3 g
	VI Mature (fertilized)	Nidamental glands are white and thick covering all the digestive gland. Oviducts are green and filled with oocytes. The ovary is large and yellowish. Individuals are fertilized and spermatophores are evident based on gills	Functional maturity	Same criteria used in the macroscopic characteristics

Continued

Table 5. *Continued*

Maturity stages	Macroscopic characteristics	Developmental phases of the reproductive system	Proposed criteria
VII Mature (spawning)	Nidamental and oviductal glands are white. Oviducts are enlarged and empty with remaining oocytes. The ovary has decreased in size and still has few mature oocytes		Same criteria used in the macroscopic characteristics

I. argentinus into intermediate maturity stages that complement criteria suggested in previous studies (Nigmatullin, 1989; Brunetti, 1990; Ortiz *et al.*, 2011). For these species, DT analysis applied to conventional measures of reproductive systems proved to be a useful tool to develop new and simple criteria for the classification of maturity stages, with known misclassification error, that is easy to use in the field and independent of observer training (Tables 4 and 5). Moreover, the present approach could be easily applied to classify other species using maturity scales and existing biological data.

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