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Seasonal patterns of investment in reproductive and somatic tissues in the squid *Loligo forbesi*

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Abstract – Despite many previous studies on the life history of the squid *Loligo forbesi*, the roles of internal and external factors in growth and maturation have not previously been investigated in detail. The present study takes advantage of the availability of statistical techniques, namely generalised additive models, which permit simultaneous evaluation of the effects of multiple explanatory variables while avoiding the need to assume linear relationships. This has the further advantage that the size of body parts can be entered directly into the models rather than expressed as indices (e.g., gonado-somatic index). The present analysis is based on measurements taken from monthly market samples during 1989-1991 and aims to infer patterns of reproductive and somatic investment though comparison of large numbers of individuals at different (post recruit) life-cycle stages. Results indicate that, once the effect of body size is removed, gonad weight in both sexes is affected by month and digestive gland weight, consistent with seasonal triggering of maturation, and with energy for gonad growth being derived from food. There is also evidence of a negative relationship between somatic and ovary growth in females, possibly indicating remobilisation of somatic tissue to grow the ovary. In males, relationships between variables tended to have more complex forms, probably related to the existence of two or more growth patterns leading to different sizes at maturity. As found in previous work, maturation begins around 1−2 months earlier in males, suggesting that sensitivity to external triggers is controlled by a sex-dependent internal factor.

Key words: Life-history / Maturation / Generalised additive models / Squid / *Loligo forbesi*

Résumé – Tendances saisonnières de l'investissement reproductif et somatique chez le calmar, *Loligo forbesi*. La vie du calmar *Loligo forbesi* a déjà fait l'objet de nombreuses études, cependant l'action des facteurs internes et externes sur la croissance et la maturation n'avait jusqu'à présent pas été analysée en détail. Cette étude s'appuie sur une méthode statistique, en l'occurrence les modèles additifs généralisés, qui permet d'évaluer les effets de multiples variables explicatives, tout en évitant d'avoir à faire l'hypothèse de relations linéaires. Cette méthode possède l'avantage de pouvoir intégrer directement la taille des organes dans les modèles, plutôt que d'y faire référence à travers des indices (comme l'indice gonado-somatique ou le rapport poids de la glande digestive / poids du corps). L'analyse, présentée ici, repose sur des mensurations faites durant la période 1989-1991 et vise à mettre en évidence divers types d'investissement reproductif, à travers l'analyse d'un grand nombre d'individus recrutés et observés à des stades différents parmi les recrues.

1 Introduction

In common with many squid species, *Loligo forbesi* (Cephalopoda: Loliginidae) has an annual life-cycle and is semelparous (Lum-Kong et al. 1992; Pierce et al. 1994a; Collins et al. 1995a). Rocha et al. (2001) defined its reproductive mode as "intermittent, terminal spawning", in which egg-laying occurs in batches, there is no post-spawning regeneration of the gonad and the animal dies shortly after the completion of spawning.

Because of their short life span and rapid growth rates, cephalopod growth and abundance are thought to be especially sensitive to changes in environmental conditions such as seawater temperature (Rodhouse et al. 1992; Boyle and Pierce 1994; Waluda and Pierce 1998; Waluda et al. 1999; Pierce et al. 2004b). Previous research has suggested that environmental temperature can influence somatic growth rates and final body size, both in captivity and in the natural environment, with accelerated growth rates observed with increasing water temperatures (Jackson et al. 1997; Forsythe et al. 2001; Jackson and Moltschaniwskyj 2002; Forsythe 2004).

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Both intrinsic and extrinsic factors are thought to be involved in control of the maturation process in cephalopods: at some point during the growth process the optic gland becomes sensitive to light, after which long day lengths can inhibit maturation. High temperatures can result in rapid completion of the life cycle and smaller adult size, while good food supplies may delay spawning and lead to larger adult size. The precise role of each of these factors differs between species (Mangold 1987).

In *L. forbesi*, although maturation and spawning display a clear seasonal (winter) peak in Scottish (UK) waters, the roles and relative importance of intrinsic (e.g., body size, nutritional state) and extrinsic (e.g., day length, temperature) factors in controlling the onset of maturation have not previously been investigated.

In common with many loliginid squid, there are at least two different size-at-maturity classes in male *L. forbesi* throughout its range (Guerra and Rocha 1994; Moreno et al. 1994; Porteiro and Martins 1994; Collins et al. 1995b; Boyle et al. 1995), most likely related to alternative mating strategies: by analogy with other loliginids, large males probably guard females at the spawning grounds while the small males are "sneakers" (Hanlon and Messenger 1992). Regardless of the selective value, this indicates that there is probably no single body size at which the onset of maturation occurs in males. Indeed, Collins et al. (1999) provide evidence of three or more size modes at maturity in males and at least two in female *L. forbesi*, and considerable variability in size-at-age has also been noted for other loliginid spp. (Semmens and Moltschaniwskyj 2000).

Collins et al. (1995c) reported a relative decline in head and mantle mass associated with maturation in both male and female *L. forbesi* specimens collected from Irish waters, having removed the linear effect of body length. Ho et al. (2004) found a decline in mantle muscle instantaneous growth rates with increasing gonad size in females, suggesting that energy previously used for somatic growth was being directed towards reproductive growth. Similar conclusions have been drawn with regard to the squid *Loligo gahi* (Guerra and Castro 1994). However, size effects may be non-linear and the question remains as to whether there is mobilization of somatic tissue or simply rechanneling of ingested energy into growing gonads. In the case the ommastrephid squid *Illex argentinus*, Hatfield et al. (1992) argued that, although mantle mass of mature female squid declined relative to mantle length, nutrient reserves for maturation were derived from food rather than from somatic tissue. Additional difficulty arrises when interpreting body weight in mature males, as a result of the process of continuous production and release of spermatophores during the life stages of maturity and mating.

Previous research on the life-cycle of *L. forbesi* has normally described the maturation process using the gonadosomatic index (GSI) (Porteiro and Martins 1994; Rocha and Guerra 1994, 1995; Boyle et al. 1995; Collins et al. 1995c). Similarly, the digestive gland index has often been used as a proxy for nutritional state in cephalopods, as the weight of this organ reflects the intensity of recent feeding (e.g., Houlihan et al. 1998). However, the use of index functions to describe the growth of organs, because such indices are ratios, may mask underlying trends in the sizes of different body components, particularly if there is any allometric growth (Thorpe 1976) or non-linear growth. Recently developed univariate smoothing techniques, such as generalised additive modelling (GAM), can serve to indicate relationships between several variables in circumstances when linear regression analysis is not appropriate (i.e., when non-linear relationships between variables are evident) (Fox 2000, 2002; Hastie and Tibshirani 1990; Quinn and Keough 2002).

Ideally, relationships between (say) gonad weight, nutritional status and body length would be examined by following the growth of individual animals. However, inferences about such relationships can also be derived from population samples. Differences between male and female squid might be expected, since female *L. forbesi* grow larger gonads and accessory organs than do males (e.g., Boyle et al. 1995) and presumably therefore invest more energy in the maturation process. Furthermore, previous evidence suggests that males mature slightly earlier in the year than females (Pierce et al. 1994a).

This study uses GAMs to describe relationships between maturation (measured by gonad and accessory reproductive organ size), somatic investment (measured by mantle weight), nutritional status (measured by digestive gland weight) and season in *L. forbesi*, explicitly accounting for effects of body size by including mantle length as an explanatory variable. Thus, the questions asked are: (a) what controls the onset of maturation – season, size, nutritional state or some combination of these factors; (b) does somatic investment continue throughout life, does it stop, or is energy stored in the mantle mobilised to grow gonads; (c) how do the sexes differ in terms of the time course and cost of reproductive investment?

2 Materials and methods

2.1 Data

An existing data set representative of the squid population in Scottish waters was used for this analysis. The original database was composed of measurements taken from monthly samples of "mixed" squid (boxes containing squid unsorted by size) from the commercial market in Kinlochbervie on the west coast of Scotland, as well as additional material from commercial landings and research trawling (the latter by the Scottish Executive Environment and Rural Affairs Department (SEERAD) vessel "Scotia"). Detailed biological data were typically collected from approximately 30 males and 30 females from each sample; for further details of sample processing, see Pierce et al. (1994a), who used these data to describe the basic life history of the species. The data used for the present analysis comprised: capture date, dorsal mantle length (*DML*, mm), sex, gonad and accessory reproductive organ weight (for males: spermatophoric complex weight and testis weight; for females: ovary weight, nidamental gland weight and oviducal complex weight), mantle weight and digestive gland weight. We use the abbreviation "reproductive weight" for the combined weights of gonads and accessory reproductive organs. The full-scale monthly data collection programme ran from July 1990 to June 1991, with some additional

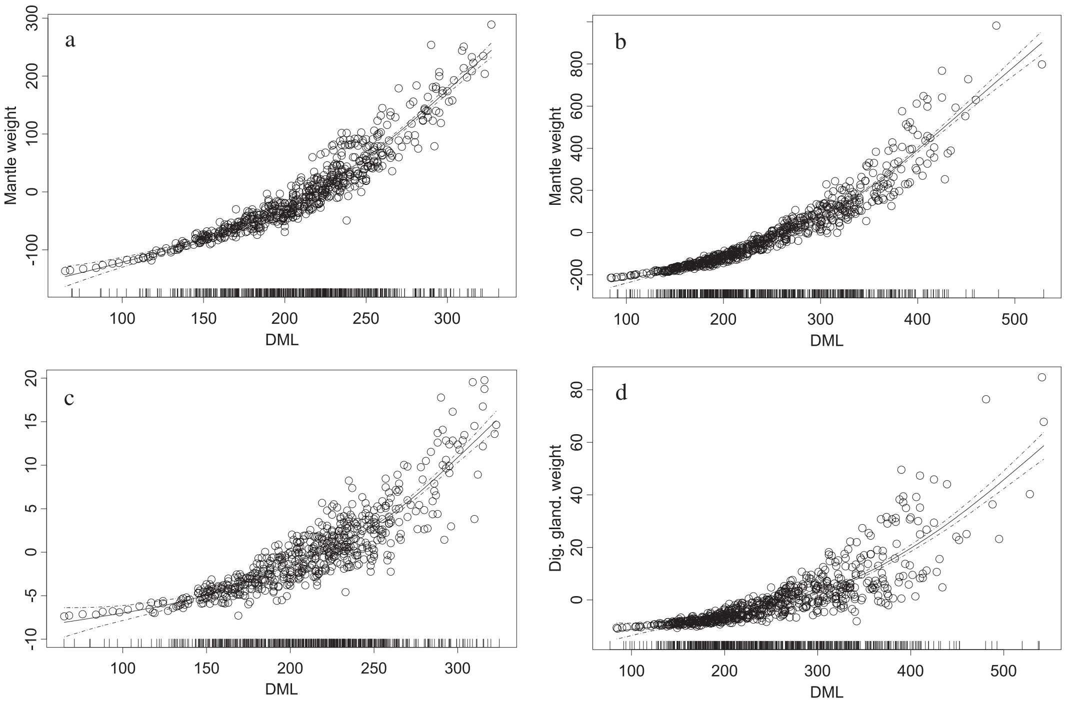

Fig. 1. Scatterplots with fitted additive model (AM) smoothing curves for the relationships between mantle weight and dorsal mantle length-*DML* in (a) females and (b) males, and between digestive gland weight and dorsal mantle length (*DML*) in (c) females and (d) males.

monthly data collected between December 1989 and October 1991. More recent sampling (to 2001, see Young et al. 2004) had more restricted objectives and involved collection of fewer measurements; thus the 1989-91 data set represents essentially the only "complete" data available for the present analysis. Since the questions asked relate to the extrinsic and intrinsic factors that normally induce maturation, the age of this data set is not considered to be an issue.

2.2 Analysis

Data were stored in an MS Access database. Data were extracted for all *L. forbesi* individuals caught in Scottish waters with measured values for all variables. A total of 1409 individuals were used for further analysis (790 males and 619 females) among samples collected from December 1989 through October 1991.

All statistical analyses were carried out using the Minitab (Ryan et al. 1985) and Brodgar v2.4.1 (`www.brodgar.com`) software packages. Data were analysed separately for males and females. Bivariate scatter plots and dotplots (Cleveland 1993) and regression analyses were used to identify outliers. Suspect points were tested against established length-weight relationships and four specimens (representing 0.28% of the data set used) with recorded values considered to be implausible (and therefore most likely representing errors in the data recording process) were discarded from the data set prior to further statistical analysis.

To quantify factors affecting reproductive investment, two alternative response variables were used, gonad weight and "reproductive weight" (as defined above). Putative explanatory variables were month, mantle length, mantle weight and digestive gland weight. In an attempt to obtain additional insights into investment in somatic tissue we also fitted models using mantle weight as the response variable and gonad weight included amongst the explanatory variables. Examination of the scatter of data (e.g., increasing variance with increasing means) and diagnostic plots indicated that it was reasonable to assume a Poisson distribution for the response variables. As the models were found to be over-dispersed, a quasi-Poisson distribution was ultimately used.

Preliminary analysis indicated strong co-linearity between mantle weight, digestive gland weight and mantle length. Therefore, prior to fitting GAMs to gonad and reproductive weights, we removed the effect of mantle length on these variables i.e., we effectively used size-adjusted values for these variables, with the size-adjusted variable taking positive values when weight was higher than expected for a given body length. Scatterplots indicated that it was reasonable to assume a normal (Gaussian) distribution for the response variable but that relationships were non-linear. Additive models (AM) were therefore fitted to the mantle weight–mantle length and digestive gland weight–mantle length relationships (see Fig. 1), and residuals from both AMs subsequently used in place of the original values for mantle weight and digestive gland weight. The residuals were uncorrelated with the other explanatory variables. Similarly, due to high co-linearity, prior to fitting GAMs to mantle weight data for males (but not for females), it was found to be necessary to derive residuals for the gonad weight – mantle length relationship (see Fig. 2).

Finding the optimal GAM model involves two aspects, namely the selection of explanatory variables and the degrees of freedom of the smoother for each explanatory variable. The variable month has only 12 values, and to avoid

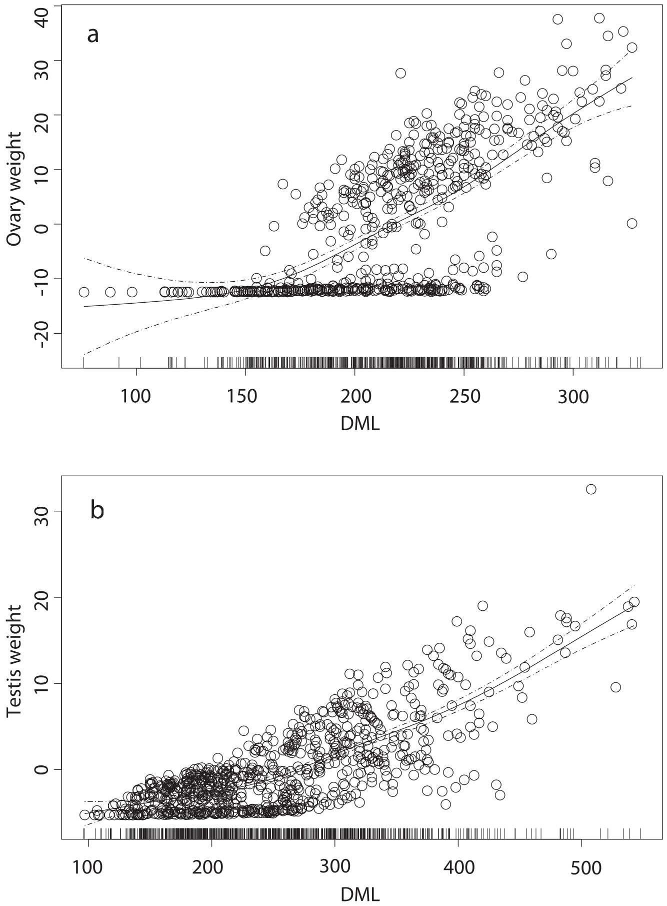

Fig. 2. Scatterplots with fitted AM smoothing curves for the relationships between (a) ovary weight versus *DML* in females (in this case, the smoother was not used in further analysis (see text)) and (b) testis weight versus *DML* in males.

over-smoothing we decided to use 4 degrees of freedom for this term. The optimal degrees of freedom for the other smoothers were found by using cross-validation (Wood 2000, 2004; Fox 2000, 2002). In this method, each observation is left out in turn and predicted by the model. This leads to a predicted error for each observation. The optimal degrees of freedom for each smoother are then found by minimising the sum of all prediction errors. To avoid over- or under-smoothing, the results from the cross-validation method were verified by applying F tests (Fox 2002) in which deviances of two models with different degrees of freedom were compared. In most cases, the F test gave similar results. In the cases where it did not, we used the results from the F tests.

3 Results

3.1 Females

3.1.1 Ovary weight

The optimal GAM model for gonad (ovary) weight contained smoothing functions for month ($df = 4$) and *DML* ($df = 4$), and size-adjusted digestive gland weight was fitted as a parametric component (see Table 1). The smoother for the partial effect of month (Fig. 3a) indicates that the most strongly negative effect occurred in September to October and the most strongly positive effect in May. Although no straightforward interpretation is possible in terms of day length or temperature effects on maturation, the strong role of extrinsic factors is clear. The partial effect of *DML* (Fig. 3b) increases monotonically with increasing ovary weight but is non-linear, with

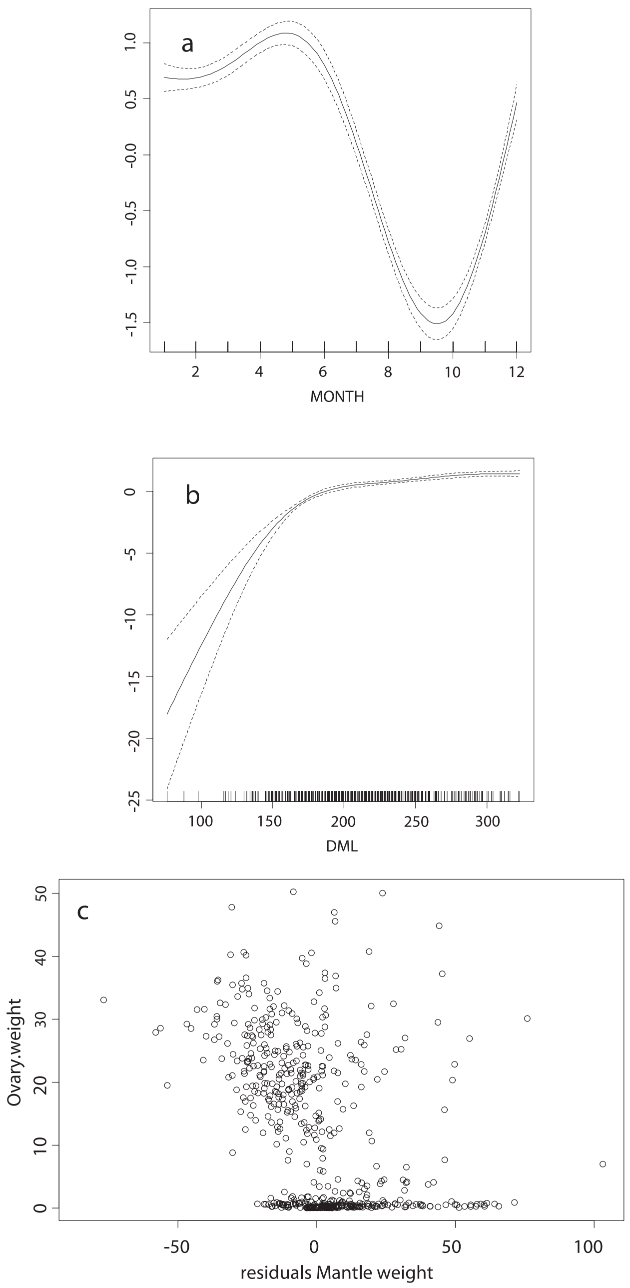

Fig. 3. GAM smoothing curves fitted to partial effects of explanatory variables on gonad (ovary) weight in females. Gonad weight is represented as a function of (a) month and (b) *DML*. Dashed lines represent 95% confidence intervals around the main effects. Also shown is (c) the scatterplot for gonad weight against residuals of mantle weight. Although there is an apparent trend, once effects of other explanatory variables were taken into account there was no significant effect.

Table 1. Summary of optimum generalised additive models. A quasi-Poisson distribution was assumed for all response variables. The explanatory variables mantle weight and digestive gland weight were always size-adjusted, i.e. we used residuals from an additive model relating them, separately, to mantle length (indicated by *). When gonad weight was used as an explanatory variable, it was size-adjusted for males (again indicated by *) but not for females. For each combination of sex and response variable, the table summarises the model fit, and the dispersion parameter, deviance explained and the AIC value. For explanatory variable fitted as parametric (linear) terms, the regression coefficient (ε) and t-value and associated probability value are given. For smoothers, degrees of freedom (*df*), Chi-squared values and associated probabilities are given. For all explanatory variables and smoothers shown, the associated probability value (p) was less than 0.001 unless specified otherwise.

Sex	Response variable	Month	DML	Mantle weight	Digestive gland weight	Gonad weight	Dispersion	Deviance Explained	AIC
Female **(*df*; χ^2)**	Gonad weight	4; 584.8	4; 412.3	-	$\varepsilon = 0.0378$ * $t = 3.89$	-	3.31	80.9%	3.06
“	Reproductive weight	4; 693.3	4; 617.0	-	$\varepsilon = 0.0213$ * $t = 2.60$ $p < 0.01$	-	6.58	84.9%	6.11
“	Mantle weight	4; 36.8	6; 4 879	-	$\varepsilon = 0.0078$ * $t = 4.17$	2; 48.0	1.34	96%	1.37
Male **(*df*; χ^2)**	Gonad weight	4; 276.6	7; 471.4	6 *; 54.7	4 *; 71.7	-	1.19	69.6%	1.34
“	Reproductive weight	4; 304.4	7; 644.1	7 *; 52.7	6 *; 62.2	-	1.2	77.7%	1.33
“	Mantle weight	4; 117	4; 11 665	-	6 *; 135	6 *; 23	2.16	98.2%	2.24

a point of inflection occurring around 170 mm *DML*, and positive effects of size seen only at *DML* > 170 mm. This suggests that attainment of a minimum body size is a prerequisite for maturation. Size-adjusted digestive gland weight had a positive linear effect on ovary weight.

Size-adjusted mantle weight did not have a significant effect on ovary weight. Visual examination of the scatterplot of ovary weight against residuals for mantle weight (Fig. 3c) suggested that high gonad weights occurred more frequently for negative residual values. However, once effects of month, *DML* and digestive gland weight were included in the model, mantle weight had no significant effect on ovary weight.

3.1.2 Reproductive (gonad + accessory reproductive gland) weight

The optimal GAM model for reproductive weight contained smoothing functions for month ($df = 4$) and *DML* ($df = 4$), and size-adjusted digestive gland weight was fitted as a parametric component (see Table 1). Almost identically with the above results for ovary weight alone, the smoother for the partial effect of month on reproductive weight (Fig. 4a) indicates that the most strongly negative effect occurred in September to October and the most strongly positive effect in May. Similarly, the partial effect of *DML* (Fig. 4b) also increases monotonically with increasing reproductive weight and is non-linear, with a point of inflection occurring around 170 mm *DML*. Positive effects of size were seen only at *DML* > 170 mm. Size-adjusted digestive gland weight had a positive linear effect on reproductive weight. Size-adjusted mantle weight did not have a significant effect on reproductive weight.

3.1.3 Mantle weight

The optimal GAM model for mantle weight contained smoothing functions for month ($df = 4$), *DML* ($df = 6$) and ovary weight ($df = 2$), and size-adjusted digestive gland weight was fitted as a parametric component (see Table 1). The smoother for the partial effect of month (Fig. 5a) indicates a seasonal effect, with the most strongly negative effect in April and the most strongly positive effect in the autumn/winter months. The partial effect of *DML* (Fig. 5b) increases almost linearly with increasing mantle weight, with positive effects of size seen at *DML* > 200 mm. The partial effect of ovary weight (Fig. 5c) on mantle weight was negative, with larger (heavier) ovaries corresponding to lower mantle weights. This suggests that weight (i.e., energy) previously invested in somatic tissues is diverted for use in sexual maturation in female squid, although an equivalent effect was not apparent in the model for ovary weight (Sect. 3.1.1 above). Size-adjusted digestive gland weight had a positive effect on mantle weight.

3.2 Males

3.2.1 Testis weight

The optimal GAM model for gonad (testis) weight contained smoothing functions for month ($df = 4$) and *DML*

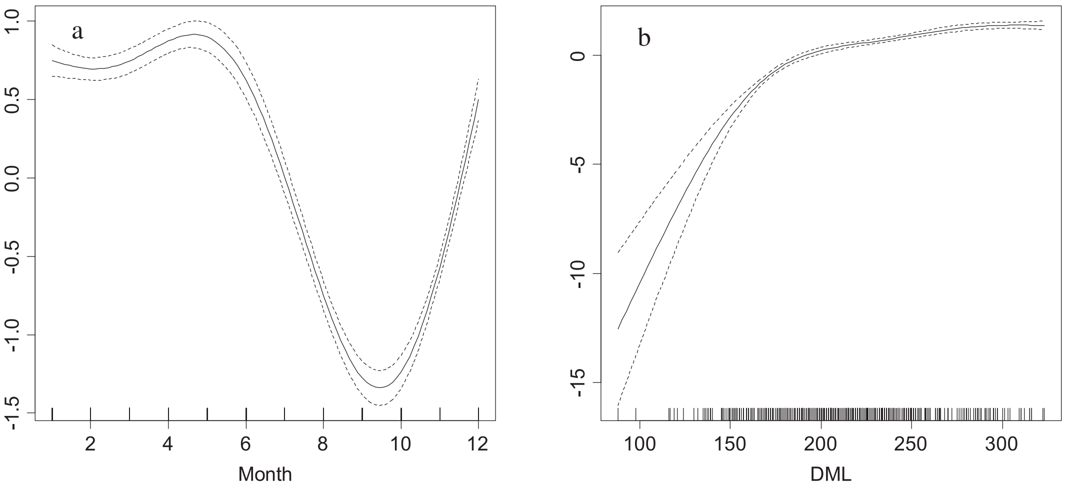

Fig. 4. GAM smoothing curves fitted to partial effects of explanatory variables on reproductive weight in females. Reproductive weight is represented as a function of (a) month and (b) *DML*. Dashed lines represent 95% confidence intervals around the main effects.

($df = 7$), and both size-adjusted mantle weight ($df = 6$) and digestive gland weight ($df = 4$) (see Table 1). The smoother for the partial effect of month (Fig. 6a) indicates that the most strongly negative effect occurred during the end of June – beginning of July and the most strongly positive effect occurred February to March. Comparing these results with those for females (Fig. 3a), the earlier onset of maturation in males than females is evident.

The shape of the partial effect of *DML* (Fig. 6b) indicates the possibility of at least two sizes at maturity (which may be related to the higher degrees of freedom required than for the equivalent relationship in females), with the first point of inflection occurring around 170 mm *DML* (as was seen for female animals) and a second occurring at around 400 mm *DML*. Comparing this with the equivalent relationship for females (Fig. 3b), which shows an asymptote at around 200 mm *DML*, it is evident that gonad growth spans a wider range of body sizes.

Size-adjusted mantle weight (Fig. 6c) had a negative effect on testis weight for both low and high mantle weights, with a positive effect at intermediate mantle weights. This form of relationship may be a function of there being multiple size-at-maturity relationships present in the population. Size-adjusted digestive gland weight had a generally positive and linear effect on gonad weight, although the relationship appears to change for the (largest) animals on the right-hand edge of the distribution (Fig. 6d).

3.2.2 Reproductive organ (gonad + accessory gland) weight

The optimal GAM model for reproductive organ weight contained smoothing functions for month ($df = 4$) and *DML* ($df = 7$) and both size-adjusted mantle weight ($df = 7$) and digestive gland weight ($df = 6$) (see Table 1). The smoothers (Fig. 7) had very similar shapes to those for testis weight, suggesting that the testis and accessory reproductive structures are grown essentially simultaneously.

3.2.3 Mantle weight

The optimal GAM model for mantle weight contained smoothing functions for month ($df = 4$) and *DML* ($df = 4$) and both size-adjusted mantle weight ($df = 6$) and digestive gland weight ($df = 6$) (see Table 1). The smoother for the partial effect of month (Fig. 8a) indicates that the most strongly negative effect occurred around April and the most strongly positive effect occurred in September to October. The partial effect of *DML* (Fig. 8b) increases monotonically with increasing mantle weight, its simplicity indicating that the basic mantle-weight-body length relationship is probably identical regardless of size-at-maturity. The effect of size-adjusted testis weight on mantle weight is complex (Fig. 8c). It could be interpreted as reflecting two sizes at maturity, with two distinct peaks in mantle weight occurring at two different testis weight ranges. The smoother for the effect of size-adjusted digestive gland weight indicates a generally positive relationship between digestive gland weight and reproductive weight (Fig. 8d).

4 Discussion

GAM models have recently been employed to quantify patterns in cephalopod abundance and maturity (Bellido et al. 2001; Pierce et al. 2005). The use of GAM in this study allowed the visualisation of the often non-linear relationships between explanatory variables and growth / maturation parameters. By avoiding the use of indices such as GSI or DGI, we argue that effects of body size are more satisfactorily accounted for, allowing the underlying relationships between somatic (mantle) growth, maturation (gonad growth) and recent feeding (digestive gland weight) to be separated.

Seasonality

As expected, gonad weight in both males and females followed a clear seasonal pattern, suggesting the presence of a seasonal trigger for maturation, with males maturing a few

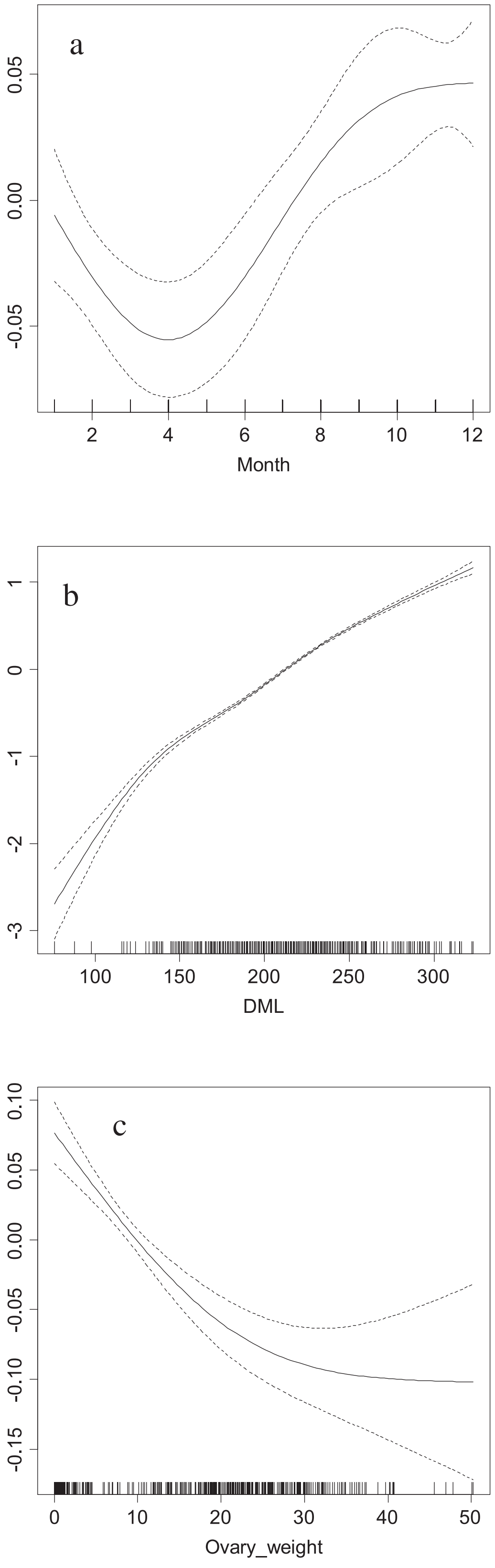

Fig. 5. GAM smoothing curves fitted to partial effects of explanatory variables on mantle (eviscerate) weight in females. Mantle weight is represented as a function of (a) month, (b) *DML* and (c) gonad weight. Dashed lines represent 95% confidence intervals around the main effects.

months earlier than females, as has been previously reported (e.g., Lum-Kong et al. 1992). This sex-related difference could arise, for example, if sensitisation of the optic gland to day length, as suggested by Mangold (1987) was triggered at different times in males and females. In any case, the sex difference points to the role of an intrinsic factor in triggering maturation.

There was, as would be expected, a seasonal pattern for mantle weight in both male and female specimens, an indication of *L. forbesi*'s seasonal growth during its one-year life cycle.

Body size

In females, gonad weight appears to reach an asymptote at around 200 mm mantle length, and this could be interpreted as indicating that mantle length continues to increase after gonad growth stops. However, in a mature individual, ovary weight will tend to decrease as eggs pass into the proximal oviduct and, ultimately, are released. Thus the asymptote in our composite picture (combined data from 619 females) may indicate the outcome of the opposing effects of larger females tending to have larger gonads and being more likely to have started spawning.

In males, gonad weight continued to increase with increasing mantle length (*DML*) up to large body sizes, which could be interpreted as indicating that growth and maturation proceed simultaneously throughout life. However, the existence of more than one size mode at maturity in males (e.g., Boyle et al. 1995; Collins et al. 1999), coupled with the presence of inflection points in the fitted relationship, suggests that the form of the relationship is a consequence of superimposing two or three asymptotic curves, and also possibly reflective of the effects of continuous production and release of spermatophores in mature animals.

Nutritional status

The generally positive relationship, in both sexes, between gonad weight and (size-adjusted) digestive gland weight points to a positive link between recent feeding and gonad growth, as would be expected if energy for gonad growth is derived primarily from feeding as suggested by Hatfield et al. (1992). It is also possible that a good nutritional state itself provides a trigger for sexual development.

The positive effect of digestive gland weight on mantle weight in both sexes suggests that increased feeding supports more rapid somatic growth at a given body size.

Work on captive cephalopod specimens has also yielded information on the effect of nutritional state on growth and maturation. However, the effect of nutrition is seen to vary among cephalopod species, with some species experiencing a decrease in feeding during the final stages of maturation (e.g., *Sepia dollfusi*, Gabr et al. 1999a), while others continue to feed at stable rates or increase their nutritional load during reproductive maturation (e.g., *Sepia pharaonis*, Gabr et al. 1999b).

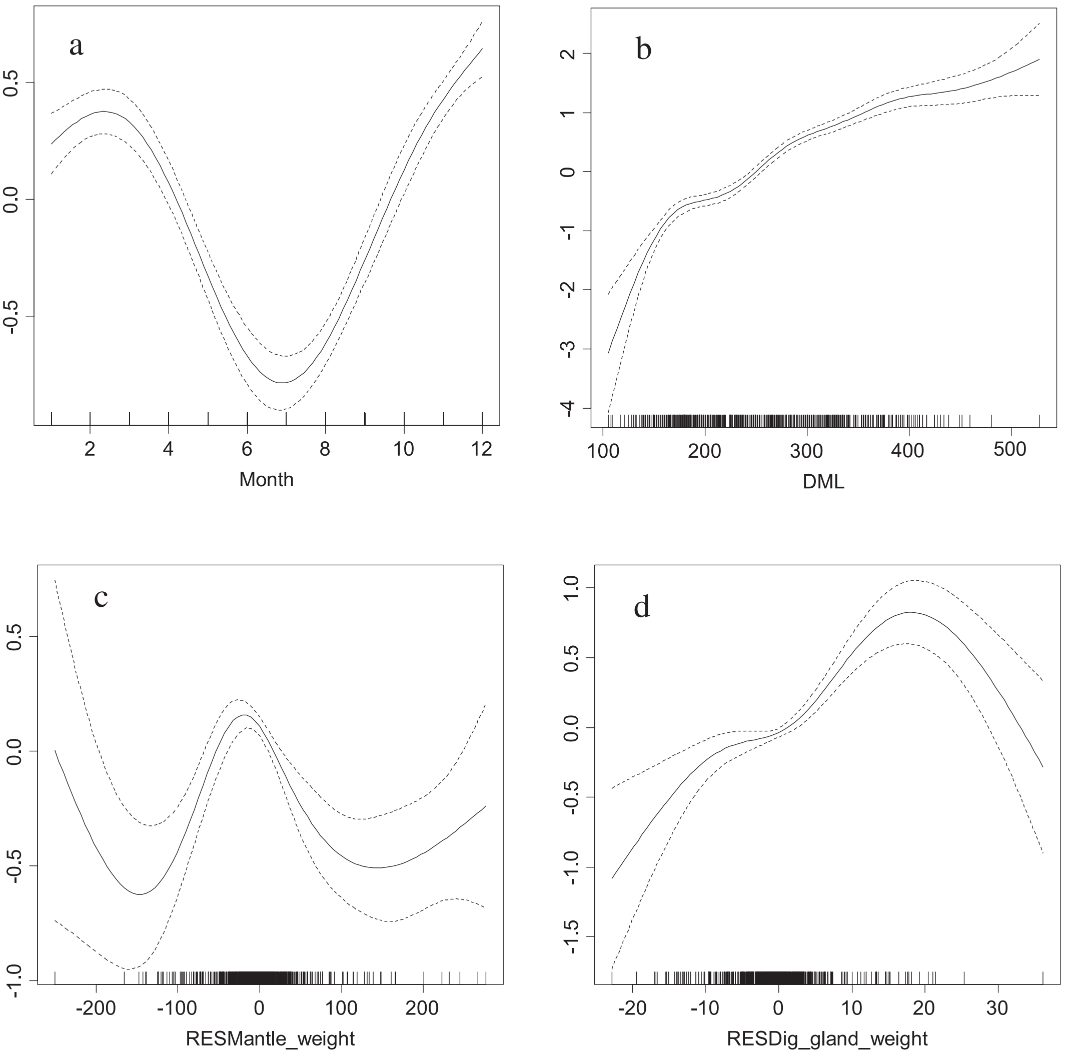

Fig. 6. GAM smoothing curves fitted to partial effects of explanatory variables on gonad (testis) weight in males. Gonad weight is represented as a function of (a) month, (b) *DML*, (c) residual of mantle weight and (d) residual of digestive gland weight. Dashed lines represent 95% confidence intervals around the main effects.

Somatic growth versus maturation

Some cephalopod species clearly exhibit decreasing somatic growth rates with increasing sexual maturation (Arkhipkin and Silvanovich 1997; Arkhipkin et al. 1998).

In the present study on *L. forbesi*, when effects of *DML*, month and nutritional state are factored out, there remained a negative effect of gonad size on mantle weight in females, with lighter mantles associated with large gonads, appearing to imply mobilisation of mantle tissue to produce ovary tissue. However, the analysis of ovary weights, conversely, revealed no effect of mantle weight in females. The relationship between mantle weight and gonad weight was apparent in both sets of analyses for males but was, however, more complex in form.

Complexities in observed relationships between variables

Regarding the complex forms seen in the relationships between gonad weight, mantle weight and digestive gland weights in males (Figs. 4 and 6), firstly trends at the (data poor) edges of the data distributions should be treated cautiously. However, the u-shaped relationship between mantle weight and testis weight, and the point of inflection in the testis weight – digestive gland weight smoothing curve, both corresponded with a high density of covariate points (sampled organism data points) along the x-axis, possibly suggesting that there is an additional embedded trend that requires further analysis to describe accurately. It seems likely that this is related to the presence of multiple size-modes at maturity but it will be necessary to follow the growth and maturation trajectories of individual squid to confirm this.

Accessory reproductive structures

Analysis of the combined "reproductive weight" data for gonad tissue and accessory reproductive organs, as compared to analysis of gonad weights alone, yielded almost identical results for females and very similar results for males. However, the inclusion of the accessory organ weights did reveal small, imbedded trends that were missing from the gonad weight

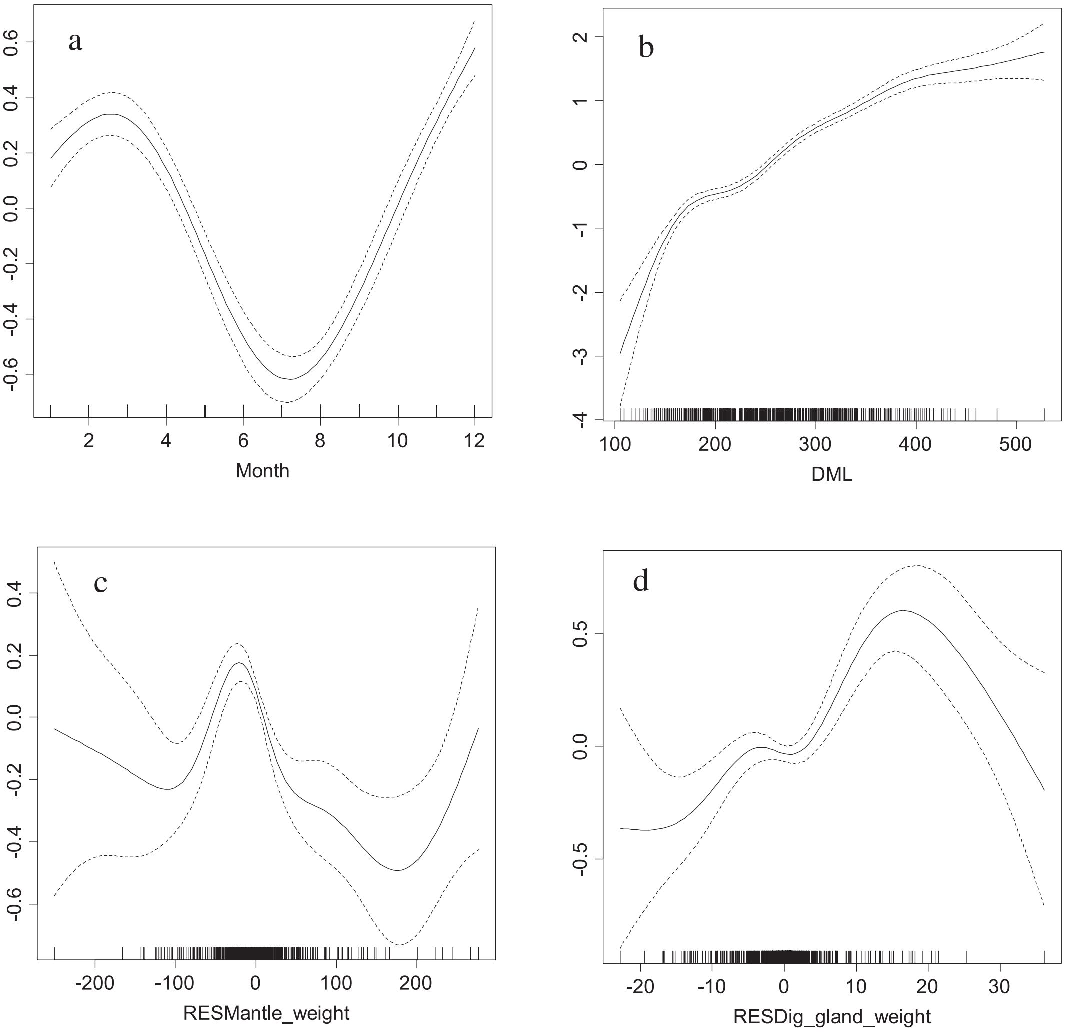

Fig. 7. GAM smoothing curves fitted to partial effects of explanatory variables on reproductive weight in males. Reproductive weight is represented as a function of (a) month, (b) *DML* and (c) residual of mantle weight. Dashed lines represent 95% confidence intervals around the main effects.

analysis alone. This shows that changes in the accessory organs can also be used to identify trends in maturation and growth in *L. forbesi*.

From populations to individuals

Caution is needed when attempting to infer individual growth patterns from population samples. In males, it has been shown that the presence of two (or more) size modes at maturity complicates the form of relationships between variables, with the population picture representing a composite of several underlying relationships. There may also be individual variation in the amount of energy invested into somatic and reproductive development.

One important explanatory variable that we were unable to examine in this analysis was age. Squid statoliths display growth increments that are thought to be daily, and this has been validated indirectly by Collins et al. (1995a), who showed that the average age of successive monthly samples increased by around 30 days. There is generally thought to be a weak link between age and length in squids, with early growth rates (related for example to ambient temperature) having marked effects on final adult size (e.g., Forsythe 2004) and life-span, however, for some loliginid species, reproductive maturation appears to be associated more with body size than with individual age (Jackson et al. 1997).

It would be beneficial if further research took into account the relative age of animals for which morphometric and reproductive data are available, to provide a more detailed analysis of the relationship between reproductive and somatic growth in *L. forbesi*, as differences in age at maturity, in relation to hatching period, have been noted for other cephalopod species (e.g., Jackson and Moltschaniwskyj 2002).

While population studies such as the present work can provide important clues, ultimately, captive rearing experiments will be needed to fully tease apart the different factors controlling somatic and reproductive investment in *Loligo forbesi.*

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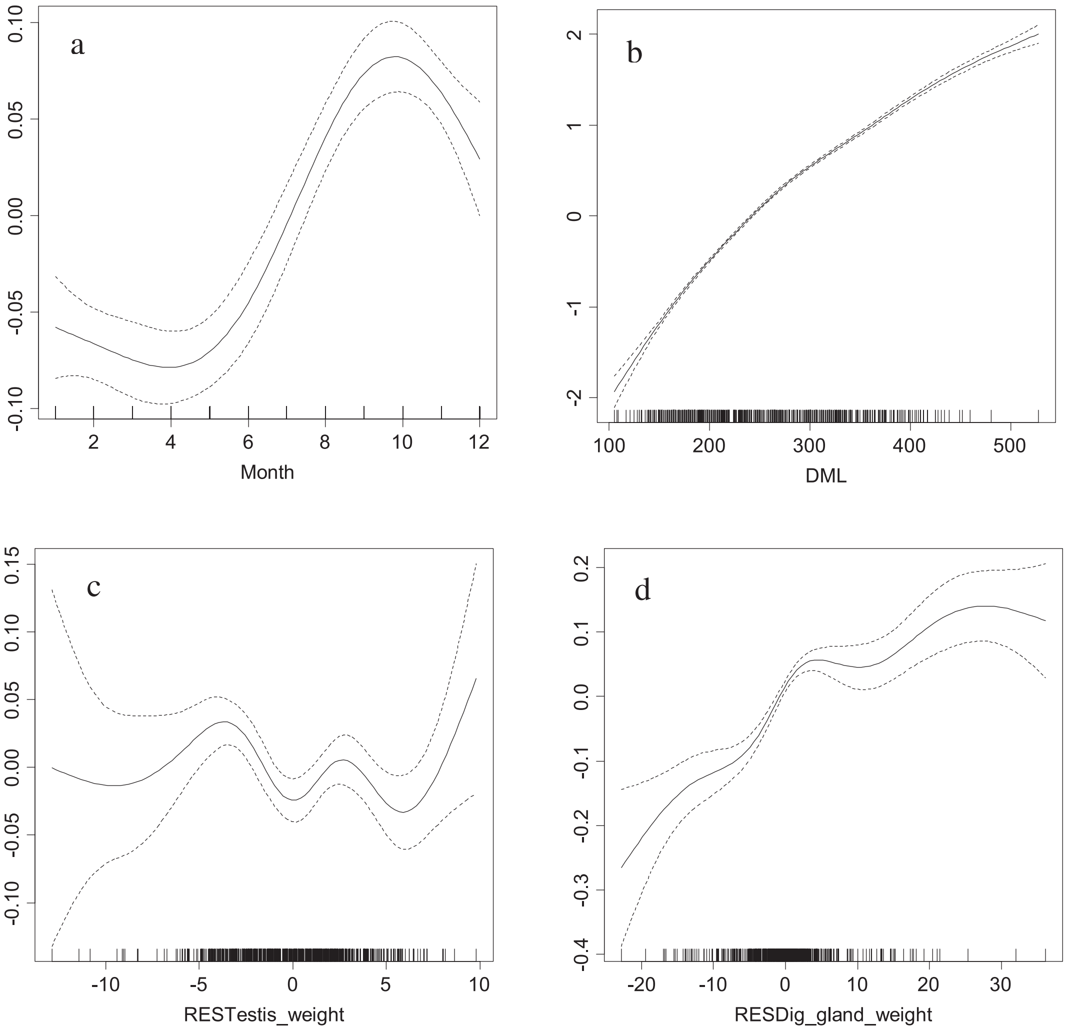

Fig. 8. GAM smoothing curves fitted to partial effects of explanatory variables on mantle (eviscerate) weight in males. Mantle weight is represented as a function of (a) month, (b) *DML*, (c) residual of gonad weight and (d) residual of digestive gland weight. Dashed lines represent 95% confidence intervals around the main effects.

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