

Optic gland enlargement and female gonad maturation in a population of the octopus *Eledone cirrhosa*: a multivariate analysis

P. R. Boyle and R. S. Thorpe

Department of Zoology, University of Aberdeen; Aberdeen AB9 2TN, Scotland

Abstract

Eleven principal component analyses were performed on morphometric data from a series of monthly samples of the octopus *Eledone cirrhosa* (Lamarck) collected from March 1978 through January 1979 in the Aberdeen (Scotland) area. This technique has allowed a partitioning of general growth effects from variation in the reproductive system. Optic gland size varies as a reproductive character together with ovary and oviducal glands. Optic gland size is not clearly associated with other cerebral organs, the brain and optic lobes. The association between the reproductive characters developed during the onset of the main period of reproductive maturation in the early summer of 1978 and persisted for the remainder of that year. This result supports the experimental evidence linking optic gland enlargement with female gonad maturation and suggests that this process occurs normally in the field population.

Introduction

Evidence that the process of gonad maturation in octopods is under the control of a substance(s) produced from the optic glands was first published by Wells and Wells (1959) for *Octopus vulgaris*. The optic glands are a pair of small bodies lying on either side of the brain with an innervation from the subpedunculate/dorsal basal area of the supraoesophageal brain mass. Sited on the optic stalk connecting the brain to the optic lobe of either side, they are spatially grouped with the cerebral tissues. The association between optic gland enlargement and female gonad maturation was revealed as a consequence of the many brain lesion experiments performed at the Zoological Station, Naples. It was noticed that in those individuals in which the experimental lesion affected the innervation of the optic glands or severed the optic tract, the female became precociously sexually mature within a few weeks (Boycott

and Young, 1956; Wells and Wells, 1959). Maturation in these octopods is essentially a process of gonad growth and associated enlargement of the oviducal glands and oviducts (Wells, 1960).

Subsequent experiments with this system in *Octopus vulgaris* have shown that the presence of the gland is essential for sexual maturity and that the secretion increases the rate of protein synthesis in the ovary for yolk production (Wells *et al.*, 1975). Male sexual maturity is also determined by optic gland secretion (Wells and Wells, 1972a). Transplants of optic gland material from one octopus to another stimulate the recipient regardless of the sex of the donor and it appears that implants from other octopods (*O. macropus*, *Eledone moschata*) are equally effective (Wells and Wells, 1975). The removal of optic glands from maturing or mature individuals causes the reproductive system to regress (Wells and Wells, 1972a, b; Wells *et al.*, 1975) and the octopod begins to feed again and grow (Wodinsky, 1977), although mating behaviour is not affected (Wells and Wells, 1972b). Clearly, the optic gland secretions have profound effects on the physiology of octopods and the system is thought to apply throughout the Coleoidea (for reviews see Wells and Wells, 1977a, b).

Apart from the general observation that the optic glands of sexually mature octopods collected from the sea appear enlarged and pigmented (Wells and Wells, 1959), there is no quantitative evidence for the operation of this system in the field or in non-experimental individuals. Additional functions for the optic glands, based mostly upon fine structural evidence, have also been proposed (Froesch and Mangold, 1976; Mangold and Froesch, 1977). The suggestion of these authors for a catabolic function of the glands introduces some uncertainty over the cause of their changed size and appearance.

Morphometric data for the octopus *Eledone cirrhosa* collected at Aberdeen in recent years provide an opportunity to test the general experimental hypothesis that there is a relationship between optic gland enlargement and gonad maturation on a field population. A bivariate

approach to the problem, for example by simply correlating optic gland size with that of the ovary, is unprofitable because in general there is broad correlation between all of the body parts and the overall size of the octopod, and with a sufficient sample size a significant correlation can be obtained between the mass of any two body parts. To overcome this difficulty we have used the multivariate technique of principal components analysis to compare the size variation of the optic gland with that of other parts of the body.

Materials and methods

Material

Methods for the capture and maintenance of live *Eledone cirrhosa* (Lamarck) at Aberdeen have been described elsewhere (Boyle, 1981). The individuals used in this study were a sub-set of those collected in 1978 and 1979 from within 50 km of Aberdeen, Scotland, and already examined during investigations of growth (Boyle and Knobloch, 1982 b) and reproduction (Boyle and Knobloch, 1982 a, 1983).

The bodies of octopuses kept for analysis were weighed on a beam balance to the nearest 1 g after water in the mantle cavity and superficial mucus had been drained away. They were then wrapped individually in polythene bags and stored deep-frozen. After thawing, each octopus was washed briefly to remove mucus and then re-weighed to the nearest 100 mg. This, the second weighing, was taken as the total body weight of the octopus for all subsequent purposes. The mean loss of weight after freezing was 4% (\pm SE 0.2) of the fresh weight and the correlation between fresh weight and thawed weight was $r=0.997$ ($N=574$). This weight loss is assumed to have occurred equally from all tissues. All weights used in this paper are tissue wet weights after freezing.

Four categories of body parts were separated by dissection from each thawed octopus as follows:

Somatic body parts: mantle (musculature cut cleanly from the back of the head); arms (cut as far towards their bases as possible).

Cardiac body parts: systemic heart (vessels trimmed short); branchial hearts (both, weighed separately then combined, vessels trimmed short).

Cerebral body parts: brain (supra- and sub-oesophageal parts separately dissected but weighed together); optic lobes (both, weighed together).

Reproductive body parts: ovary (without oviducal glands and oviducts), oviducal glands (both, weighed together, oviducts trimmed short).

Each body part was weighed separately on an electronic balance to a degree of accuracy (100 mg to 0.1 mg), depending on the absolute weight. The approximate locations of these body parts are shown in Fig. 1.

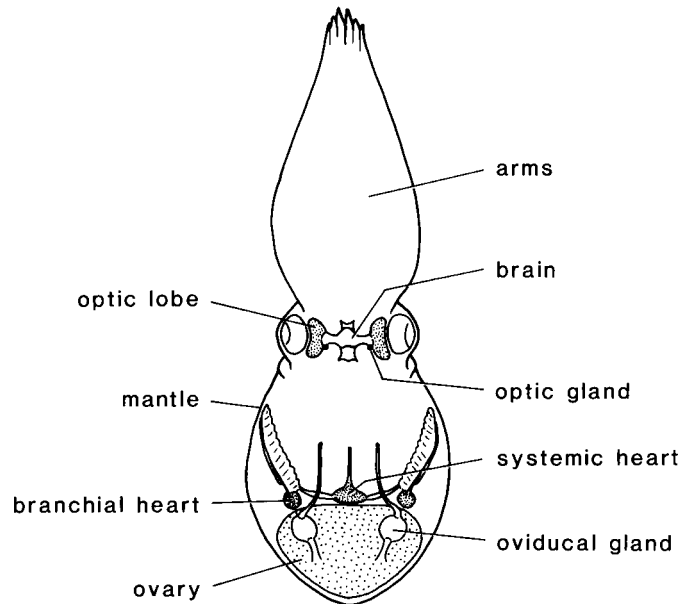


Fig. 1. *Eledone cirrhosa*. Diagrammatic plan view (from dorsal surface) showing body parts referred to in this paper. Not to scale

In addition, both optic glands were trimmed cleanly from the optic stalk with insect scissors and immediately sealed into micro-blood sampling tubes. The weight of the tube and optic glands was then recorded (to 0.1 mg) and the pre-determined weight of the tube was subtracted. This procedure reduced the errors due to water loss involved in routine weighing of such small masses of tissue. Finally, the separate weights recorded for the parts of each octopus were added together and then subtracted from the initial (thawed) total weight. This remainder is due mainly to the gut, digestive gland and gills together with the eyes, parts of the head and buccal mass, and throughout this paper is referred to as "remnant".

The full list of the body parts considered and their sequence in the analysis is as follows: remnant, mantle, arms, systemic heart, branchial hearts, brain, optic lobes, optic glands, ovary, oviducal glands. Each of these now forms a character in the principal components analysis.

Data for individuals caught between March 1978 and January 1979 have been subjected to analysis on a monthly basis. This period is a section of a longer data series and was selected because it showed a clear predominant size ("year") class of octopuses (Boyle and Knobloch, 1982 b). The sample size varied by month, but all females for which a complete data set was recorded have been included (total $n=280$), even though they may have been from a different "year class". With few exceptions, the individuals in this sample had not been held in aquarium conditions for significant periods of time.

Biometric methods

Principal component analysis was used to investigate the character interrelationships. This multivariate technique is

described in many standard texts (e.g. Morrison, 1967) and is widely used by biologists to investigate character variation within and between populations, as shown by the references in Thorpe (1976, 1980, 1981, 1983 a, b).

We were interested in the relationships of the characters (body parts), independent of the magnitude of their individual variances, so the "eigen-values" and vectors were extracted from a correlation matrix. Since we wished to elucidate the development of character relationships over time, the vectors and values were obtained from each monthly sample independently. If growth is having a predominant influence on the character interrelationships, i.e., causing generally high correlations between all characters, then principal component analysis can sometimes define a growth vector and a series of growth-independent vectors. If one wishes to investigate the character interrelationships independent of growth influences, then this can be done by studying the growth-independent vectors.

The character relationships can be deduced from the sign and magnitude of the coefficients (character loadings) in a particular eigen-vector. For example, an eigen-vector (of a correlation matrix) that represents "general size" will have coefficients of the same sign and similar magnitude for each character. For a normalized vector (in which the sum of squares equals unity), this magnitude will be the square root of the reciprocal of the number of characters. In this case we have 10 characters, so a hypothetical general size vector will have 10 coefficients of 0.316. In the actual "size" vector, characters with a loading of less than 0.316 are contributing less to general size than the other characters.

Given that the eigen-vectors are normalized and that the order of the characters is as above, we can construct other hypothetical vectors in a similar manner and then compare them to the actual vectors. For example, a hypothetical "reproductive" vector with equal high loadings for three putative reproductive characters (i.e., optic glands, ovary and oviducal glands) and no loadings for the other characters will be 0, 0, 0, 0, 0, 0, 0, 0.577, 0.577, 0.577. Similarly, if we hypothesise a cerebral vector which proposes the optic glands as a cerebral component together with the brain and optic lobes then we have 0, 0, 0, 0, 0.577, 0.577, 0.577, 0, 0.

The agreement between an actual vector and a hypothetical vector is given as the sum of their cross-products, i.e.,

$$\cos \theta = \sum (h_i \cdot a_i),$$

where h_i and a_i are the i th element of the hypothetical and actual vector, respectively. This value, a cosine, varies from unity for complete agreement (zero angle between the actual and hypothetical vector) to zero for complete disagreement (90° between the actual and hypothetical vector).

The eigen-vector associated with the largest eigen-value is referred to as the first vector, whilst the eigen-vector associated with the second largest eigen-value is referred to as the second vector, and so on.

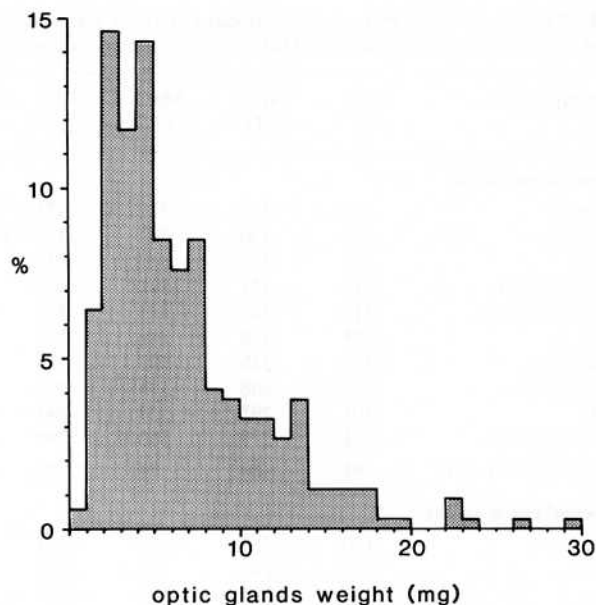


Fig. 2. *Eledone cirrhosa*. Percentage frequency distribution of weights of glands in sample taken in 1978 ($n=342$; from Boyle and Knobloch, 1982 b)

Results

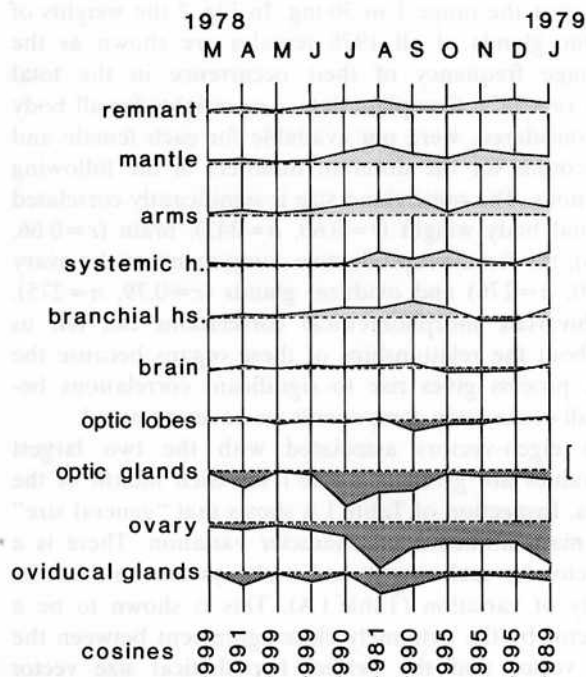
The weight of the optic glands of *Eledone cirrhosa* varied widely over the range 1 to 30 mg. In Fig. 2 the weights of the optic glands of all 1978 females are shown as the percentage frequency of their occurrence in the total sample ($n=342$). Complete data, i.e., weights for all body parts considered, were not available for each female and this accounts for the different numbers in the following correlations. The optic gland size is significantly correlated with total body weight ($r=0.60$, $n=342$), brain ($r=0.66$, $n=326$), the female reproductive components of the ovary ($r=0.70$, $n=276$) and oviducal glands ($r=0.79$, $n=275$). Such bivariate morphometrical correlations can tell us little about the relationships of these organs because the growth process gives rise to significant correlations between all of the body components we have examined.

The eigen-vectors associated with the two largest eigen-values are given in Table 1 for each month of the analysis. Inspection of Table 1A shows that "general size" is the main influence on character variation. There is a size vector for each month and it always accounts for the majority of variation (Table 1A). This is shown to be a size vector by the extremely close agreement between the actual vector and the perfect hypothetical size vector (Fig. 4: I). The cosine of the angle between the actual and the hypothetical vector is always higher than 0.98 (Fig. 3), and in spring and early summer it generally shows practically perfect coincidence¹, i.e., $\cos \theta=0.999$ for March, May and June.

¹ Bearing in mind that even a perfect set of 0.316 values would still give a cosine of 0.999 rather than 1.000

Table 1. *Eledone cirrhosa*. Principal component analysis for each month (March, 1978 – January, 1979); sample size in parentheses. Normalized eigenvector coefficients ($\times 1000$) from a matrix of correlation between body parts

| Body components | Mar. (7) | Apr. (14) | May (13) | June (23) | July (33) | Aug. (40) | Sept. (28) | Oct. (34) | Nov. (47) | Dec. (24) | Jan. (17) |
|--------------------------------|-------------|--------------|-------------|--------------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|
| (A) First eigen-vector | | | | | | | | | | | |
| Remnant | 322 | 332 | 321 | 323 | 345 | 352 | 334 | 331 | 344 | 352 | 344 |
| Mantle | 324 | 330 | 322 | 327 | 350 | 371 | 347 | 333 | 359 | 346 | 336 |
| Arms | 321 | 333 | 322 | 328 | 346 | 358 | 367 | 323 | 351 | 351 | 339 |
| Systemic heart | 324 | 321 | 319 | 319 | 322 | 356 | 336 | 360 | 328 | 322 | 353 |
| Branchial hearts | 317 | 327 | 322 | 330 | 345 | 342 | 362 | 341 | 302 | 306 | 342 |
| Brain | 305 | 326 | 316 | 327 | 321 | 325 | 330 | 303 | 306 | 308 | 325 |
| Optic lobes | 322 | 326 | 306 | 317 | 309 | 320 | 284 | 301 | 305 | 316 | 322 |
| Optic glands | 305 | 268 | 311 | 305 | 205 | 253 | 256 | 305 | 298 | 296 | 288 |
| Ovary | 301 | 295 | 307 | 294 | 275 | 176 | 239 | 251 | 248 | 252 | 190 |
| Oviducal glands | 321 | 279 | 316 | 290 | 315 | 253 | 277 | 302 | 307 | 301 | 291 |
| Percentage variance | 91 | 84 | 94 | 87 | 79 | 64 | 63 | 71 | 64 | 75 | 77 |
| (B) Second eigen-vector | | | | | | | | | | | |
| Remnant | 120 | -269 | -296 | -324 | -52 | -2 | -200 | -193 | -233 | -88 | -125 |
| Mantle | 186 | -296 | 65 | -256 | 12 | -102 | -248 | -279 | -259 | -198 | -275 |
| Arms | 130 | -285 | -144 | -270 | -150 | -194 | -181 | -306 | -264 | -213 | -247 |
| Systemic heart | 6 | -295 | 18 | -73 | -304 | -173 | -204 | 32 | -133 | -301 | 21 |
| Branchial hearts | -440 | 272 | -88 | -18 | -146 | -204 | -121 | 69 | 91 | -338 | -35 |
| Brain | -568 | -72 | 103 | -188 | -248 | -150 | -87 | -330 | -94 | -30 | -153 |
| Optic lobes | 255 | 51 | -588 | -173 | -251 | -183 | -118 | -210 | -259 | -36 | -202 |
| Optic glands | -374 | 501 | -78 | 435 | 587 | 424 | 496 | 357 | 299 | 375 | 296 |
| Ovary | 269 | -30 | 555 | 457 | 531 | 608 | 550 | 542 | 630 | 609 | 738 |
| Oviducal glands | 376 | 580 | 457 | 537 | 331 | 524 | 490 | 460 | 470 | 435 | 386 |
| Percentage variance | 4 | 8 | 2 | 8 | 9 | 18 | 20 | 16 | 13 | 13 | 13 |

**Fig. 3.** *Eledone cirrhosa*. Coefficients for the first eigen-vector for each month. An equal contribution for each character at 0.316 in the hypothetical size vector is represented by horizontal dashed line (see "Results"). The contribution (coefficient) of each character in the actual size vector is shown by continuous line. The difference between the actual and hypothetical coefficients is stippled. The cosine between the actual and hypothetical size vector for each month is given at base of figure. Scale bar on right shows a difference of 0.1 between actual and hypothetical eigen-vector coefficients

Even though every month shows an unequivocal size vector, a close inspection of the character loadings, i.e., eigen-vector coefficients, in Fig. 3 indicates that from summer onwards a set of apparently "reproductive" characters, i.e., optic glands, ovary and oviducal glands, tend to contribute to the size vector slightly less than the other characters. That is, their contribution is slightly under the par value of 0.316.

The biological cause of the difference in size of the specimens is that they are at different growth stages. Consequently we could refer to the "size" vector as a "growth" vector if we wished to infer causation. This growth vector gives a slight indication of two temporal stages. There appears to be a stage of pure growth in the pre-reproductive period in the early part of the year followed by a period, from the summer onwards, when a set of putative reproductive characters become slightly disassociated with general growth.

The optic glands tend to have high loadings (eigen-vector coefficients) in the vector associated with the second largest eigen-value (Table 1B). Inspection of this second vector shows two points very clearly. First, the optic glands are associated with the ovary and oviducal glands in a clear "reproductive" vector and second, that this "reproductive" vector develops over time. This is most apparent when one investigates the agreement between the actual second vector and the hypothetical reproductive vector, i.e., 0, 0, 0, 0, 0, 0, 0, 0.577, 0.577, 0.577, as illustrated in Fig. 4: II a. This figure shows clearly that in early spring there is no "reproductive" vector. As more individuals are

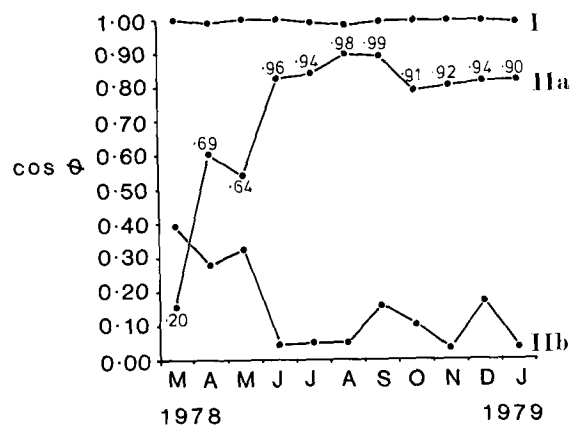


Fig. 4. *Eledone cirrhosa*. Relationship between actual and hypothetical vectors over time. Vertical axis represents cosine between the actual and hypothetical vectors, with complete disagreement ($\cos \theta = 0.00$) at the base rising to perfect agreement ($\cos \theta = 1.00$). I: Relationship between first eigen-vector and hypothetical size vector (0.316, 0.316, 0.316, 0.316, 0.316, 0.316, 0.316, 0.316, 0.316, 0.316); IIa: relationship between second eigen-vector and hypothetical reproductive vector (0, 0, 0, 0, 0, 0, 0, 0, 0.577, 0.577, 0.577), correlation coefficient between the hypothetical reproductive vector and the actual vector for each month is shown; IIb: relationship between second eigen-vector and hypothetical cerebral vector (0, 0, 0, 0, 0, 0.577, 0.577, 0.577, 0, 0)

recruited into a reproductive mode throughout late spring a "reproductive" vector quickly develops, reaches its peak in late summer and is maintained from then on.

The component scores from this reproductive vector result from the contrast between pre-reproductive or incipient reproductive individuals and those individuals with enlarging or enlarged reproductive organs, i.e., optic glands, ovary and oviducal glands. The close agreement between the hypothetical reproductive vector and the actual second vector clearly supports the hypothesis that the optic gland is primarily a "reproductive" organ. Optic gland variation can be seen to be very closely linked with variation in the obviously reproductive organs of ovary and oviducal glands once the influence of growth is separated out.

On the basis that the optic glands are spatially associated with the brain and optic lobes one can erect the alternative hypothesis that the optic glands are linked with these two body parts in a "cerebral" vector. We tested this hypothesis by plotting the agreement between the actual second vector and the hypothetical "cerebral" vector (i.e. 0, 0, 0, 0, 0, 0.577, 0.577, 0.577, 0, 0). It can be clearly seen (Fig. 4: IIb) that there is no agreement between the hypothetical cerebral vector and the actual second vector, and that once in the reproductive period (early summer onwards) these two vectors are almost orthogonal. The hypothesis that the second vector is a "cerebral" vector can therefore be rejected.

From inspection of the remaining vectors it is apparent that no vector consistently appears to be a cerebral vector. Occasionally, the third vector (representing between 4 and 6% of the variation) has fairly high loadings on the three

"cerebral" characters. However, the coincidence between the actual third vector and the hypothetical "cerebral" vector is never close. On four occasions the angle (θ) between them only reached 47° to 56° , and on one occasion it reaches 40° . The hypothesis that size variation in the optic glands is primarily or substantially "cerebral" can therefore be rejected.

Discussion

Principal component analysis of the sizes of these body parts of *Eledone cirrhosa* has given a clear result showing that the variation in size of the optic glands is associated with the size of the female gonad. Taken together with the extensive experimental work conducted on this interaction in *Octopus vulgaris*, we believe that this result provides convincing evidence to show that the optic glands are related to gonad size in the field population.

It is probable that it is the population structure during the period of the analysis, with its very predominant single size class, which has allowed these relationships to be identified. In this relatively homogeneous, but unselected, sample, general growth is obviously the major influence on the size of the body parts. Sexual maturation, which involves a considerable investment of resources in gonad growth, proves to be the second major influence on the size of body parts.

Before March 1978 our sample size was not large enough to permit this type of analysis. From February 1979 onwards the pattern of reproductive character association we have described begins to break down. We suggest that the loss of a consistent reproductive character association in late winter is due to the low levels of maturation at that time, and later in the year, when rate of maturation in the population is increasing, the pattern is then confused by year-class heterogeneity. The agreement between the 11 separate analyses for the period indicates the reliability of the technique.

The onset and development of the association between the reproductive characters should not be equated simply with a breeding season. Some individuals can approach sexual maturity at any time of the year and the process of maturation is probably rather rapid. There is an increase in the mean levels of indices of sexual maturity throughout spring and early summer which reached a peak in July 1978 (Boyle and Knobloch, 1983), but this reflects the recruitment of increased numbers of individuals into the "reproductive mode" rather than a general maturation of the whole population. The lack of synchrony in individual maturation is an obstacle to any suggestion that environmental cues might trigger the activity of the optic glands.

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