Age, growth and mortality of *Loligo vulgaris* wild paralarvae: implications for understanding of the life cycle and longevity

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Running title: Ageing and mortality of *Loligo vulgaris* wild paralarvae

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ABSTRACT

26   Age, growth and mortality were estimated for the first time in wild paralarvae of the common squid, *Loligo vulgaris*, by examining growth increments in the statoliths of 273 animals collected off the Ría de

28   Vigo (NW Spain, NE Atlantic Ocean). Hatching occurred all year round, with a main peak during late spring and a secondary peak during early autumn for the period 2003-2005. Paralarval size varied from

30   1.26 to 7.58 mm and their abundance decreases abruptly as they grow. Statolith increments were clearly visible without grinding in almost all specimens, allowing a reliable estimation of age. Results indicate

32   that the paralarvae are planktonic during, at least, three months. Growth in ML during this period fitted an exponential equation. The instantaneous relative growth rates (G) were 2.11, 2.15 and 1.82 % ML day^{-1}

34   for 2003, 2004 and 2005, respectively. No significant differences in size at age were found between the three years of sampling. Taking into account the growth rates estimated for the whole cycle of *L. vulgaris*,

36   we suggest that the life-span of this species was previously underestimated by 7-8 months, and that a realistic life-span for this species could be about 24 months instead 12 months. The underestimation of

38   age in adults is due to the proximity of the rings deposited during the paralarval and early juvenile stages, which prevents accurate reading of the total number of growth increments in later stages. The estimated

40   instantaneous rate of total mortality (Z) was 9.6, 5.3 and 4.8% day^{-1} for 2003, 2004 and 2005, respectively. These rates are consistent with those expected for a high fecundity species (*sensu* Caddy)

42   and provide support for the idea that *Loligo vulgaris* is a batch spawner with a total fecundity greatly in excess of the number of ripe eggs present in a mature female at any one time. Eye diameter (ED) was

44   found to be a reliable and rapid way of estimating ML and age. Plausible explanations of why the smallest newly hatched paralarvae from Galician waters are half of size that those from the western Mediterranean

46   are discussed.

INTRODUCTION

50   Although many planktonic paralarvae of cephalopods, such as those of loliginid squids,

are relatively large individuals which physically resemble the adults and are capable of relatively

52   rapid movement (Boletzky 1974), the presence of these stages is notably rare in the plankton

samples collected on continental shelves worldwide (Boyle & Rodhouse, 2005). This could be

54   partially due to the inadequacy of the sampling methods, which until now have been unable to

collect representative samples of these animals, possibly due to their patchy distribution

56   (Piatkowski 1998, González et al. 2005). Even in studies focused on the spawning areas of

loliginid squids, which are concentrated and in which it would therefore be expected to find

58   relatively numerous swarms of paralarvae in the water column, very poor results have been

obtained (Collins et al. 2002). Another limitation that prevents carrying out ecological studies of

60   these young stages is the uncertainty of species identification (Sweeney et al. 1992). These are

the main reasons why, although the adult phase of the main exploited cephalopods is relatively

62   well known, studies focused on paralarval stages are rather scarce in comparison with studies of

larvae of other invertebrates and fishes (Boletzky 2003).

64   Poor sampling of the paralarvae of many cephalopod species, even those commercially

exploited, has at least two negative consequences. First at all, the lack of information may

66   preclude correct interpretation of the adult life cycle. Understanding the timing of spawning, the

paralarval distribution, their age and growth, and their mortality rates, is essential to obtain

68   accurate views of the location, abundance and life cycle of the adult populations. Secondly, the

scarcity of paralarvae explains why cephalopod paralarval surveys are not widely used for

70   fisheries assessment purposes (see Boyle & Rodhouse 2005 for review).

        The number of individuals that reach a specific stage of the life cycle of any species, or

72   recruitment, is a biological parameter of paramount importance to understand biomass

fluctuations in adult populations. This is particularly important in species, such as the majority of cephalopods, for which the biological characteristics - short life-cycle (1-2 years), rapid growth to maturity, spawning once at the end of their life-cycle, and their ecological opportunism - result in labile populations, in which there is a complete turnover of biomass every one or two years (Guerra 2006). Recruitment success is related to both biotic factors and environmental conditions. Two recent studies undertaken in Galicia, Northwest Spain on *Octopus vulgaris* (Otero et al. 2007, in press) emphasised the importance of studying the influence of the oceanographic features on the spawning strategy and the paralarvae ecology to understand natural variability in recruitment events, especially in a geographic area which constitutes the northern boundary of the Iberian-Canary current upwelling system (Álvarez-Salgado et al. 2003).

The common squid *Loligo vulgaris* Lamarck, 1797 is a fast-growing cephalopod that inhabits temperate waters of the Eastern Atlantic from the North Sea and British Isles (55ºN) to northern Namibia (20ºS), and the Mediterranean Sea (Guerra 1992). Although many studies have been carried out on subadults and adults throughout its range (see Boyle 1983, Boyle and Rodhouse, 2005 for reviews) and particularly in the Galician waters (Guerra & Rocha 1994, Rocha et al. 1994, Rocha & Guerra 1999), little is known about abundance, distribution, age, growth and mortality of the early stages of development, except for the few data available on abundance and distribution of wild paralarvae in northwestern Atlantic Spanish waters (Rocha et al. 1999, González et al. 2005) and those involving captive animals.

From laboratory experiments, daily increment deposition in *L. vulgaris* statoliths was validated (Villanueva 2000 a). The effect of temperature on embryonic and post-hatching growth as well as on statolith increment deposition rate was shown to be important in this species (Villanueva 2000 a, b, Villanueva et al. 2003). Furthermore, it was observed that interactions among other abiotic factors, like photoperiod and light intensity, also affect the deposition rate in statoliths (Villanueva et al. 2007).

98   Although several studies have been carried out to estimate age and growth of wild

Mediterranean and north-eastern Atlantic *L. vulgaris* populations, based on reading the daily

100   growth increments on their statoliths (Natsukari & Komine 1992, Arkhipkin 1995, Bettencourt et

al. 1996, Raya et al. 1999, Rocha & Guerra 1999), in all cases the analysis was undertaken

102   employing only subadults and adults, but never including the planktonic phase. This gap could

represent an important bias in the interpretation of the real age and growth rate of *L. vulgaris*

104   because the increments deposited during the early stages of development are more difficult to

read in larger statoliths, which is thus an impediment to correctly elucidate the demographic

106   dynamics of this species.

To accurately estimate mortality rates is difficult in short-lived species, and a high

108   proportion of the estimates of natural mortality currently used in stock assessment are based on

empirical relationships originally developed for fish and are applied to an unspecified part of the

110   life history of the species (Caddy 1996). Survival under controlled conditions has been reported

for several species of loliginid squid (e.g. Yang et al. 1986, Hanlon et al. 1989, Villanueva

112   2000a). However, Bigelow (1992), who estimated mortality for the oegopsid squid *Abralia*

trigonura based on growth increments in statoliths of a few wild paralarvae, noted the complete

114   lack of estimates of mortality from field data, and this remains true.

The aim of this paper is to assess the use of statolith microstructures in studying some

116   demographic parameters of the early stages of wild *L. vulgaris* paralarvae and to evaluate the

implication of the results in relation to our understanding of the demographics of post-paralarval

118   populations. This study examines paralarval growth in length and in weight per year, changes in

some morphometric and meristic characters of the paralarvae with growth (thereby identifying

120   morphometric parameters that can be used to accurately estimate mantle length in damaged

paralarvae), hatching season, age and growth differences between year-classes, and mortality in

122   different paralarval year classes.

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Collection of wild planktonic paralarvae. A total of 47 biological surveys was undertaken onboard the R/V *Mytilus* in Galician waters, NW Spain (Fig. 1), an area of wind-driven upwelling, from January 2003 to October 2005. The surveys varied in periodicity between years but methodology was otherwise consistent. Plankton sampling was undertaken on a monthly basis during 2003, on a fortnightly basis between May and October in 2004 and twice a week in July and late September-early October in 2005. Four transects of 2.8 km were covered in each survey with average bottom depths ranging from 26 to 85 m (Fig 1). Due to the low number of cephalopod paralarvae obtained from the inner transect (T1, Fig. 1), during each monthly survey in 2003, it was substituted by a deeper one (110 m, T5) in 2004 and 2005. Zooplankton samples were collected by towing, near-bottom and at the surface, using a 750 mm diameter bongo net equipped with 375 μm mesh. At a ship speed of two knots, the bongo net was first lowered and stabilised near the bottom for a period of 15 min and subsequently hauled up at 0.5 m s^{-1}. The net was then redeployed to collect samples in surface waters. The Bongo net was equipped with a current meter, to allow calculation of the volume of water filtered during each haul, thus permitting an estimation of paralarval abundance (Nº / 1,000 m^3), and a depth meter to help identify the water strata sampled by the bongo nets during each haul.

The zooplankton samples were fixed onboard with 4% buffered formalin. After 24 hours they were transferred to 70% alcohol. Paralarvae of *L. vulgaris* were separated and later identified in the laboratory according to Fioroni (1965), Sweeney et al. (1992) and reference collections of *L. vulgaris* paralarvae hatched under rearing conditions.

Measurements on paralarvae. A total of 376 individuals was measured. Paralarvae damaged during collection (N=9) were discarded from the present study. The wet body weight

148   (BW) of the whole paralarvae was obtained to the nearest 0.1µg using a Sartorius MC 210P precision balance. Using a Nikon SMZ 800 stereomicroscope, the following measurements were

150   made to nearest 0.01 µm, following Roper and Voss (1983): total length (TL), dorsal mantle length (ML), ventral mantle length (VML), mantle width (MW), eye diameter (ED), longest arm length

152   (AL) and tentacle length (TeL). Numbers of suckers on arms and tentacles were also counted.

154   **Age determination.** The mantle and funnel of the paralarvae were removed to access the statoliths. The statoliths were visible as opaque structures within the statocysts. Statoliths were

156   removed with fine dissecting needles (0.2 mm tip diameter) under a stereomicroscope. All statoliths were measured (to 0.01 µm), following the terminology of Clarke (1978), from the end of the dorsal

158   dome to the tip of the rostrum (statolith length, SL) and across the widest part of the dorsal dome (statolith width, SW). The method applied for ageing the paralarvae involved mounting the statolith

160   on a microscope slide, using Crystalbond, with the anterior concave side uppermost. The growth increments of most of the statoliths were clearly visible due to its relative transparency. In a few

162   cases, particularly the oldest paralarvae, this was not the case and the statoliths were ground, first on the anterior surface, then turned over and ground on the posterior surface. The statoliths were then

164   turned over so that the anterior surface was uppermost. This grinding of both surfaces in the sagittal plane results in the production of a relatively thin statolith section. Increments were determined

166   along the axis of maximum statolith growth with a NIS Elements D 2.30 image analysis system interfaced with a Nikon compound microscope (400× magnification). Counts were obtained semi-

168   automatically: putative increments were detected automatically by computer software from an enhanced image but final identification of increments was carried out manually. In a few of the

170   larger paralarvae, increments were not clearly identifiable near the outer margin of the ground surface, and in this case, the number of increments missed was estimated by extrapolation from the

172   adjacent area (González et al. 2000). An age-length (ML) key was estimated for each year. Hatching

       date was back-calculated from the date of capture and the age of each specimen.

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       Growth and mortality data. Instantaneous relative growth rate (G, % ML day^{-1}) for each year

176   was calculated, using only the animals for which age was estimated from daily growth

       increments on the statolith, following Forsythe and Van Heukelem (1987) as:

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$$G = \frac{Ln\ ML_2 - LnML_1}{t_2\text{-}t_1}\ x100$$

180   where ML is the dorsal mantle length (µm) at time t (days). The ML_1 and ML_2 were the average

       mantle length of individuals within the initial and final 10-day periods, respectively. Linear,

182   exponential and power models were fitted to the data.

       The instantaneous rate of total mortality (Z) for the years 2003, 2004 and 2005 was

184   calculated using simple catch curves (Ricker 1975). The paralarvae collected were grouped into age

       classes of equal breadth (10 days), and plotted against the natural logarithms of the frequency of

186   occurrence for successive age-classes.

       We chose the day as the unit of time to express mortality rates following Caddy (1996),

188   who indicated that, although it is habitual practice in stock assessment, it is rather obvious that it

       is not very practical to express instantaneous rates of mortality on an annual basis in short-lived

190   species.

192   **Statistical analysis.** Differences between years in weight vs ML, ML vs age and survivorship vs

       age were analysed using generalised additive models. In the first case both variables were

194   transformed, since the underlying relationship is expected to approximate to a power function. In

       the latter two cases, a better approximation to a Gaussian distribution and homogeneity of

196   variance was achieved by log-transforming the response variable. Differences   between   years

were determined by fitting separate smoothers (for the effect of the main explanatory variable,

198   i.e. ML or age) for each year, as well as including year as a factor. This model was then

compared with a model with a common smoother for all three years, using an F test. This

200   approach is equivalent to but more robust than the option of including linear interaction terms.

Since the sampling months differed between years, models of weight-at-length and length-at-age

202   which included month as an explanatory variable were also explored. In addition, since

conditions experienced around the time of hatching may be critical we also substituted hatching

204   month for catch month. For the survivorship model, data were the numbers of animals surviving

to a given age and sample size was insufficient to make separate calculations for each month. All

206   GAMs assumed a Gaussian distribution for the response variable and were fitted using

BRODGAR software (see Zuur et al. 2007).

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RESULTS

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Correlates of growth of wild paralarvae

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A total of 385 *L. vulgaris* paralarvae was collected during the three-year sampling period.

214   Their size (dorsal mantle length, ML) varied from 1.26 to 7.58 mm for the whole period studied.

Of these, 73% were small paralarvae ranging from 1.50 to 3.0 mm ML. Abundance decreased

216   with increasing size once the animals reached around 2.0 mm ML (Fig. 2).

The relationships between ML and the five morphometric characters of the paralarvae

218   measured were all linear and showed high determination coefficients (Table 1), the highest value

being for the eye diameter (Fig. 3). The relationship between the tentacle length (TeL) and the

220   number of suckers was also linear, with sucker count increasing from 4 suckers at 0.5 mm TeL to

40 suckers at 3.50 mm TeL.

222   Figure 4a illustrates the ML-BW relationships for the three year of sampling. Initial

exploration of GAMs including month as a continuous explanatory variable indicated that

224   differences between months were non-significant. However, weight-length relationships differed

significantly between years (i.e. there was a significant interaction between effects of year and

226   ML), the model with separate smoothers for the effect of ML on weight in each year being a

significant improvement on the model with a common smoother. In all three years the

228   relationship between log-transformed weight and log-transformed ML was close to linear (Table

2, Fig. 4b).

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Age and growth

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The statolith increments were clearly visible without grinding in almost all specimens,

234   allowing a reliable estimation of age (Fig. 5). Statoliths belonging to 273 paralarvae, for which

ML ranged from 1.40 mm and 7.58 mm, were read. An exponential model was the best fit to the

236   growth of the paralarvae up to 80 days of age (Fig. 6a). The best estimates of instantaneous

relative growth rate (G) for the wild paralarvae of *L. vulgaris* between 1.4 and 7.6 mm ML in

238   Galician waters were 2.11, 2.15 and 1.82 % ML day^{-1} for 2003, 2004 and 2005, respectively. The

eye diameter was found to be a reliable parameter to estimate the age of the animals (N=376;

240   R^2=0.80) and thus, it was used in those damaged paralarvae where it is difficult to measure the

ML.

242   Initial GAM fits revealed a marginally significant tendency for length-at-age to be

smaller later in the year so month was retained. The final model included a weak negative effect

244   of month but no significant interannual variation. The model with separate smoothers for each

year was not significantly better than one with a common smoother (see Table 3, Fig. 6b).

246   Inclusion of hatching month rather than month of capture in the model results in almost no

change in the overall model; the significance of hatching month was P=0.032 as compared to

248   P=0.047 for capture month.

        Splitting the months into spring-summer (April-September) and autumn-winter (October-

250   March), there was a significant interaction between the effects of age and season (the length-age

        relationship was less linear in spring and summer, P=0.004), although the main effect of season

252   was then not significant. If the year was divided according to hatch month there was no such

        interaction.

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Hatching season

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        Hatching of *Loligo vulgaris* paralarvae occurred all year round with a main peak located

258   in late spring-early summer and a secondary one in early autumn. Figure 7a represents the

        annual hatching season comprising the *Loligo vulgaris* paralarvae collected during the period

260   2003-2005. On the other hand, the oldest animals were caught from September through

        December (Fig 7b). This suggests relatively less hatching occurs in the last quarter.

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Mortality of planktonic paralarvae

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        Figure 8 shows the number of specimens per age class (10 days interval), and the catch curve for

266   the period 2003-2005. The instantaneous rate of total mortality (Z) was 9.6, 5.3 and 4.8% day^{-1}

        for 2003, 2004 and 2005, respectively. GAM results indicated that survivorship at age was

268   higher in 2004 than in 2003 and there was also a significant interaction between year and age

        effects: comparison of shapes of smoothers suggests that the main difference was in survival up

270   to the age of 6 days (see Table 4, Fig. 9).

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DISCUSSION

274   The relatively high number of paralarvae of *L. vulgaris* and *O. vulgaris* (Otero et al. in press) collected in Galician waters shows that, among the sampling methods employed to collect

276   cephalopod paralarvae (see Boyle & Rodhouse, 2005 for a review), the bongo net is one of the most successful.

278   The smallest hatching size of *L. vulgaris* paralarvae from Galician waters was only around half the size of those collected in the Mediterranean Sea (Boletzky 1979, Turk et al.

280   1986). We considered the possibility that the difference in size could be explained if the smaller paralarvae were *Alloteuthis* spp, the only other loliginid species present in this geographic area

282   (Guerra 1992). Subadult and adult *Alloteuthis* are caught mainly in winter and summer months in Galician waters (Arnaiz 2005). However, annual landings of *Alloteuthis* in Galicia ranged from

284   0.8 to 13 mt during the period 1997-2007, representing the 0.9% of the total *L. vulgaris* catch in weight for the same period (Arnaiz 2005, www.pescadegalicia.com). The possibility of these

286   small paralarvae being *Alloteuthis* was rejected because the loliginid paralarvae we collected had two rows of red chromatophores in the tentacles instead of one, as occurs in *Alloteuthis* (Fioroni

288   1965).

      Another possible explanation of the small size of the paralarvae in waters of Galicia,

290   compared to those of the Mediterranean, might be that they experienced some type of shrinkage due to the stress of the sampling or due the procedures of fixation and storage. Nevertheless, the

292   studies undertaken have demonstrated that paralarvae of this species do not experience any shrinkage due to stress when towing is performed or due to the fixation and preservation

294   processes (González unpublished data). However, oceanographic parameters, such as lower sea temperature in northeastern Atlantic waters, could lead *Loligo vulgaris* to hatch at smaller sizes

296   in these waters. Thus, Moreno et al. (2009) indicated the importance of the SST, which, in

Portuguese waters, was revealed as the most important environmental factor affecting

298   distribution, reinforcing the role of temperature in *L. vulgaris* life history traits (Moreno et al.

2005).

300   The difference in hatching size of *L. vulgaris* (Boletzky 1979, Turk et al. 1986, present

paper), as well as for *Octopus vulgaris* (Villanueva 1995, Otero 2007), between Mediterranean

302   and Galician waters could indicate also genetic differences. Using microsatellites DNA,

Cabranes et al. (2008) detected a significant subpopulation structuring in *Octopus vulgaris*

304   consistent with an isolation-by-distance model of low levels of gene flow, and Perez-Losada et

al. (2002) obtained similar results for *Sepia officinalis*. Although loliginid squid are more mobile

306   than both octopus and cuttlefish, the Atlantic and the Mediterranean have been isolated several

times through the course of history, perhaps associated with substantial environmental changes

308   in the latter (Cabranes et al. 2008).

The biological plasticity reported for *L. vulgaris* and other short-lived loliginid species

(Boyle & Rodhouse 2005), could explain also why paralavae hatch at smaller sizes in Galician

310   waters. There is presently little prospect of identifying which environmental factors might

account for this phenomenon. However, small paralarvae of *L. vulgaris* were also observed in

312   adjacent areas, e.g. a similar size range of paralarvae was collected from 1986 onwards in

Portuguese waters (Moreno et al. 2009).

314   Among the morphometric parameters measured in the paralarvae of *L. vulgaris* in

Galician waters, the ML and the ED were closely correlated. Because the eye is almost always

316   intact in the paralarvae captured by nets, it is a reliable, accurate and rapid way of estimating

paralarval ML and age, especially when the mantle of paralarvae are damaged during capture.

318   According to our results, paralarvae of *L. vulgaris* hatch throughout the year in Galician

waters with peaks on spring and early autumn. This agrees with the studies undertaken by

320   Moreno et al. (2009) in Portuguese waters and also by Rocha and Guerra (1996), who observed

322   that the reproductive period of the species in Galician waters extends throughout the year with a season of intensive spawning from December to April. On the other hand, the dissimilarity

324   between the hatching periods of *L. vulgaris* herein indicated and *O. vulgaris* (Otero el al. 2007) in the same geographical area might imply that the squid has evolved differentially in this

326   ecosystem to hatch inside the strongest months of upwelling. Conversely, *O. vulgaris* main peak of hatching is located outside these months, thus presumably avoiding offshore transport of

328   hatchlings and ensuring that the presence of the planktonic paralarvae coincides with a high density of mesozooplankton. In the case of *O. vulgaris*, these conditions occurred during the

330   relaxation of upwelling events when nutrient salts are consumed to produce biogenic matter, which is retained in the system and transferred through the food web (Otero et al. 2008, in press).

332   Comparisons between several hard structures revealed that analysis of growth increments in statoliths remains the best way to estimate age in squids (e.g. González et al. 2000).

334   Nevertheless, validation is necessary to confirm that the deposition of their growth increments is daily, a premise that was demonstrated in the case of *L. vulgaris* (Villanueva 2000a). In statoliths

336   of both juveniles and adults of the majority of the species, there is an area close to the nucleus where increments cannot be clearly discerned due to being very close together and the presence

338   of a thick wing with an amorphous crystallization. This could lead to an underestimation of adult age and hence introduce a bias into the interpretation of maturity and mortality data (González et

340   al. 2000, Hendrickson & Hart 2006). These issues underline the importance of applying ageing techniques to statoliths of wild squid paralarvae because the increments read in a paralarval

342   statolith would later be obscured once the squid becomes a juvenile. The advantage of readings in paralarval statoliths, at least during the first few months or so, is that grinding of this hard

344   structure is not necessary – although it remains a difficult and time-consuming technique–.

           The first age estimates for wild *L. vulgaris* paralarvae, presented here, indicated that this

346   species inhabits the plankton for about three months (up to 9 mm ML) in Galician waters. The

paralarvae disappear from the meso-zooplankton fraction as they grow, mainly due to the high

348   mortality rate during that period and because the survivors become nektonic.

This is the first time also that the instantaneous relative growth rates (G) have been

350   estimated for the wild paralarvae of loliginid squids. The overall G for squid up to three months

old, estimated from animals collected in Galician waters, is within the range of values obtained

352   by Villanueva (2000a) in culture-based studies of paralarvae from this species in the

Mediterranean. He found that G ranged from 1.16 ML day^{-1} in winter to 2.81 ML day^{-1} in

354   summer. Our results are also consistent with the G values of 1.07-2.75 ML day^{-1} obtained by

Turk et al. (1986), also using cultured squid from the Mediterranean. The lower value obtained

356   by Turk et al. (1.07) coincides with the results from Boletzky (1979), also for Mediterranean

animals but reared at lower temperatures and with less variety and lower density of food

358   organisms. However, our data differ from the age and growth rates estimated from statolith

analysis by Natsukari & Komine (1992) for oldest wild Mediterranean animals of above 60 mm

360   ML. This discrepancy could be explained because the G and age of the small animals (below 60

mm ML) estimated by Natsukari and Komine were calculated using an exponential model fitted

362   only to larger animals.

If growth rates estimated in the present work for the first 90 days of life of the paralarvae

364   (2.15, 2.11 and 1.82 ML day^{-1} in 2003, 2004 and 2005, respectively) remained constant

throughout the life of the animal, squids of 92 mm ML would reach this size from 191 to 226

366   days of age, whereas individuals of 383 mm ML would reach this size from 257 to 305 days.

However, as shown in other loliginid and oegopsid squids (Natsukari & Komine 1992, González

368   et al. 1996, Boyle & Rodhouse 2005), the value of G (ML day^{-1}) decreases with increasing age.

Rocha & Guerra (1999) estimated ages ranging from 167-382 days for *L. vulgaris*

370   varying from 92 to 383 mm ML, with estimated G ranging from 0.53 to 0.84 % ML day^{-1}.

Considering the highest of these G values (0.84), which is very close to that G (0.81) we

372   estimated for the last period (age 70-90 days) of the wild paralarval stage, squids of 7 mm ML (three months of age) would reach 92 mm ML after 306 days, and 383 mm ML after 476 days.

374   These calculations suggest that Guerra & Rocha (1999) underestimated the age of juveniles and adult *L. vulgaris* by 6-7 months. However, since that the former rate is still high and considering

376   the decrease of the growth rate throughout the animal life cycle, a realistic life span for this species could be about 24 months instead 12 months as indicated by those authors. Similarly, the

378   age of *L. vulgaris* could also have been underestimated by Bettencourt et al. (1996) for animals from south Portugal and by Arkhipin (1995) for animals from the west Saharan shelf. These

380   underestimates of age and lifespan in adult squid presumably reflect the above-mentioned proximity of the rings in part of the statolith deposited during the paralarval stages and imply that

382   this has not previously been adequately taken into account. This issue has important consequences for our understanding of the life history of the species, and also has implications

384   for stock assessment, especially when we have to deal when models that explain the relationships between oceanographic parameters and the early life cycles (when the mortality is extremely

386   high), which have to consider two years before the catches of the larger animals instead one year before.

388   Laboratory studies (see Forsythe and Van Heukelen, 1987 for a review, Hatfield et al. 2001) have consistently shown that the growth in body weight of loliginid and benthic octopods

390   occurs in two phases over the life cycle. The first phase is exponential in form with a constant rate of growth between 4 and 8 %, depending on the species. The second phase is logarithmic

392   and lasts until near the end of the life cycle (Forsythe & Van Heukelen, 1987). Our data showed that the growth in body weight also fits an exponential equation ($BW_{(\mu g)} = 0.653e^{0.0584Age}$;

394     $R^2=0.834$) with a G=6.29% BWday^{-1} for the whole period sampled. These data agreed with the revision made by Forsythe and Van Heukelem (1987). However, we preferred to use the growth

396   in ML because the accuracy of this measure is much higher than for the wet body weight (BW).

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On the other hand, it has been shown that, in any case, one of the most important variables

398   affecting growth rates is temperature (Forsythe, 1993; Hatfield, 2000; Hatfield et al., 2001). This

could be one of the explanations for the differences in growth rate (expressed in ML day^{-1})

400   between the different sampling years.

The estimates of mortality for the planktonic period of life in paralarvae of *L. vulgaris* are

402   the first available for myopsid squid and closely agree with the estimate by Bigelow (1992) for

the oegopsid squid *Abralia trigonura*, also based on growth increments in statoliths. The rate of

404   mortality we estimated corresponds to the instantaneous rate of total mortality (*Z*). We cannot

presently determine which part of that mortality corresponds to natural mortality (M) and which

406   to fishing mortality (F), although paralarvae are evidently too small to be retained by normal

commercial nets and it may therefore be reasonable to view it as equivalent to natural mortality.

408   The rates of mortality calculated in the present work for the three years of sampling (9.6, 5.3 and

4.8% day^{-1} for 2003, 2004 and 2005, respectively) for *L. vulgaris*, a species in which fecundity

410   varies widely, ranging from 782 to 21,885 ripe oocytes per female in Galician waters (Guerra &

Rocha 1994), are similar to those estimated theoretically (M = 6.75) for species of high fecundity

412   (eggs per female = 200,000) in the interval between 64 and 153 days according to the 7

gnomonic intervals into which Caddy (1996) divided 1 year. Our results are, however,

414   considerably higher than those corresponding to a species of low fecundity (2.47 for females that

spawn about 135 eggs), also estimated by Caddy for the same interval of time. In Galician

416   waters, the paralarvae live in the plankton for at least 90 days, which falls within the interval

presented in the model developed by Caddy (1996). Our estimates indicate that there is a high

418   natural mortality in planktonic paralarvae. This phase of life represents 12-13 % of the life cycle

of *L. vulgaris*, and there is no reason to suppose that the paralarval mortality rate could be

420   extrapolated to the rest of its cycle. Interestingly, these high mortality rates in paralarvae indicate

that *L. vulgaris* should be considered within the category of species with high fecundity,

422 according to Caddy (1996). Although this is not obvious from the numbers of ripe oocytes found

in a female at any one time (Rocha & Guerra 1996), the potential fecundity of this species is

424 much higher, ranging from 28,500 to 74,200 oocytes, when the total oocyte stock is considered

(Laptikhovsky 2000). This high number of oocytes could all be spawned in different batches

426 over the extended spawning period of the species, which shows clear signs of being an

intermittent terminal spawner (Rocha & Guerra, 1996).

428 On the whole, we found that *Loligo vulgaris* wild paralarvae remain in the plaktonic

phase for about three months, growing at high rates and showing no significant interannual

430 variation. However, mortality differs significantly between years, influenced by several

parameters, among which oceanography is potentially one the most important. Due to the

432 scarcity of studies regarding the ecology of wild paralarvae, and especially its relation to

physical and chemical oceanographic parameters, we should encourage further studies to

434 advance in the knowledge of the early stages of development, a critical point of the cephalopod

life cycle.

436

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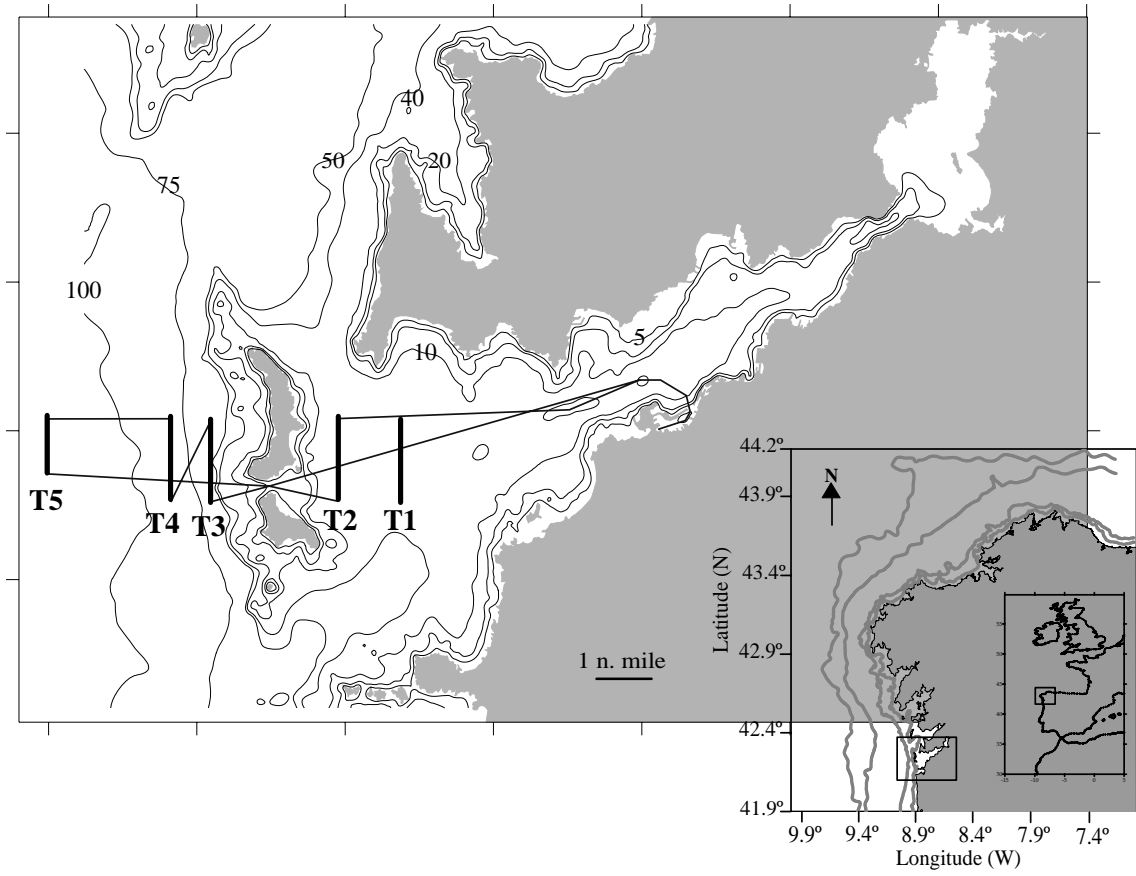544 conditions off Galician waters (NW Spain). J Plankton Res 21:21–33

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Figure Legends

568 Fig. 1. Map of the study area indicating the four plankton transects were performed in 2003 with average

bottom depths ranging from 26 to 85 m. Due to the low number of cephalopod paralarvae obtaining

570 in the inner transect (T1) during 2003, it was substituted by a deeper one (110 m, T5) in 2004 and

2005.

572 Fig. 2. Dorsal Mantle Length (DML, in mm) distribution of the *Loligo vulgaris* paralarvae collected from

2003 to 2005.

574 Fig. 3. Dorsal Mantle Length (µm) vs Eye diameter (µm) relationship.

Fig. 4. a) Dorsal Mantle Length (µm) vs Body Weight (BW in µg) relationships in the three years of

576 sampling. Symbols, 2003: cross; 2004: squares; 2005: triangles. b) Smoother for the partial effect of

DML on BW in 2005. Both BW and DML were log-transformed. Dashed lines indicate 95%

578 confidence limits.

Fig. 5. Light micrograph of a statolith from a 1.9 mm DML paralarvae. Growth increments (days) are

580 clearly visible without grinding. The hatching increment is indicated.

Fig 6. a) Dorsal Mantle Length (µm) at age (days) for the period 2003-2005. b) Smoother for the partial

582 effect of age on DML (DML was log-transformed). Dashed lines indicate 95% confidence limits.

Fig 7. (a) Hatching season for the *L. vulgaris* paralarvae based on the mean abundance (number of

584 individuals per 1000 m^3) for the period 2003-2005. (b) Monthly mean age of the paralarvae

collected from 2003 to 2005.

586 Fig 8. Logarithms of number of *Loligo vulgaris* wild paralarvae (LnN) of successive age periods (age in

days) in samples from the Ria de Vigo. The catch curve equations for each sampling year following

588 the decrease of fishing rate are given. The instantaneous rate of total mortality (Z) corresponds to

the slope of the regressions.

590 Fig 9. Smoothers for the partial effect of age on survivorship in each year. Survivorship was log-

transformed. Dashed lines indicate 95% confidence limits: (a) 2003, (b) 2004 and (c) 2005.
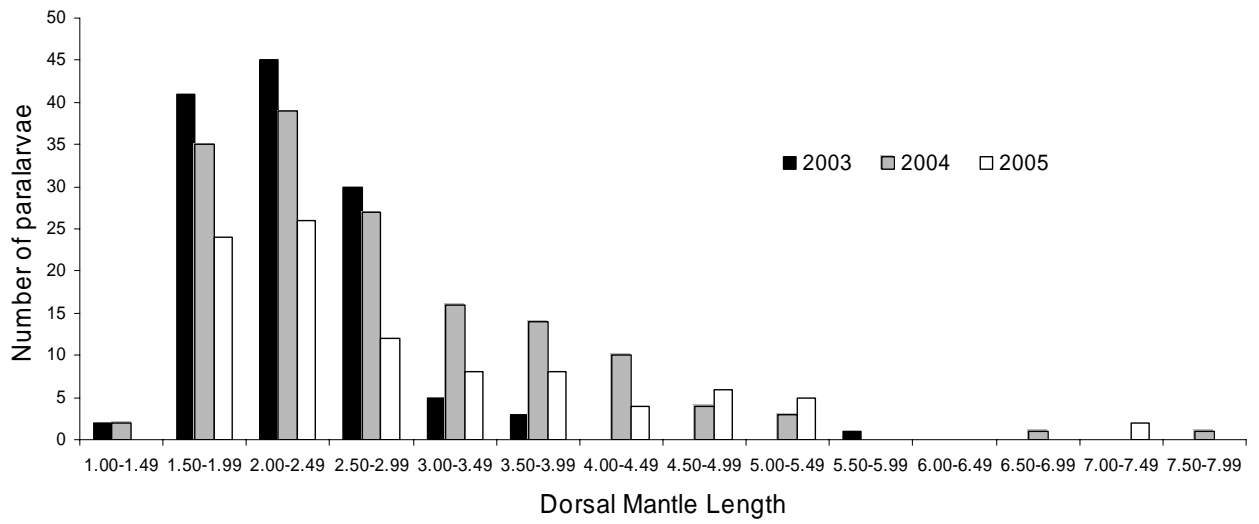
592   Fig. 1.

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Fig. 2.

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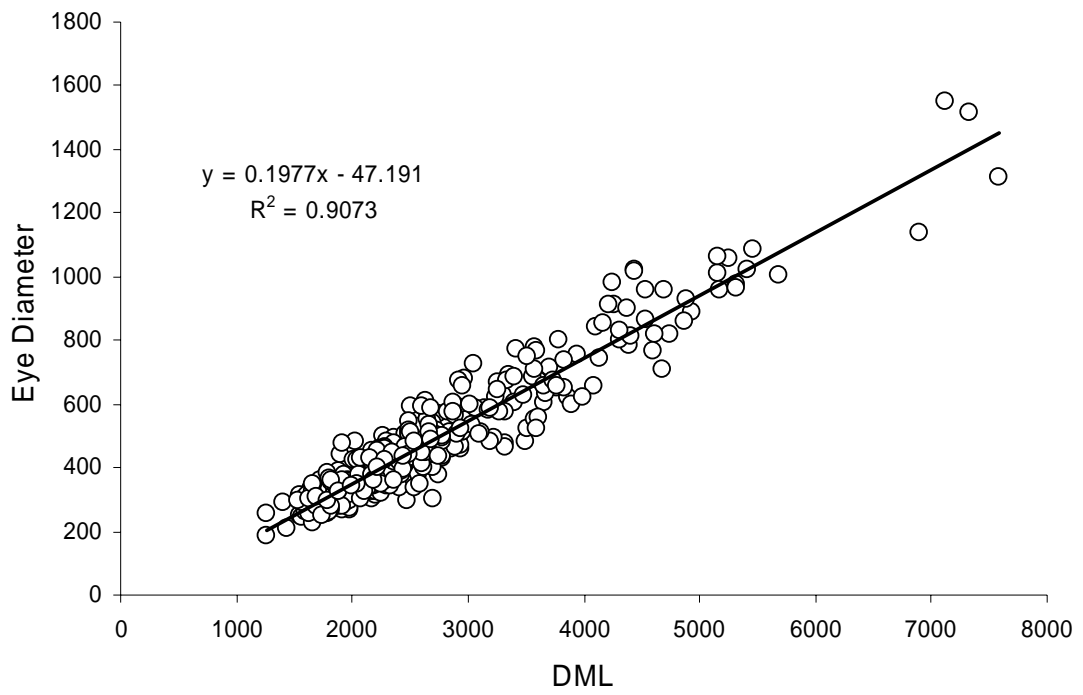606

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Fig. 3.

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The chart shows a scatter plot with "Eye Diameter" on the y-axis (ranging from 0 to 1800) and "DML" on the x-axis (ranging from 0 to 8000). The equation displayed is:

$$y = 0.1977x - 47.191$$
$$R^2 = 0.9073$$

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Fig. 4.

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$y_{2003} = 6E\text{-}10x^{2.7792}$
$R^2 = 0.831$

$y_{2005} = 2E\text{-}10x^{2.9411}$
$R^2 = 0.9395$

$y_{2004} = 2E\text{-}09x^{2.6497}$
$R^2 = 0.9338$

640

Fig. 5.

642

Fig. 6.

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$y_{03}= 1718.4e^{0.0232x}$
$R^2 = 0.7885$

$y_{04} = 1787.3e^{0.0205x}$
$R^2 = 0.8809$

$y_{05}= 1767.3e^{0.0191x}$
$R^2 = 0.9201$
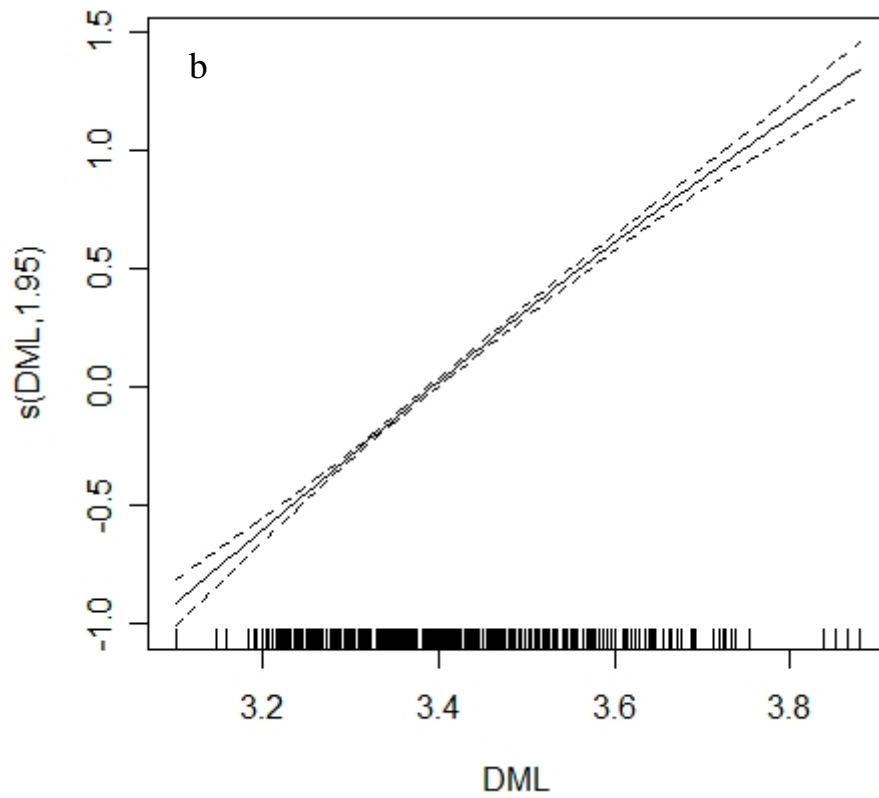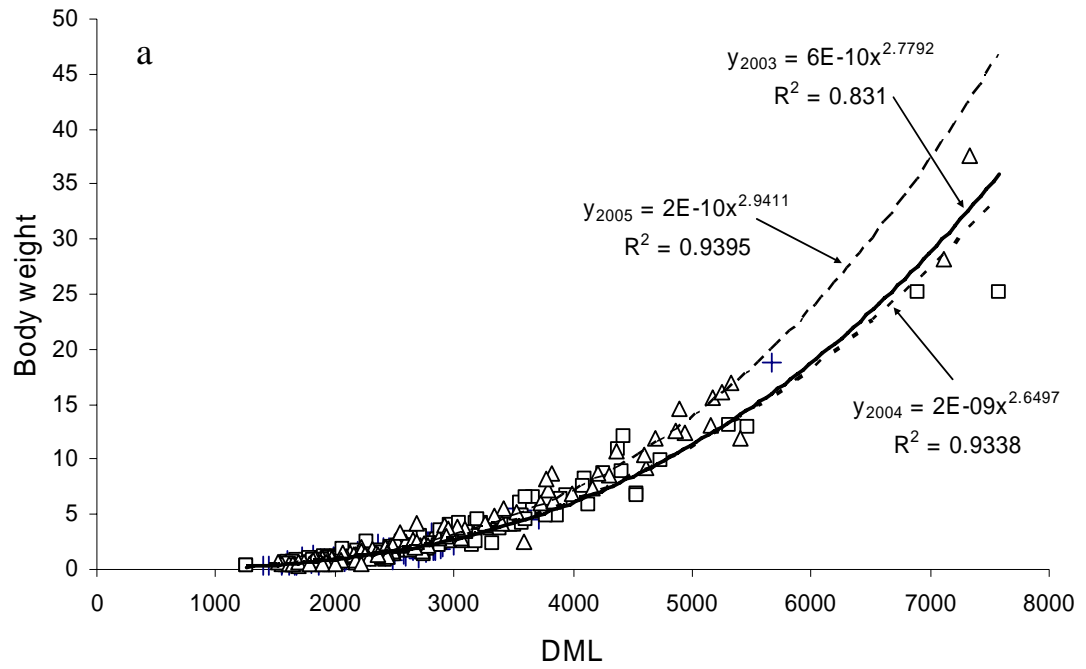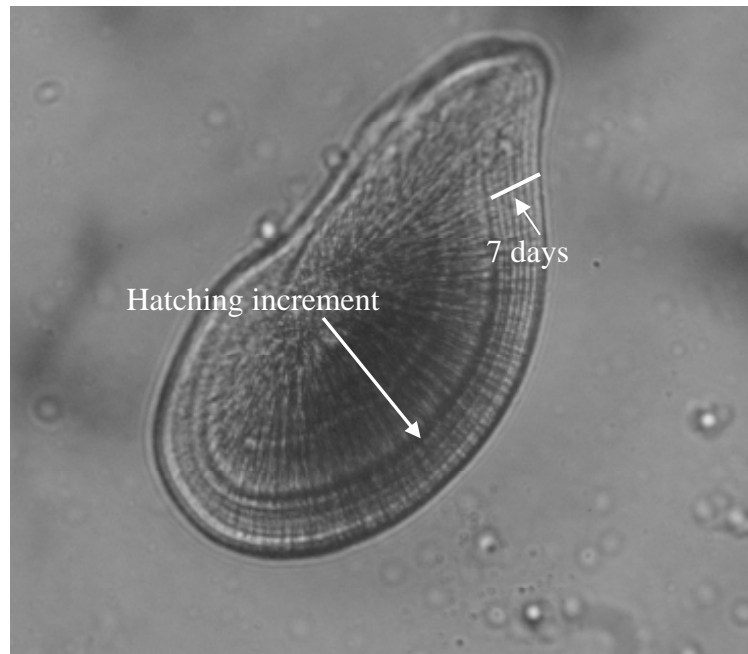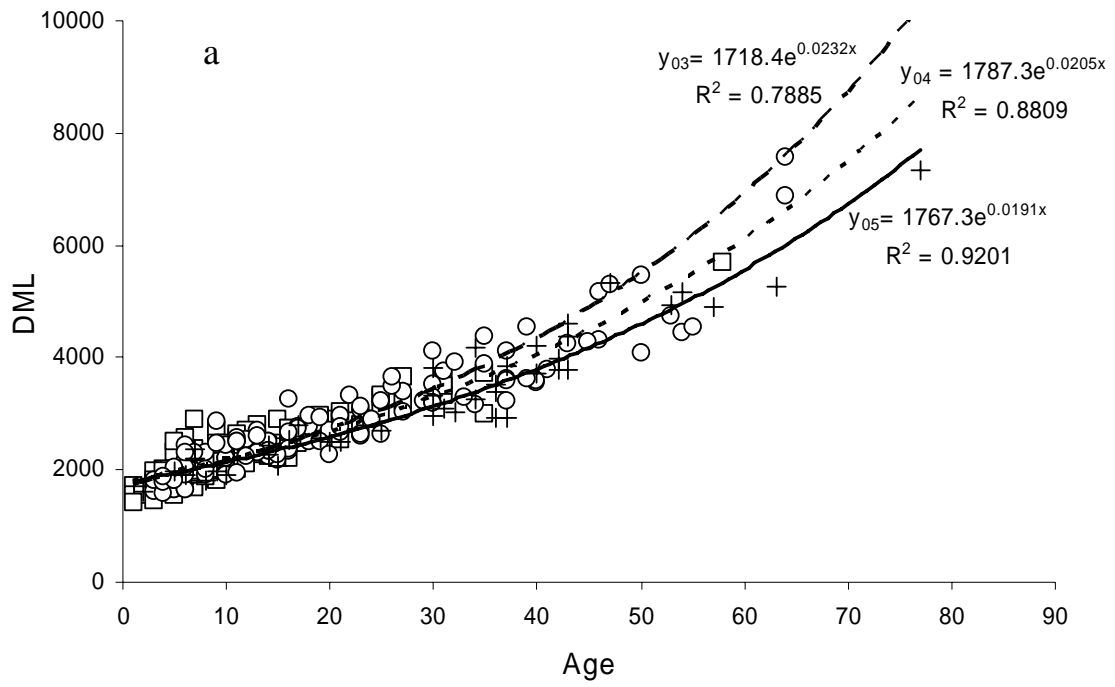
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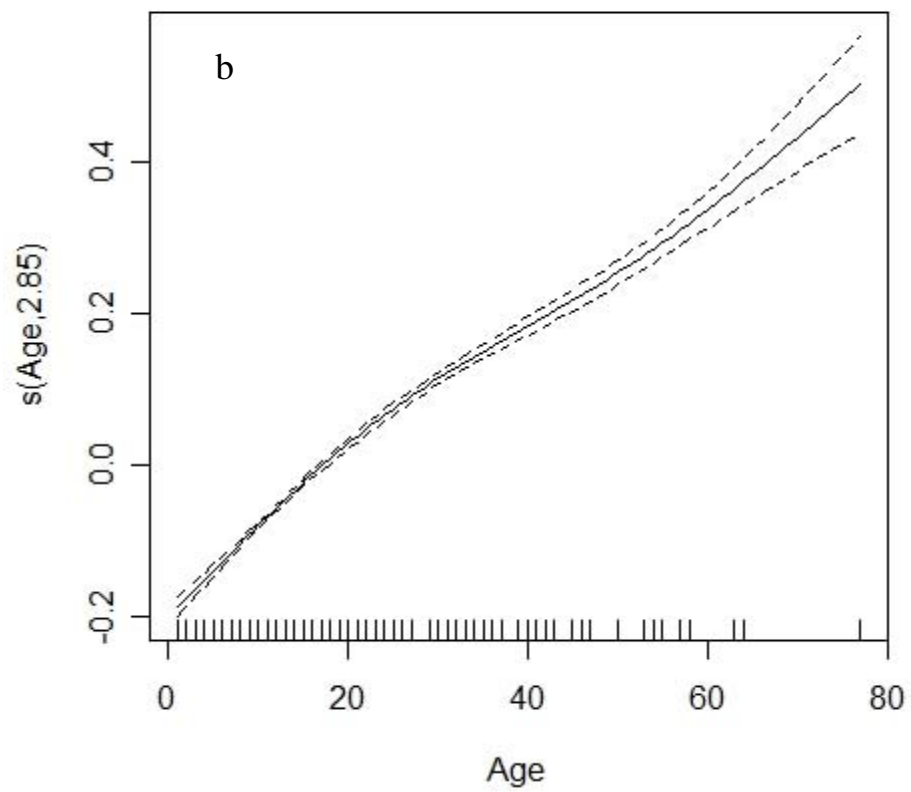
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Fig.7.

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b

686   Fig 8.

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Figure content — plot of Ln N versus Age with the following labelled regressions:

$y_{04} = -0.5289x + 4.6237$
$R^2 = 0.922$; n = 152

$y_{05} = -0.4811x + 4.1309$
$R^2 = 0.891$; n = 98

$y_{03} = -0.9607x + 5.3131$
$R^2 = 0.945$; n = 126

Y-axis: Ln N
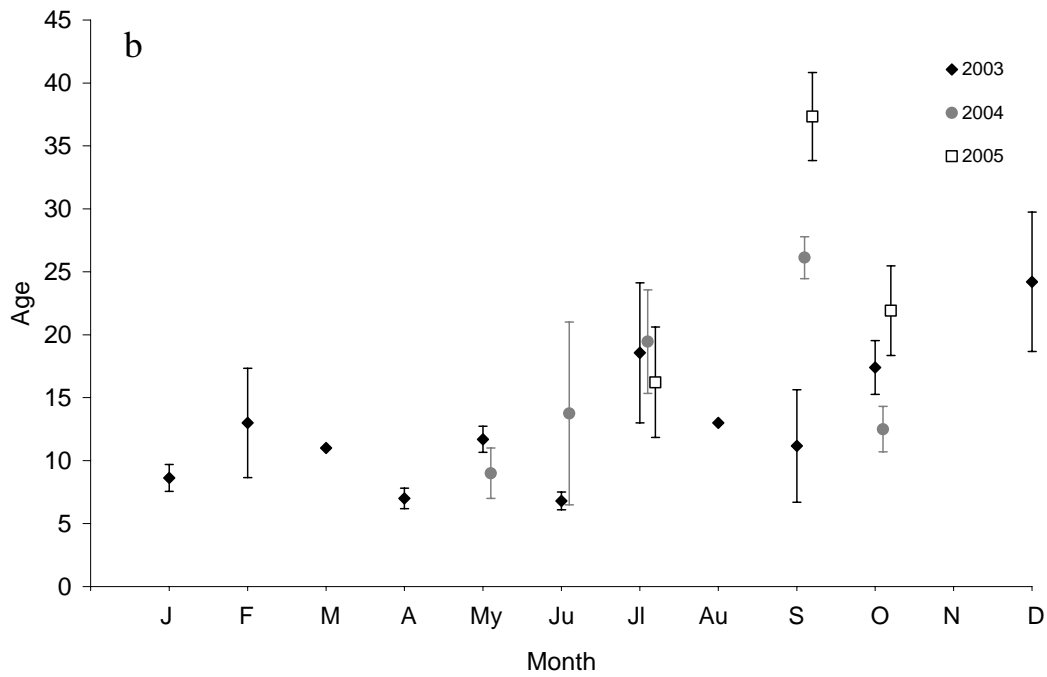X-axis: Age (1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, 91-100)
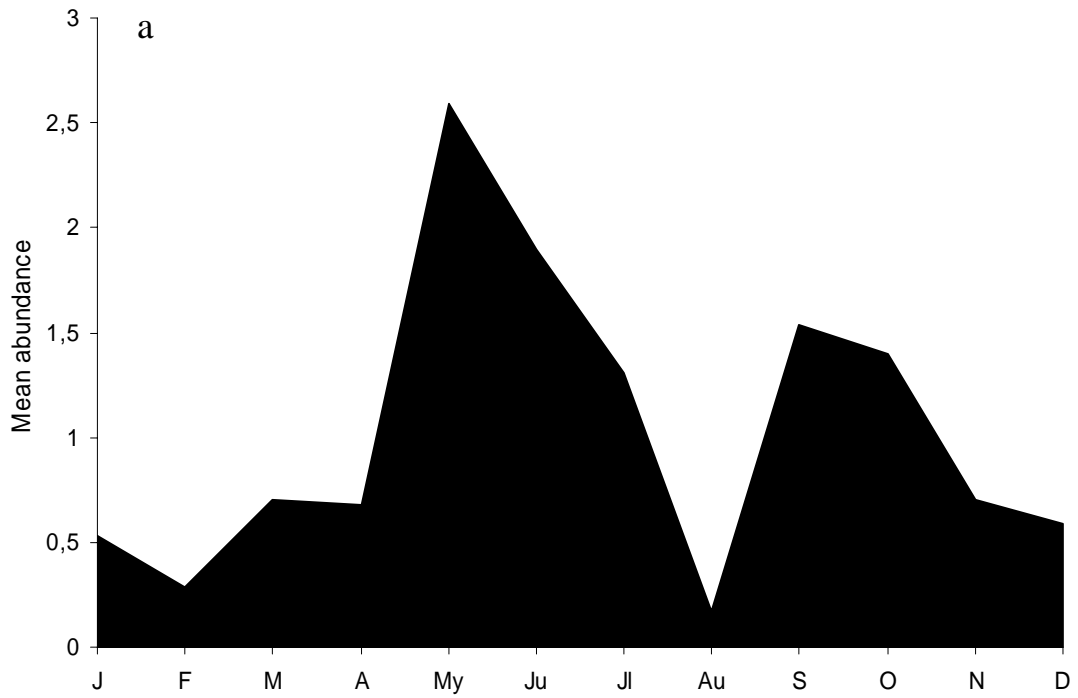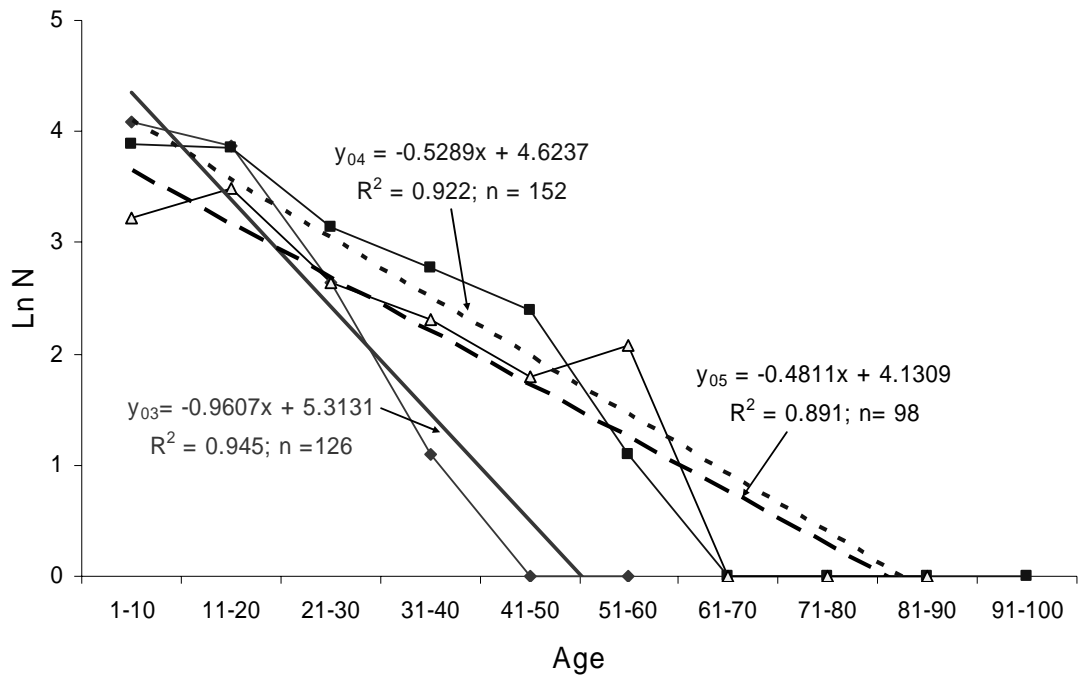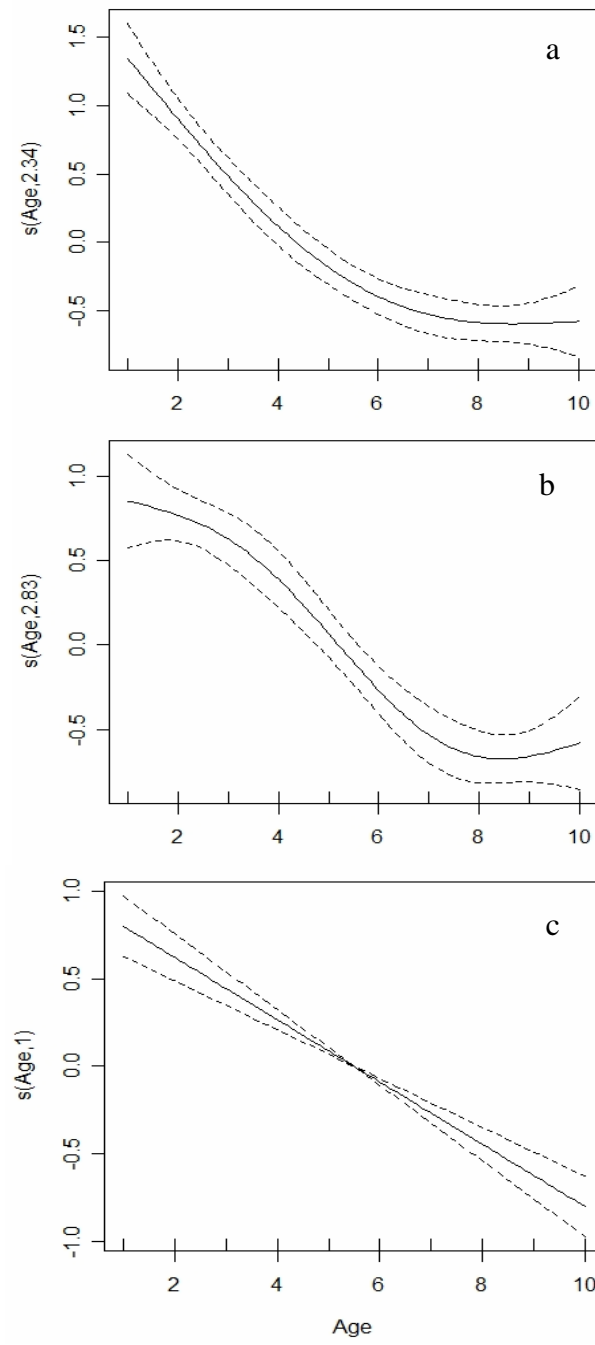
Fig. 9

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698   Table 1. *Loligo vulgaris*. Equations of the relationships between the dorsal mantle length and

the remaining measurements made to the paralarvae (n=376).

700

Equation	Fit	R^2
ED = 0.198 DML – 47.19	Linear	0.9073
VML = 0.812 DML – 174.14	Linear	0.9019
TL = 0.531 DML – 256.19	Linear	0.8716
AL = 0.328 DML – 255.14	Linear	0.8499
MW = 0.445 DML – 334.15	Linear	0.7777

702 Table 2. *Loligo vulgaris*. GAM results for body weight in relation to DML and year. Body

weight and DML were log-transformed and a Gaussian GAM fitted (n=345). The model with

704 separate smooth terms for DML in each year explained 92.5% of deviance and was

significantly better than the model with a single smoother for DML (F=4.993, P=0.0115)

706

Explanatory variable	Fit	Coefficient or Df	Statistic	Probability
Year 2	Linear	0.0323	t = 2.075	0.0387
Year 3	Linear	0.0351	t = 2.095	0.0369
DML year 1	Smoother	Df = 1.44	F = 213.1	<0.0001
DML year 2	Smoother	Df = 1.00	F = 1508.9	<0.0001
DML year 3	Smoother	Df = 1.95	F = 552.8	<0.0001

708   Table 3. *Loligo vulgaris*. GAM results for ML in relation to age and year. DML was log-transformed to improve normality and a Gaussian GAM fitted (n=271). The model explained
710   88.9% of deviance.

712

Explanatory variable	Fit	Coefficient or Df	Statistic	Probability
Month	Linear	-0.00250	t = -1.995	0.0471
Age	Smoother	Df = 2.85	F = 645.6	<0.0001

714

716   Table 4. *Loligo vulgaris*. GAM results for survivorship in relation to age and year. Survivorship was log-transformed to improve normality and a Gaussian GAM fitted (n=30).

718   Year 1 is 2003, year 2 is 2004 and year 3 is 2005. The model with separate smooth terms for age in each year explained 94.6% of deviance and was significantly better than the model

720   with a single smoother for age ($F = 5.090$, $P = 0.0063$)

Explanatory variable	Fit	Coefficient or Df	Statistic	Probability
Year 2	Linear	0.3021	t = 3.877	0.0009
Year 3	Linear	-0.2011	t = 2.581	0.0175
AGE year 1	Smoother	Df = 2.34	F = 50.00	<0.0001
AGE year 2	Smoother	Df = 2.83	F = 38.38	<0.0001
AGE year 3	Smoother	Df = 1.00	F = 85.95	<0.0001

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