Aquatic Living Resources

Yolk utilization, metabolism and growth in reared *Loligo vulgaris reynaudii* paralarvae

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Abstract – To understand the mechanisms that influence recruitment of the commercially important chokka squid *Loligo vulgaris reynaudii*, knowledge of its early life history is required. This paper evaluates the influence of food supply on yolk utilization, metabolism and growth of paralarvae. Eggs collected on the spawning grounds were incubated and the paralarvae reared in the laboratory under "fed" and "starved" conditions for 22 d at 16 ± 1 °C. Some paralarvae lasted 42 d in the laboratory. Mantle length (ML), wet and dry weights (WW and DW) and yolk weight (YW) were measured daily from samples of ~30 (10−51) paralarvae from each group. Yolk weight was estimated using image analysis to determined yolk volume. Three methods (growth model, O_2 consumption rates and yolk utilization rates) were used to estimate metabolic rates. Input parameters included daily mean wet weight of paralarvae and temperature. Mean ML, WW, DW and YW at hatching were found to be 2.3 mm, 1.86 mg, 0.45 mg and 0.21 mg, respectively. The experiment revealed that daily yolk utilization rates were 86 and 95% d^{-1} for fed and starved paralarvae respectively, and that the yolk reserve was almost exhausted 3−4 d after hatching. Starved paralarvae survived for 6 days (with 80% mortality), while fed paralarvae attained a growth rate of 7.8% body WW d^{-1} over the first 22 days after hatching. Results illustrate that temperatures on the chokka squid spawning grounds allow paralarvae to grow at the fastest rates possible without being subjected to a growth "slow down" caused by a high temperature dependent imbalance between sustaining high metabolic and commitment high feeding rates.

Key words: Squid / Paralarvae / Temperature / Growth / Metabolic rate / Feeding rate

Résumé – Utilisation du vitellus, métabolisme et croissance chez des paralarves du calmar *Loligo vulgaris* reynaudii. Afin de comprendre les mécanismes qui influencent le recrutement du calmar chokka, *Loligo vulgaris reynaudii*, qui représente une ressource commerciale importante, il est nécessaire de connaître les stades initiaux de son cycle de vie. Cette étude concerne l'influence de l'apport de nourriture sur l'utilisation du vitellus, sur le métabolisme et sur la croissance des paralarves. Les oeufs ont été récoltés sur les sites de ponte puis ils ont été incubés et les paralarves élevées au laboratoire. Les conditions d'alimentation et de jeûne sont testées pendant 22 jours à 16 ± 1 °C. Quelques paralarves ont survécu jusqu'à 42 jours. La longueur du manteau (ML), le poids du vitellus (YW), le poids humide et sec (WW et DW) sont mesurés tous les jours, sur environ 30 (10−51) paralarves de chaque groupe. Le poids du vitellus est estimé par l'analyse d'image en mesurant son volume. Trois méthodes (modèle de croissance, taux de consommation d'oxygène et le taux d'utilisation du vitellus) sont utilisées pour estimer le taux métabolique. Le poids moyen humide des paralarves et la température ont été choisis comme paramètres d'entrée. Les moyennes des variables ML, WW, DW et YW à l'éclosion sont 2,3 mm, 1,86 mg, 0,45 mg et 0,21 mg, respectivement. Les tests montrent des taux journaliers d'utilisation de vitellus de 86 et 95 % pour les paralarves, respectivement nourries et à jeun, et des réserves en vitellus quasiment consommées en 3−4 jours après l'éclosion. Les paralarves à jeun ont survécu pendant 6 jours (80 % de mortalité), alors que les paralarves nourries présentent un taux de croissance de 7,8 % de WW j^{-1} pendant les 22 jours après l'éclosion. Les résultats montrent que la température dans les sites de ponte des calmars permet aux paralarves de croître le plus rapidement possible, sans toutefois subir de ralentissement de croissance, comme une température élevée peut l'entraîner par déséquilibre entre métabolisme et capacité d'alimentation.

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1 Introduction

The commercially important chokka squid (*Loligo vulgaris reynaudii* d'Orbigny, 1845) is found on the continental shelf off the west and south coasts of South Africa (Augustyn 1990). Of concern has been the high variability in biomass and catches, which is thought to be linked to the dynamics of the complex marine environment off South Africa (Roberts 2005).

Understanding the mechanisms underpinning the biomass dynamics and concomitantly recruitment of chokka squid has become a major focus of research, and has led to ocean interaction studies on squid spawning behaviour (Roberts 1998), egg development (Oothuizen et al. 2002), ecology (Roberts and Sauer 1994; Roberts 2005), larval transport (Roberts and van den Berg 2002, 2005) and fisheries (Roberts 1998). In particular, it was suggested that a mismatch of 100−200 km exists between the main spawning grounds on the eastern Agulhas Bank and the place of greatest copepod abundance near the "cold ridge" on the central Agulhas Bank (Roberts 2005). The latter location is thought to be optimal for survival of the paralarvae.

This situation is not unique and, although on a smaller scale, is similar to that observed for some of the ommastrephid squid life cycles e.g. *Illex illecebrosus*, *I. argentinus*, and *Todarodes pacificus* (Coelho 1985; Hatanaka et al. 1985). Ocean current measurements (Roberts 2005) however, have indicated that this (apparent) geographical separation between place of optimal egg incubation and optimal paralarvae feeding may be overcome by the existence of a westward flowing shelf current on the eastern Agulhas Bank. It was postulated that passive drift in this current would take paralarvae about 4−6 days to reach the abundant food region near the "cold ridge".

A more recent study (Roberts and van den Berg 2005) has added greater complexity to the "westward transport hypothesis", and shown that paralarvae hatched along the inshore region of the Tsitsikamma coast (<100 m), which is closer to the "cold ridge" (∼50−100 km), are mainly transported eastwards away from the "cold ridge" into a region deemed to be less abundant in zooplankton. It is possible that these paralarvae in a round about way end up in the vicinity of the "cold ridge" via mechanisms such coastal upwelling which move the paralarvae offshore and into the westward bound greater shelf current, but this means more time passively drifting in a "limited" food domain. Also, very importantly, Roberts (2005) showed that the "cold ridge" and its associated greater copepod production shows interannual variability, and is especially weak during El Niño years. This variability was quantitatively linked to recruitment (biomass) and catches — strengthening the "westward transport hypothesis".

Fundamental to this hypothesis and the associated "cold ridge–copepod maximum" variability is the aptitude of chokka squid hatchlings to survive periods of "limited" food availability during passive transport. As a preliminary step in understanding paralarval survival and recruitment, this paper investigated the effect of food supply on hatchling rate of yolk utilization, survival, growth and metabolism in reared *L. v. reynaudii* paralarvae.

2 Material and methods

2.1 Egg collection and experimental design

On 21 November 2003, laid eggs were collected by SCUBA divers at a depth of 22 m on the spawning grounds in St. Francis Bay, South Africa, and air freighted in their original seawater to the Mariculture Laboratory at the University of Rio Grande in southern Brazil. Water temperature throughout the 17 h journey was maintained at approximately 15 ± 1 °C. The eggs were incubated in a closed recirculation seawater system consisting of 6 round tanks (0.6 m diameter $\times$ 0.4 height), an activated carbon filter (0.8 m length $\times$ 0.4 m width $\times$ 0.3 m height) and a filter bed (1.8 m length $\times$ 0.6 m width $\times$ 0.3 m height), holding a volume of 1100 L (see Vidal et al. 2002a, for details).

The egg cases were initially kept in one tank and acclimatized to 16 °C until hatching on the 8 December. One thousand same day hatchlings (referred to as Day 0) were then placed into each of four tanks. The experiment comprised of a "fed" and "starved" group reared simultaneously in separate tanks with each having a replicate tank (i.e. 2 $\times$ fed tanks and 2 $\times$ starved tanks).

The main prey organisms offered as live food were *Artemia* sp. *nauplii* enriched with SUPER SELCO (INVE®), wild zooplankton (mainly copepods, *Acartia tonsa*, *Temora turbinata*, *T. stylifera, Oncea* spp. and *Paracalanus* spp.) and juveniles of the mysid *Metamysidopsis elongata atlantica*. Zooplankton and mysids were collected by plankton tows at Cassino Beach (32° 14' S; 52° 10' W). Food was supplied at 08:00, 11:00, 14:00 and 18:00 each day (for details on feeding regimen see Vidal et al. 2002a). The experiment was conducted in natural seawater at 16 ± 1.0 °C (mean 16.3 °C) with a salinity above 33 and a photoperiod of 10 h light vs. 14 h dark.

Mortality was determined daily by counting the number of dead paralarvae in each tank. Survival was calculated as the percentage of live paralarvae left in each tank vs. the initial number (1000) and excluded the paralarvae sampled for data collection.

2.2 Measurement of yolk volume, mantle length, and wet and dry weight

Daily samples of ∼30 (range 10−51) paralarvae were obtained from both tanks of each group during the first seven days after hatching and on days 10, 14, 17 and 22. These were anesthetized with magnesium chloride and two visual images (dorsal and ventral) of each paralarva taken using an *Olympus SZX-9* dissecting microscope at 8 $\times$ magnification with the ocular replaced by a *SONY DXC-197A* camera. Measurements of mantle length (ML) and of the internal anterior and posterior yolk sacs from each paralarva were obtained by image analysis using *NIH-Image* public domain software (1.61 version). ML was obtained from the dorsal images and measurements of the yolk sac from the ventral images (Fig. 1).

Volume of the yolk sack was estimated by superimposing standard geometric forms (cylinder, cone and rotational ellipsoid) onto the shape of the yolk sac (Fig. 1; see Vidal et al. 2002b, for details). Total yolk volumes (anterior + posterior yolk sacs) determined for each paralarva (Fig. 1) were
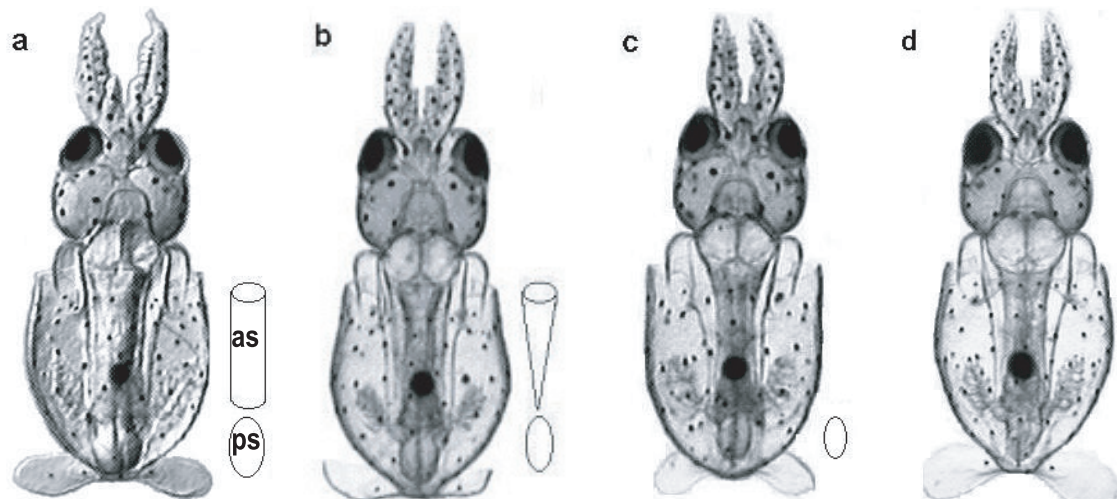
Fig. 1. *Loligo vulgaris reynaudii*. Paralarvae from the fed group are depicted in ventral view at 0 (a), 1 (b), 2 (c) and 4 (d) days after hatching. The anterior (as) and posterior (ps) yolk sacs are visible. The volumes of the yolk sacs were estimated using standard geometric forms (cylinder, cone and rotational ellipsoid).

then converted to wet weight when multiplied by a density of $1.036 \, \text{mg mm}^{-3}$ (O'Dor et al. 1986). Yolk weights were converted into calories using the yolk value of $1.71 \, \text{kcal g}^{-1}$ measured by Giese (1969) for *L. opalescens*.

After images of the yolk sac were taken, wet and dry body weights were determined from the same paralarvae using a *Sartorius R160P* microbalance with a precision of 0.00001 g (for methodology details see Vidal et al. 2002b). Yolk volumes and body weight measurements were made at the same time each day (14:00–16:00) to keep time intervals constant.

2.3 Growth rate

Wet weight was used as an indicator of somatic growth as the yolk reserve was absorbed. For starved paralarvae growth was expressed as an instantaneous growth rate over their survival time. For fed paralarvae, growth rates were calculated up to Day 22 from hatching using the standard exponential function (Forsythe and Van Heukelem 1987):

$$WW = WW_0 \exp^{bd}.$$

Where WW is the daily mean body weight in mg of wet weight, WW_0 is the initial WW, b is the instantaneous growth rate in % body $WW \, \text{d}^{-1}$ and, d is age in days post-hatching. Wet weights were log-transformed and plotted against age.

2.4 Rate of yolk utilization

An exponential function provided the best fit for the relationship between yolk weight and age.

$$W_y = W_{y0} \exp^{bd}.$$

Where W_y is the daily mean yolk weight (mg), W_{y0} is the initial yolk weight obtained at Day 0, b is the rate of yolk utilization and d is age in days post-hatching. Yolk weights were

log-transformed and plotted against age. Differences between groups on regression slopes were tested by ANCOVA (Zar 1996).

2.5 Metabolic rates

Standard metabolic rates and feeding rates were calculated using the daily mean wet weight of paralarvae and temperature according to the following equations (O'Dor and Wells 1987):

$$MR = a \, W^b \, c^T.$$

Where, MR is metabolic rate expressed in kcal d^{-1}, W is the daily mean wet weight of the paralarvae in g, T is temperature in °C and a, b, and c were derived from a multiple linear regression where $a = 0.0043$, $b = 0.96$ and $c = 1.187$, and

$$FR = a \, W^b \, c^T.$$

Where, FR is feeding rate expressed in kcal d^{-1}, W is the daily mean wet weight of the paralarvae in g, T is temperature in °C and $a = 0.058$, $b = 0.79$ and $c = 1.082$. Growth rates were obtained by subtracting MR from FR (i.e. $G = FR - MR$).

Empirical measurements of oxygen consumption rates by Hurley (1976) for *L. opalescens* paralarvae at 15 °C were used to calculated standard metabolic rates for *L. v. reynaudii*. These were compared with metabolic rates calculated by the O'Dor and Wells (1987) model shown above. It is important to note here that a comparison of Hurley's wet:dry ratio of 5.4 ± 0.2 ($n = 9$) with that of Vidal (2000) i.e. 4.2 ± 0.5 ($n = 282$) for the same species at 16 °C, as well as that obtained in the present study for *L. v. reynaudii* i.e. 4.3 ± 0.6 ($n = 309$), suggests that Hurley (1976) overestimated wet and dry weights of paralarvae, and consequently, obtained too high oxygen consumption rates. Overestimation could have been caused by the small sample size ($n = 9$) and inaccurate weight measurements due to water trapped inside the mantle cavity of paralarvae (Vidal et al. 2002b).

In view of this, the ratio of 4.3 (wet:dry weight) was used to recalculate Hurley's rates i.e. mean metabolic rate obtained by Hurley for a hatchling = $3\ \mu l\,O_2\,mg^{-1}\,DW\,h^{-1}$ divided by 4.3 yielded $0.697\ \mu l\,O_2\,mg^{-1}\,WW\,h^{-1}$. Assuming 4.6 kcal is equivalent to $1\ L\,O_2$ (or $0.0046 \times 10^{-3}\ kcal\,\mu l^{-1}$) (O'Dor and Wells 1987), and there are no major diel changes, this resulted in $3.21 \times 10^{-3}\ kcal\,g^{-1}\,WW\,h^{-1}$ or $77 \times 10^{-3}\ kcal\,g^{-1}\,WW\,d^{-1}$. Mean daily wet weights of paralarvae were then multiplied by this last value to obtain mean daily metabolic rates in $kcal\,d^{-1}$.

3 Results

3.1 Hatching and survival

Hatching took place mainly at night and the early morning. Embryos positioned at the distal part of the egg case (i.e. opposite to extremity attached to the sand) hatched first often in consecutive order. Newly hatched paralarvae were observed to swim near the water surface, but then moved deeper 2–3 days later. It is possible that the initial rise in the water column might be caused by buoyancy derived from the temporary greater yolk weigh to body weight ratio (10–14%) (Vidal et al. 2002b).

The chokka squid paralarvae were kept alive for 42 days in the tanks which is a new record for this species. Mortality seems to have been influenced by two factors. Firstly, many paralarvae hatched with abnormally rudimentary fins. This presumably affected feeding success (Vidal et al. 2002a) which would account for the high mortality rates observed within the first 10 days. The cause of this abnormality is unknown, but could be related to the lengthy transport period between South Africa and Brazil. Secondly, large amounts of fecal pellets produced by a benthic cyclopoid copepod later caused deterioration in the water quality. Its presence was first noticed on Day 27 when mortality rates began to increase. Survival rates decreased from 25–31% on Day 31 to less than 5% on Day 42 when the experiment was terminated. It is likely that this unidentified copepod was introduced into the tanks with the wild zooplankton assemblage offered as food to the paralarvae.

3.2 Rate of yolk utilization

Most of the yolk mass was absorbed exponentially in the first three days after hatching for both starved and fed paralarvae (Fig. 2). Note that the latter group had higher yolk utilization rates between Day 0 and Day 1.

The daily yolk utilization rates expressed by the slopes of the exponential function between yolk weight and age were calculated to be 86% for the fed group and 95% for the starved group. No significant differences were found when the data were log-transformed and the slopes compared by ANCOVA ($F = 0.102$; 1, 8 df; $p > 0.25$). In terms of weight this meant that, of the 0.21 mg (average) of yolk at hatchling, an amount of 0.18 mg (86%) was consumed by fed paralarvae over the first 24 h with only 0.03 mg of yolk remaining for the second day.
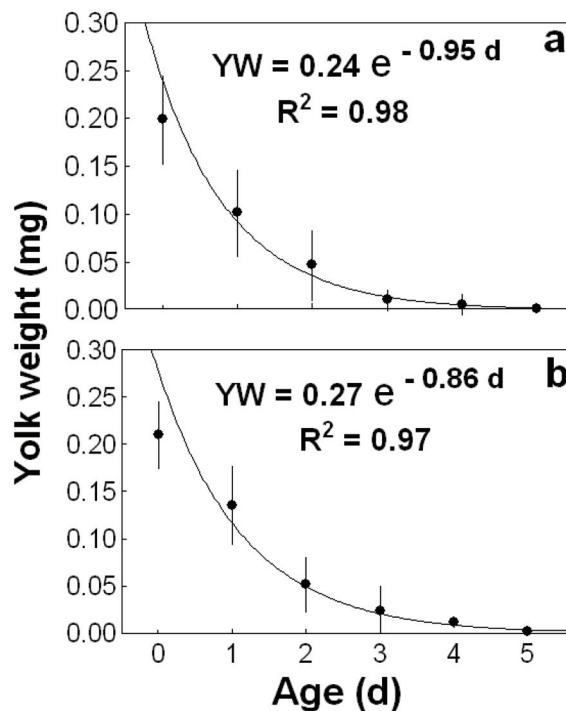

Fig. 2. *L. v. reynaudii*. Yolk utilization (measured in terms of weight) of (a) starved and (b) fed paralarvae reared at $16 \pm 1\ °C$. Age is expressed as days post-hatching and yolk weight values are means of 10–51 paralarvae $\pm$ SD. Most starved paralarvae died on Day 6.

3.3 Observed growth

During the first 2 days, weight loss was experienced in both the fed and starved paralarvae as a result of yolk utilization (Fig. 3). This resulted in a body mass decrease of 18.4% of body $WW\,d^{-1}$ for the fed paralarvae. The effects of food availability, however, became apparent on Day 3 when the mean weight of the fed paralarvae became significantly higher ($t = 2.9$; 36 df; $p < 0.01$) compared to those starved. From Day 3 to Day 6 body mass of fed paralarvae increased at a rate of 12.1% of body $WW\,d^{-1}$, while body mass of the starved paralarvae steadily decreased losing 16.7% of body $WW\,d^{-1}$ (Fig. 3). By Day 6 the fed paralarvae were 66.3% heavier than those starved and had recovered their original hatching weight culminating in an overall "no net growth" phase. As shown in Figure 4, after the "no net growth" phase growth was exponential at a rate of 7.8% body $WW\,d^{-1}$ (or 9.8% body $WW\,d^{-1}$ if the negative initial phase of growth is not considered). Interestingly, this "no net growth" phase has also been observed in *L. opalescens* paralarvae (Vidal et al. 2002b).

3.4 Theoretical influence of temperature on metabolic, feeding and growth rates of paralarvae

Estimated metabolic rates of paralarvae between Day 0 and 22 were obtained using the model cited in O'Dor and Wells (1987) and the temperature range of 0–30 °C (Fig. 5a). As expected, metabolic rates increased exponentially with temperature. A noticeably sharp increase was observed between 20 and 30 °C. For a given age, each 5 °C increase in
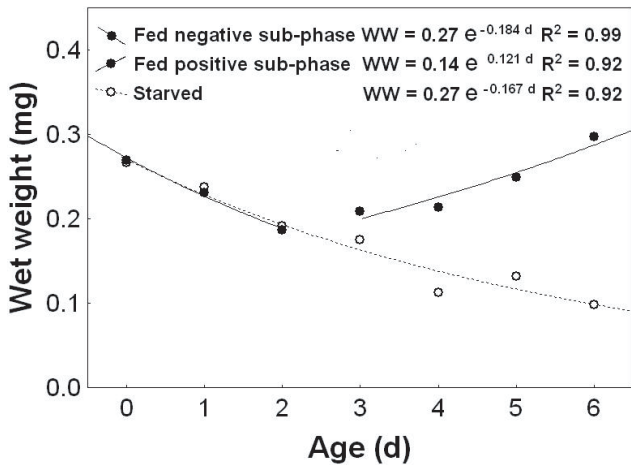
Fig. 3. *L. v. reynaudii*. Growth of starved and fed paralarvae at 16 ± 1 °C expressed as wet weight versus age. Age is expressed as days pos-hatching. Values are means of 15–51 paralarvae (fed, black circles) and 6–41 paralarvae (starved, open circles).
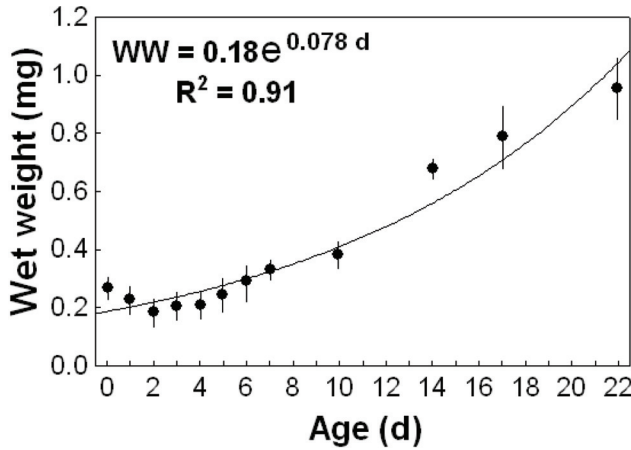

Fig. 4. *L. v. reynaudii*. Growth of fed paralarvae reared at 16 ± 1 °C expressed as wet weight (WW) versus age (in days post-hatching). Values are means of 15–51 paralarvae ± SD.

temperature produced a concomitant 2.36 times increase in the metabolic rate. This increase applied to all ages but was more evident at the higher temperatures (>15 °C) and in older paralarvae. At the same temperature, metabolic rates increased by a factor of 1.29 between Day 0–10, by a factor of 3.21 between Day 0–17 and by a factor of 4.65 between Day 0–22. This means that at 15 °C the metabolic rate of a newly-hatched paralarva (i.e. Day 0 = 0.135×10^{-3} kcal d^{-1}), increases to 0.625×10^{-3} kcal d^{-1} on Day 22 (Fig. 5a).

Also worth noting is that paralarvae in this study showed a factor of 3.37 increase in their mean *WW* between Day 0 (1.86 mg) and Day 17 (6.27 mg), which produces an equivalent increase (×3.21) in the metabolic rate during the same 17 days.

Trend lines for feeding rates (Fig. 5b) exhibited less steep slopes for increased temperature compared with those for metabolic rates (Fig. 5a). Each 5 °C increase in temperature produced an increase in feeding rate of ca. 1.5 times for all ages. At a given temperature, feeding rates of paralarvae increased 1.24 times between Day 0–10, 2.61 times between
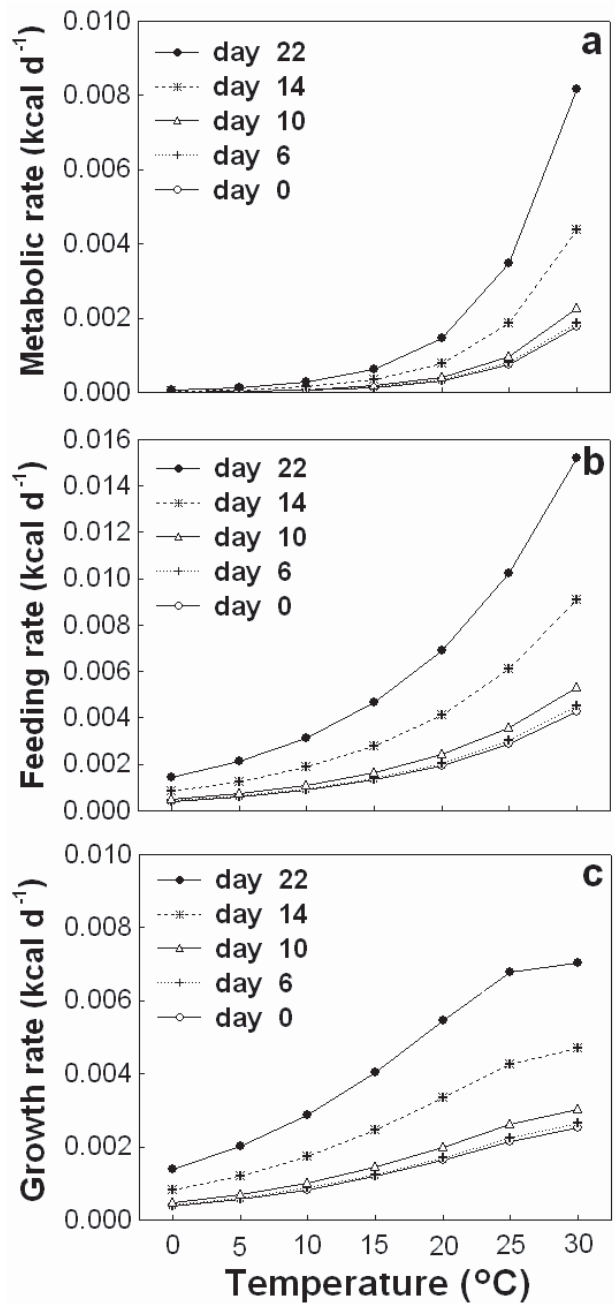
Fig. 5. *L. v. reynaudii*. Theoretical effect of temperature on (a) metabolic (MR), (b) feeding (FR) and, (c) growth (G) rates of chokka squid paralarvae for the first 22 days after hatching. Equations from O'Dor and Wells (1987) were used: $MR = a\,W^b\,c^T$; $FR = a\,W^b\,c^T$ and $G = FR-MR$, where a, b, c are constants, T is temperature and W is mean wet weight of paralarvae (see methods for details). Note the different scales.

Day 0–17, and 3.54 times between Day 0–22 (Fig. 5b), illustrating the lower slope of the curve relative to MR. By comparison, as paralarvae increased in weight by a factor of 3.37 times between Day 0–17 (already indicated above), the increase in feeding rate produced by the model (at 15 °C) was 2.61 times (i.e. from 1.32×10^{-3} to 3.44×10^{-3} kcal d^{-1}). Hence, the model produces an increase in feeding rate which is
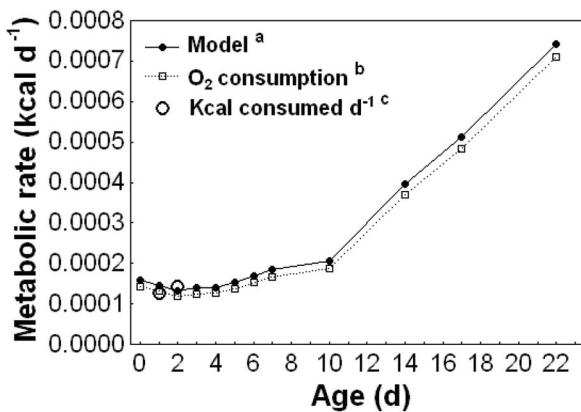
Fig. 6. *L. v. reynaudii*. Comparison of metabolic rates of paralarvae for the first 22 days after hatching estimated using three different methods: (a) O'Dor and Wells (1987) model, (b) oxygen consumption rates measured by Hurley (1976) and, (c) daily yolk utilization rates (see text for details).

proportional to the metabolic rate (ca. 1.0−1.2 times) in early paralarvae (Day 0−10), but then as paralarvae start growing exponentially, the increase in metabolic rate becomes higher than that produced for feeding rates (Figs. 5a,b).

Simulated growth rates of paralarvae (Fig. 5c) also show an increase with temperature, but interestingly, towards the higher temperatures (25−30 °C) the curves indicate a slow down in growth especially obvious in old paralarvae.

3.5 Influence of growth on metabolic rate

The influence of growth on metabolic rate was estimated using data generated from three different methods (Fig. 6). These were (1) the model cited in O'Dor and Wells (1987) using the temperature of 16 °C, (2) oxygen consumption rates measured by Hurley (1976) obtained at 15 °C, and (3) daily yolk utilization rates obtained in the present study. Yolk utilization rates were estimated in starved paralarvae between Day 0 and 1, and Day 1 and 2, since in the absence of external food, they were maintained exclusively on the energy derived from their yolk reserve. This yielded the two data points in Figure 6. It is encouraging to see that the different methods yielded such similar results, albeit, the model estimates are the higher with the two metabolic rates estimated from the yolk utilization falling between the other curves.

As before, the metabolic rates are seen to reflect the "no net growth" phase in the first 6 days after hatching with an exponential increase in metabolic rate thereafter (Figs. 5 and 6).

4 Discussion

4.1 Yolk utilization and early growth

Both fed and starved *L. v. reynaudii* paralarvae exhibited high rates of yolk utilization, most with their yolk reserve completely exhausted five days after hatching. Interestingly, the rates determined for the fed group were higher than those

measured for *L. opalescens* at the same temperature, i.e. 86% vs. 58% (yolk weight d^{-1}) (Vidal et al. 2002b), but the rates for starved paralarvae of both species were very similar i.e. 95% and 100% (yolk weight d^{-1}). Most starved paralarvae in the present study survived 6 d (with 80% mortality; Table 1), while about 10% of those remaining surviving up to 8 d.

From the determination of exponential yolk utilization rates and the calculated caloric content of the yolk (0.36×10^{-3} kcal at hatching), it was possible to compare observed with predicted survival times for the starved paralarvae. If yolk was utilized at an exponential rate of 95% d^{-1} then a survival time of one day is anticipated. This can be extended to three days if a linear yolk utilization rate is used with a metabolic rate of 0.121×10^{-3} kcal d^{-1} (Table 1; Fig. 6). Whichever is adopted, these results are not in agreement with the experiment which demonstrated that starved paralarvae can survive up to 6 days, and therefore, implies that the exponential yolk utilization rates obtained in this work are overestimated. It is perhaps significant that this is unlike *L. opalescens* for which observed and predicted survival times based on a linear yolk utilization rate were very similar i.e. ~4 d (Vidal et al. 2002b; Table 1).

Other important observations come to light when these predicted values are compared to those obtained by O'Dor et al. (1986) for *I. illecebrosus*. In their study, they assumed that the yolk reserve must be partitioned in three ways, namely: 1) material for further development, 2) energy for standard metabolism and, 3) energy for activity. This meant that, of the estimated 0.148×10^{-3} kcal contained in the yolk of *I. illecebrosus* paralarvae (Table 1), 0.016×10^{-3} kcal d^{-1} would be allocated to development, while 0.005×10^{-3} kcal d^{-1} was estimated as the standard metabolic rate of paralarvae. Using these values, the authors then predicted a survival time of 7 days. The results of the present study and that of Vidal et al. (2002b), however, show a distinct decrease in body mass during yolk utilization (i.e. an initial negative growth sub-phase shown in Fig. 3) which indicates no conversion of yolk into body mass. Instead, yolk energy is used to fuel standard metabolism and activity while the paralarvae are improving its ability to capture food. If this is considered, then the estimated metabolism of *I. illecebrosus* paralarvae should be higher, otherwise the predicted survival time based on the energy content of the yolk reserve would be 29 days using a linear yolk utilization rate at 15 °C (Table 1). Hence, by assuming that energy from the yolk reserve is allocated to development, O'Dor et al. (1986) probably underestimated the daily standard metabolism of *I. illecebrosus* paralarvae.

With respect to loliginid paralarvae, the discrepancy between the observed and predicted survival times (Table 1) could be caused by the inability of the method used to accurately quantify small amounts of yolk remaining in the 4−6 d old paralarvae. This clearly will lead to an underestimation of yolk. Another factor could be the chosen rearing temperature (16 ± 1 °C) which would cause high daily yolk utilization rates. In nature, this is about ~1 °C higher than the mean temperature experienced on the spawning grounds in the lower part of the water column (Roberts and Sauer 1994). The range about this mean is about 9−21 °C, however, in the surface layer temperatures have been known to reach 23 °C during

Table 1. Data summary of energetics in newly-hatched squid. Additional data from [§] Vidal et al. (2002b); [ℓ] O'Dor et al. (1986); [£] Roper and Lu (1979).

Species	Loligo vulgaris reynaudii	Loligo opalescens [§]	Illex illecebrosus [ℓ]
Temperature (°C)	16 ± 1	16 ± 1	15
Mantle length (mm)	2.30 ± 0.09	2.50 ± 0.28	1.50–1.60 [£]
Wet weight (mg)	1.86 ± 0.16	1.93 ± 0.35	0.15
Dry weight (mg)	0.45 ± 0.05	0.46 ± 0.07	–
Yolk content (mg)	0.21 ± 0.04	0.27 ± 0.08	0.087
Yolk content (kcal)	0.359×10^{-3}	0.450×10^{-3}	0.148×10^{-3}
Standard metabolism (kcal d^{-1})	0.121×10^{-3}	0.105×10^{-3}	0.005×10^{-3}
Mean kcal consumed d^{-1}	0.135×10^{-3}	0.140×10^{-3}	-
Observed survival time (with 80% mortality) (d)	6.0	4.0	-
Predicted survival time linear (d)*	3.0	4.3	29.6
Predicted survival time exponential (d)**	1.0	1.0	-

* based on a linear metabolic rate, ** based on a exponential yolk utilization rate.

summer (Lutjerharms et al. 1989). The lower temperatures are caused by intermittent, wind-driven, coastal upwelling which commonly expose chokka squid eggs on the seabed to temperatures of 10−12 °C and cools the surface layer to ~13−14 °C (Roberts and Sauer 1994; Roberts 2005). The common occurrence of cooler water suggests that yolk utilization rates will be lower in the ocean and therefore starvation delayed, as was observed for *L. opalescens* (Vidal et al. 2002b).

It is encouraging that the results of this rearing experiment support the early life cycle model proposed by Roberts (2005). The model notes the geographic displacement of 100−200 km between the spawning grounds of chokka squid on the eastern Agulhas Bank, where temperatures are conducive for egg development, and the "cold ridge" to the west where copepod abundance is greatest (i.e. central Agulhas Bank). It proposes that hatchlings passively overcome this distance to reach the optimal feeding grounds using the commonly observed westward shelf current (Roberts and van den Berg 2002). Calculations show that this would take 4−6 days.

Although no empirical information on the swimming abilities of paralarvae is available, this crude calculation of the transport time does agree with the results of the experiment undertaken here, and shows that even in a worst case scenario (i.e. no food available) at least 20% of paralarvae should survive for 6 d at 16 °C on their yolk reserve. It maybe, however, that some of these paralarvae reach the "point-of-no-return", a condition previously described for larval fish (Blaxter and Hempel 1963). Beyond this, recovery from starvation is shown to be impossible. Of course, to consider here are the low water temperatures commonly found on the Agulhas Bank that even under conditions of low food supply would increase survival time. This would imply that paralarvae arrive in the high food abundance region (cold ridge) in better condition and hence improve survival. This maybe the reason why good chokka squid catches and high biomass (i.e. good recruitment) are associated with summers where the mean water temperature is lower than normal (Roberts 2005).

As already indicated, the experiment clearly showed that *L. v. reynaudii* paralarvae initially decrease in body weight after hatching due to yolk utilization, but then recover the lost weight by Day 5 resulting overall in a "no net growth"

phase. This early growth pattern has also been observed for *L. opalescens* (Vidal et al. 2002b). A difference, however, is that *L. v. reynaudii* begin to recover body mass as early as Day 3, compared to six days for *L. opalescens*. The shorter duration of the "no net growth" phase in *L. v. reynaudii* means that exponential growth starts earlier with this species. Indeed, growth rates obtained for *L. v. reynaudii* paralarvae were slightly higher than those of *L. opalescens* when reared at the same temperature and similar feeding regimen during the first 20 days after hatching (7.8% vs. 7.4% of body WW d^{-1}). However, further investigation is required to confirm this over longer periods. Direct comparisons of growth rates for other reported Loliginid paralarvae studies is unfortunately not possible because of different feeding conditions, experimental duration and temperatures used (Yang et al. 1986; Forsythe and Hanlon 1989; Villanueva 2000).

4.2 Metabolic rates

Despite three different methods used to estimate the metabolic rate of chokka squid paralarvae, the results reported here are seen to be remarkably similar. Metabolic rates obtained by the model and those based on O$_2$ consumption rates showed the same trend as would be expected, since both are based on daily mean wet weight of paralarvae. The rates predicted by the model, however, are higher. Several factors could account for this: (1) limitations of the model, since it was developed incorporating metabolic features of several squid species (O'Dor and Wells 1987), (2) the temperature between the present study (16 °C) and that in which O$_2$ consumption rates were obtained (15 °C), (3) inherent specific features between *L. opalescens* and *L. v. reynaudii*, and (4) a mean rate of O$_2$ consumption was used in the present study to obtain metabolic rate in 0−22 d old paralarvae, while in reality a progressive increase in this rate should be expected with weight and age of paralarvae. The latter may have been compensated for by overestimation of the rates obtained by Hurley (1976).

Importantly, the metabolic rates based on daily yolk consumption were found to be similar to those obtained by the other two methods (i.e. Hurley 1976; O'Dor and Wells 1987). This similarity indicates that while the method used in this

study underestimated yolk content in 4−6 d old paralarvae, it did produce realistic estimations of yolk content and metabolic rate in 0−3 d old paralarvae.

4.3 Metabolism and growth potential

A distinct exponential rise in the metabolic rates of paralarvae was observed with increased temperature. This is particularly evident at temperatures >15 °C, and in older paralarvae. A similar trend, albeit with a less steep slope, was seen for the feeding rate. Growth rate on the other hand, showed a slow down especially noticeable in the older paralarvae (22 d) and between 25−30 °C. This is not unexpected since maintenance of their high metabolic demands requires high feeding rates (O'Dor and Wells 1987), and is also in line with the thinking that a break-even temperature exists for large animals, beyond which, there can not be sufficient feeding to sustain their metabolic demands (O'Dor and Wells 1987).

Together these data clearly indicate that post 3 d old paralarvae develop best in the temperature range of 15 to ~24 °C, bearing in mind that survival on the yolk reserve is prolonged at lower temperatures as already discussed above. To realize fast somatic growth, which presumably improves survival, paralarvae on the Agulhas Bank would need to be positioned mostly above the thermocline in the warmer surface layer.

5 Conclusion

The first successful rearing of *L. v. reynaudii* paralarvae from eggs has been accomplished with animals lasting 42 d in a closed recirculating system. Experimentation demonstrated that starved paralarvae can survive for 6 d at 16 °C consuming their yolk at an exponential rate of 95% d^{-1} and losing 16.7% of body WW d^{-1}. Fed paralarvae at the same temperature consumed yolk at a lower rate of 85% d^{-1}, losing 18.4% of body WW d^{-1} until Day 3, but then began regaining weight at a rate of 12.1% of body WW d^{-1} until Day 6. By then they had regained their original weight. Growth continued at a weight gain of 7.8% d^{-1}. The theoretical influence of temperature on metabolic rate, feeding rate and growth was demonstrated using a model. It is suggested that paralarvae will consume yolk at a lower rate in colder water which is common on the Agulhas Bank. Furthermore, the (theoretical) growth pattern obtained for post yolk phase paralarvae is best suited to the warmer upper mixed layer on the Agulhas Bank but temperatures should not exceed ~24 °C because a slow down in growth could be experienced. These results support the hypothesis by Roberts (2005) that chokka squid paralarvae are required to passively drift westwards in a low food domain for a few days to reach the central Agulhas Bank where the existence of the "cold ridge" supports greater copepod abundance, and hence better conditions for survival and growth.

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