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# Characteristics of survivors: growth and nutritional condition of early stages of the hake species *Merluccius paradoxus* and *M. capensis* in the southern Benguela ecosystem

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Larval mortality in marine fish is strongly linked to characteristic traits such as growth and condition, but the variability in these traits is poorly understood. We tried to identify the variability in growth in relation to conditions leading to greater survival chances for early stages of Cape hake, *Merluccius paradoxus* and *M. capensis*, in the Benguela upwelling ecosystem. During two cruises in 2007 and one cruise in 2008, hake larvae and juveniles were caught. Otolith microstructures revealed a larval age ranging from 2 to 29 days post-hatching (dph), whereas juvenile age was 67–152 dph. RNA:DNA ratios, used to evaluate nutritional condition, were above the relevant threshold level for growth. No strong coupling between growth and condition was detected, indicating a complex relationship between these factors in the southern Benguela ecosystem. *Merluccius paradoxus* juveniles caught in 2007 (the surviving larvae of 2006) had significantly higher larval growth rates than larvae hatched in 2007 and 2008, possibly indicating selection for fast growth in 2006. High selection pressure on growth could be linked to predation avoidance, including cannibalism.

**Keywords:** Benguela current, Cape hake, growth, nutritional condition, otolith, RNA:DNA ratio, survival strategy.

## Introduction

Early stages experience the highest mortality rates in the lifespan of fish. Thus, small changes can lead to strong fluctuations in recruitment. One of the major agents of mortality during these early stages is predation (Houde, 1996). Growth performance and condition have a profound impact on larval and juvenile survival as they can influence their predation vulnerability (Bailey and Houde, 1989). Hence, the measurement of larval growth rate and condition, the latter as an indicator of growth potential, can be effective for evaluating the survival chances of individual larvae.

Several methods can be used to estimate growth potential and condition of early stages of fish. Otolith microstructure analysis represents a powerful tool for estimating individual age, growth rate, and hatch dates in larvae and juveniles (Campana, 2005). The back-calculation of fish size from otoliths is an important method in field-based studies of larvae, when the mark–recapture approach is not feasible (Folkvord and Mosegaard, 2002). The fish size at an earlier time is estimated by back-calculation, assuming a constant periodicity in the formation of otolith increments and proportionality between otolith growth and body growth (Francis, 1990). The method has only been validated for adults, but is often applied to early growth trajectories of larvae (e.g. Robert *et al.*, 2007). Complementing otolith microstructure analyses, the relationship between RNA and DNA is an index for protein synthesis in a cell and an indicator of nutritional condition and starvation of fish larvae (Buckley, 1979). RNA:DNA ratio (R:D) analyses can indicate changes in growth potential and nutritional constitution over short periods of ~2 d (Clemmesen and

Doan, 1996; Caldarone, 2005; Buckley *et al.*, 2008). R:D has proved to be a powerful measure of condition in field-caught clupeid larvae (Clemmesen, 1994; Folkvord *et al.*, 1996; Dänhardt *et al.*, 2007), especially when used in conjunction with growth analyses of individual larvae (Clemmesen and Doan, 1996; Caldarone, 2005).

In this study, growth and condition of field-caught larvae and juveniles of the demersal Cape hake, *Merluccius paradoxus* Franca, 1960, and *M. capensis* Castelnau, 1861, were analysed to investigate the characteristic traits, which are important for early stage survival. The two Cape hake are the key predator species in the Benguela upwelling system and a resource of the commercial fishery in the southeast Atlantic Ocean. The size of hake individuals increases with depth, although *M. capensis* lives more inshore in shallower waters than *M. paradoxus*, with some overlap at ~200–400 m (Payne, 1989). Cannibalism is known to occur in both species in the adult stage, and larger *M. capensis* are also known to prey on smaller *M. paradoxus* where their distribution overlaps (Payne *et al.*, 1987). Spawning of the two species is thought to take place on the western Agulhas Bank from where eggs and larvae drift with the Benguela Current towards northern nursery areas (Hutchings *et al.*, 2002). Presumably, spawning takes place all year round with higher spawning activity in austral spring and summer (Grote *et al.*, 2007).

Previous studies on the growth of the two Cape hake focused only on the length–weight relationships of juveniles and adults (Kono, 1980; Prenski, 1984), and otoliths of *M. capensis* juveniles were analysed in only one study (Roux, 2006). The growth of larvae and juveniles of other *Merluccius* species has been studied in small sample sizes (mainly <100 otoliths per sampling) in European hake, *M. merluccius* (Morales-Nin and Aldebert, 1997), Argentinean hake, *M. hubbsi* (Brown *et al.*, 2004; Santos and Renzi, 2006), and Pacific hake, *M. productus* (Bailey, 1982).

Information on the early life history and recruitment of the South African species is still limited (Hutchings *et al.*, 2002; Kainge *et al.*, 2007). The gap in species-specific data on the early life stages is due to the lack of visible morphological differences between eggs and larvae of the two related species. Only two studies from one survey in 2005 (Von der Heyden *et al.*, 2007; Stenevik *et al.*, 2008) have succeeded in identifying Cape hake eggs and larvae to the species level using genetic methods. Greater knowledge about growth rates and condition of the early stages of the two Cape hake species is needed for a better understanding of their recruitment processes.

The objective of this study was to investigate how the growth rates of the larvae relate to their condition to identify the characteristic traits of those hake larvae most likely to survive until recruitment. To address the objective, analyses of genetic identity, otolith microstructure, and R:D of wild-caught hake larvae were carried out on the same individual to compare species-specific condition and growth of different larval stages. Furthermore, hatch dates of larvae and juveniles were back-calculated from otolith readings for identification of spawning time.

## Material and methods

### Sampling

Larvae and juveniles of *M. paradoxus* and *M. capensis* were sampled during three cruises: two short cruises in January and September 2007 on the RV "Dr Fridtjof Nansen" and an intense sampling cruise in September/October 2008 onboard the RV "Ellen Khuzwayo" along the west coast off South Africa (Figure 1; Table 1). The cruise in September 2007 covered a smaller area and due to restrictions in ship time, it was not possible to sample larvae over the entire spawning period, nor juveniles of the same cohort as the larvae. Juvenile *M. paradoxus* originate from a nursery area off the Orange River, which is downstream of the transport route of hake larvae from the western Agulhas Bank spawning grounds (Stenevik *et al.*, 2008; B. Grote, unpublished data). Sampling grids comprised onshore/offshore transects perpendicular to the coast spaced 15 nm apart, covering the shelf and slope (Figure 1). Along transects, stations were spaced usually 10 nm apart and only rarely up to 20 nm. However, an adaptive sampling strategy was applied while at sea, adding stations for sampling in areas of high larval abundance. Temperature was measured with a Seabird CTD probe between the surface and 10 m above the seafloor at all stations.

Fish larvae were sampled with a *Hydrobios* Multinet® plankton sampler (0.25 $m^2$ mouth area), equipped with five nets of 405 μm mesh size as standard on the RV "Dr Fridtjof Nansen", collecting plankton samples in five equal depth intervals of 50 m, each from a maximum depth of 250 m to the surface. A "SCANMAR" depth recorder with acoustic transmission to the vessel was mounted on the top of the Multinet to monitor net depth. The plankton sampler was deployed in an oblique mode at a speed of 0.5 m $s^{-1}$ with the ship maintaining a ground speed of 2 knots. Hake larvae were sorted from other plankton based on the characteristics described by Porebski (1975) and Olivar and Fortuño (1991), but not identified to the species level.

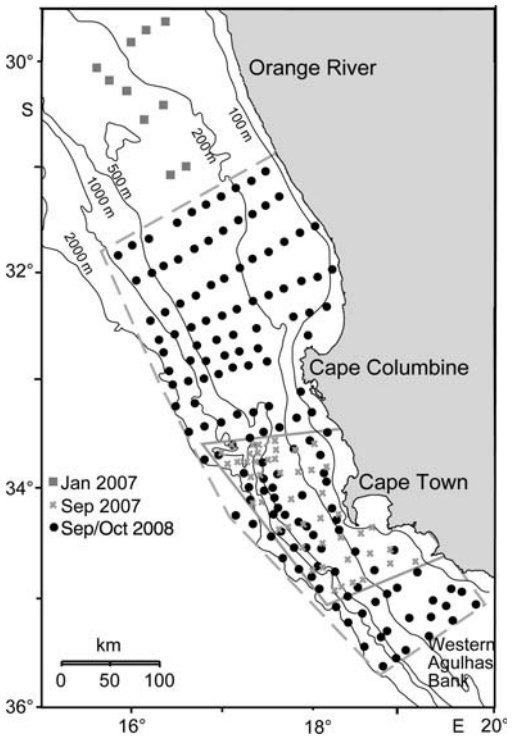


**Figure 1.** Sampling stations for hake juveniles in January 2007 (grey squares) and sampling stations and the area for larvae in September 2007 (grey line, grey crosses) and in September/October 2008 (light grey dashed line, black circles).

**Table 1.** Sampling details of three surveys indicating number of stations, number of otoliths, and R:D analyses (number in parenthesis) per survey for the two hake species and stages, latitudinal range, and ships.

| | | | *M. paradoxus* | | *M. capensis* | |
|---|---|---|---|---|---|---|
| **Sampling time** | **Latitudinal range (°S)** | **Number of stations** | **Larvae** | **Juveniles** | **Larvae** | **Juveniles** |
| 29 January–2 February 2007 | 31.2–29.4 | 42 | – | 57 | – | 2 |
| 17–21 September 2007 | 34.7–33.8 | 52 | 72 (111) | – | 6 (8) | – |
| 20 September–20 October 2008 | 36.0–31.1 | 121 | 95 (160) | – | 11 (17) | – |

Juvenile hake of the previous year class up to a size of 100 mm were collected in daytime pelagic and bottom trawls of 30 min bottom time at a speed of 3 knots. The nets had an 18.22-m mouth opening and a 32-mm codend mesh, and the dimensions of net opening were monitored with a "SCANMAR" net monitoring equipment. The total length (*TL*) of hake larvae and juveniles was measured onboard to the nearest 0.1 or 1 mm, respectively. All samples were frozen in liquid nitrogen immediately after length measurement, and always within 30 min after the haul was on board. Before further analyses in the laboratory, all larvae and juveniles were freeze-dried (*Leybold-Heraeus*, LYOVAC GT2 freeze-drier) for 24 or 48 h, respectively, and larvae were weighed to the nearest 0.1 μg (*Sartorius* microbalance MC21 S).

## Genetic identification

As larvae and juveniles of *M. paradoxus* and *M. capensis* are indistinguishable visually, mitochondrial DNA (mtDNA) was used to identify the species (Von der Heyden, 2007). Tissue homogenates obtained from R:D analyses were sufficient for genetic identification and allowed genetic identification and condition measurements on the same individual. Species-specific primers for each of the two hake species were used for amplification in polymerase chain reactions (PCRs) to screen the mtDNA of each individual larva and juvenile, and the mtDNA of previously identified adult samples of both species was used as positive and negative controls and run parallel in each PCR to check the precision of the analyses.

## Growth and age

For age and growth rate determination, sagitta otoliths of larvae and right sagitta otoliths of early juveniles (30–100 mm *TL*) were removed as quickly as possible from the collected individuals with fine needles under a dissecting microscope. Larvae were returned to the −80°C freezer immediately thereafter. Otoliths were cleaned and mounted either with epoxy resin or embedded in "Euparal" on a glass slide. Otoliths of larvae >5 mm and of juveniles were polished and ground, respectively, with silicon carbide paper of 2400 and 4000 granulation to reveal all the growth increments.

Right juvenile otoliths were polished with frequent microscopic control until the core was reached.

In this study, it was assumed that the deposition of increments in the South African species occurred daily as in the related *Merluccius* species. The otoliths were viewed at ×400–1000 magnification and digital images were taken with a Zeiss Axio Cam ICc1. The computer image-analysis system Image Pro Plus 5.1 was used to measure individual increment width and radius. Three consecutive measurements of the otolith radius were made and the average radius was calculated for each otolith. Daily increments were enumerated from the otolith core to the edge along the axis of radius measurement. Increments were counted twice by two different observers and the mean of the readings was used to estimate the specimen's age. However, if the differences exceeded 5%, the otolith readings were rejected. Of the total number of 293 otoliths, 83% ($n = 243$, 184 larvae and 59 juveniles) were used for growth analyses.

Larval and juvenile ages were analysed by counting the number of increments from a more prominent dark ring near the nucleus, which was defined as hatch check (HC), and increments were assumed to be formed daily according to Bailey (1982) and Morales-Nin *et al.* (2005). Hatching in related species usually occurs 2–6 d after fertilization, depending on ambient temperature (12–17°; Bailey, 1982; Bjelland and Skiftesvik, 2006). The age in days post-hatching (dph) was defined as the number of increments counted from the HC. For back-calculation of spawning dates, 2 d were added to the age (dph) of larvae, taking into account the egg duration time and the relatively high ambient water temperatures (15–17°C) in which eggs and larvae were caught. However, egg duration time may have been underestimated.

The relationship between larval/juvenile length and age (increment number from otolith readings) was analysed by fitting the following Laird-Gompertz equation proposed by Zweifel and Lasker (1976):

$$L_t = L_0 e^{k(1-e^{-Gt})}, \quad (1)$$

where $L_t$ is the length at age $t$, $L_0$ the size at hatching, $k$ the specific growth rate at hatching, $G$ the rate of exponential decay of the specific growth rate, and $t$ the age in days (dph).

To describe the allometric relationship between otolith size ($OR_c$) and body size ($TL_c$), we used the power function:

$$TL_c = a\, OR_c^{\,b}, \quad (2)$$

where $TL_c$ is the total length (mm) measured at capture, $a$ and $b$ the regression parameters, and $OR_c$ the otolith radius (μm) at capture. For the rearrangement of this equation, we followed the model given by Folkvord and Mosegaard (2002) for the back-calculation of total length at a given age:

$$TL_i = \left(\frac{OR_i}{OR_c}\right)^b TL_c, \quad (3)$$

where $TL_i$ is the total length (mm) back-calculated, $OR$ the otolith radius (μm) at increment $i$, $OR_c$ the otolith radius (μm) at capture, $b$ the regression parameter, and $TL_c$ the total length (mm) at catch. This back-calculation formula was used to estimate $TL_i$ of *M. paradoxus* larvae and juveniles within the first 30 d of life. Interannual differences in growth during larval life were assessed by comparing early growth trajectories among the three

years in a repeated-measures MANOVA (Robert *et al.*, 2007). Pillai's trace, a more robust statistic for unequal sample sizes, was selected as the test statistic.

Mean growth rates (*MGR*) of larvae were calculated for the last 3 d by the equation:

$$MGR = \frac{\sum_{i=k-2}^{k} TL_i - TL_{i-1}}{3}. \quad (4)$$

### RNA:DNA ratio

To estimate condition and growth potential, R:Ds of larvae were measured according to Clemmesen *et al.* (2003) and Belchier *et al.* (2004). Whole freeze-dried larvae were used in these analyses. All samples were rehydrated in Tris–sodium dodecyl sulphate (SDS) buffer (Tris 0.05 mol $l^{-1}$, NaCl 0.01 mol $l^{-1}$, ethylenediaminetetraacetic acid 0.01 mol $l^{-1}$, SDS 0.01%) for at least 15 min cooled on ice. Larvae larger than 6 mm were rehydrated for 30 min cooled on ice. Cells were disrupted by shaking in a cell mill (Mixermill MM2 by Retsch) with differently sized glass beads (diameter 2.0 mm and 0.17–0.34 mm) for 15 min for homogenization. The resulting homogenate was centrifuged at 3829 *g* (6800 rpm) at 0°C for 8 min (Sigma Laboratories Centrifuge 3–18 k). The sum of RNA and DNA in the homogenate was determined fluorometrically in a microtiter fluorescence reader (Labsystems, Fluorescan Ascent). Subsequently, the enzyme RNase was added to the samples for RNA digestion (30 min at 37°C) and the remaining DNA was measured. The RNA fluorescence was calculated by subtracting the DNA fluorescence from the total one. By using the calibration curve fitted to the standard measurements (23 s r RNA Boehringer), the amount of RNA was calculated. Following Le Pecq and Paoletti (1966), the DNA concentration was calculated using the relationship between RNA and DNA fluorescence with a slope ratio of standard DNA to standard RNA of 2.2, which adjusts for the relative fluorescence intensity difference of RNA and DNA. Minimum dry mass for which confident R:Ds were obtained was 23 μg. R:Ds were standardized with a reference slope ratio (Caldarone *et al.*, 2006). Standardized R:D values were used to calculate instantaneous growth rates ($G_i$) with an equation from Buckley *et al.* (2008), developed for both cod and haddock, as these species belong to the order Gadiformes as hake:

$$G_i = (0.0254\, srd) + (0.0037\, srd\, t) - 0.0873, \quad (5)$$

where $G_i$ is the instantaneous growth rate, *srd* the standardized R:D, and *t* the median *in situ* temperature (°C) of the depth strata in which the larva was caught. To estimate the R:D threshold level for the growth of hake larvae, the turning point from positive $G_i$ to negative $G_i$ was calculated, followed by back-calculating the related R:D of this turning point.

The separation of yolk-sac and feeding larvae was based on the examination of yolk-sac depletion or stomach contents in all larvae. Size and age of *M. paradoxus* larvae in 2008 were included in a canonical discriminant analyses (CDAs) to estimate the turning point in growth from yolk-sac to feeding larvae. The analysis derives canonical variates that have the highest possible multiple correlation with previously defined classes to maximally separate the two groups of larvae, yolk-sac and feeding larvae, based on their growth pattern.

For the identification of coherences between R:D, temperature, and growth rate, which were not visible in direct correlations, a principal component analysis (PCA) was performed on the correlation matrix, extracting components with eigenvalues >1. STATISTICA 9.0 (StatSoft Inc.) was used to perform all statistical tests.

## Results

### Age and length distribution

A high dominance of *M. paradoxus* was found in the collected larvae. To estimate age and growth rates of hake larvae, a total of 167 sagitta otoliths of *M. paradoxus* larvae and 17 sagitta otoliths of *M. capensis* larvae were analysed (Table 1). In addition, 57 sagitta otoliths of *M. paradoxus* juveniles and two of *M. capensis* juveniles from January 2007 were investigated. Otoliths of the two hake species were visually indistinguishable in the larval or juvenile stage. Hake larvae have relatively small, round otoliths, which become more oval with increasing age. The first ring after the primordium, assumed to be the HC (Alvarez and Cotano, 2005), was observed at 7.5 ± 0.8 μm (Figure 2). The regular pattern of L- and D-zones, observed from the HC to the margin of the otoliths, was interpreted as daily increments. Length of the *M. paradoxus* larvae from September 2007 used for age analysis was 1.7–11.2 mm (Figure 3a), corresponding to an age ranging from 2 to 29 dph. In September/October 2008, *M. paradoxus* larval length ranged from 2.1 to 7.2 mm (Figure 3b), corresponding to an age of 4–24 dph. *Merluccius capensis* larvae had a narrower size range in September 2007 with 2.6–3.4 mm (Figure 3a) and their age ranged from 7 to 12 dph. In September/October 2008, the size of *M. capensis* larvae was between 2.3 and 7.6 mm (Figure 3b), corresponding to an age of 6–26 dph. In 2007 and 2008, 14 and 24% of hake larvae represented the yolk-sac stage (1.8–3.0 mm), respectively. Larvae larger than 12 mm *TL* were absent from the samples, possibly indicating the avoidance of the sampling gear. In January 2007, the majority of *M. paradoxus* juveniles were between 60 and 90 mm

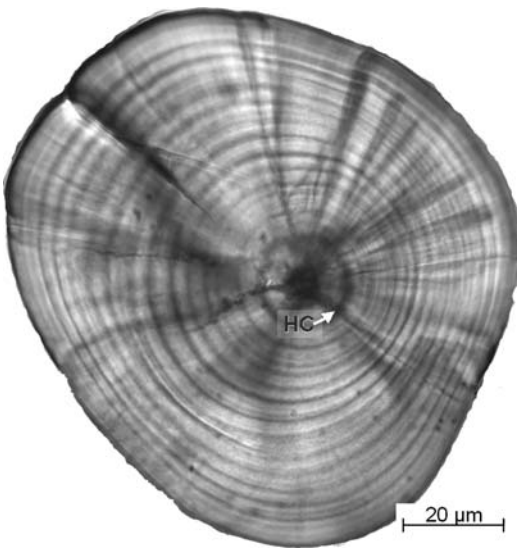


**Figure 2.** Larval otolith of hake, *M. paradoxus* (5.8 mm larval total length), with 17 daily increments at ×1000 magnification. HC, hatch check.

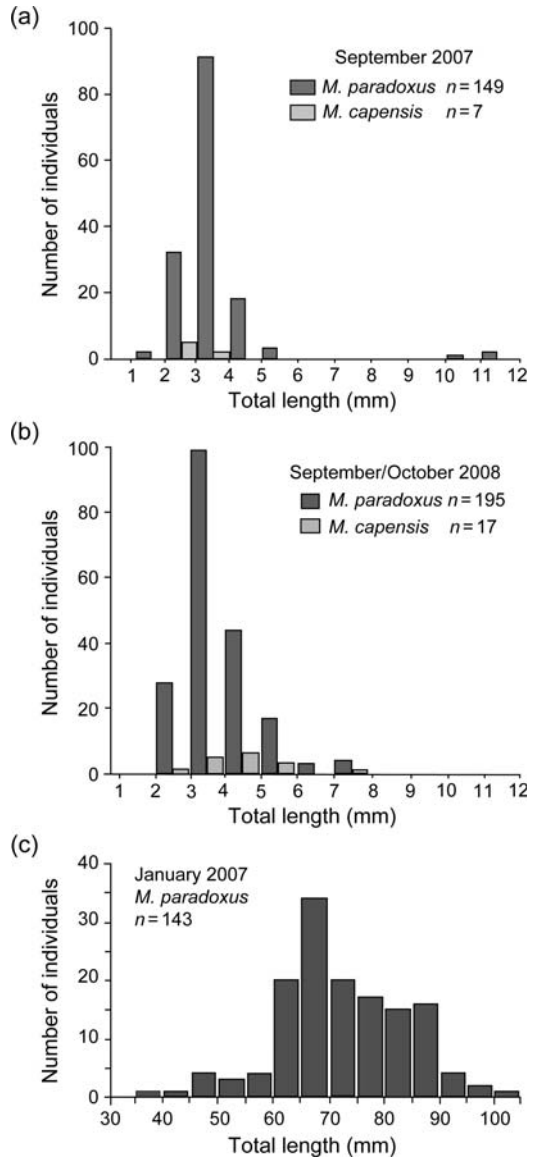


**Figure 3.** Length frequency of *M. capensis* and *M. paradoxus* larvae caught with a Multinet (405 μm) in September 2007 (a) and in September/October 2008 (b) grouped by 1 mm intervals and length frequency of *M. paradoxus* juveniles caught with a trawl in January 2007 (c).

(Figure 3c) with the whole size range from 35 to 105 mm, corresponding to an age range 67–152 dph.

Back-calculated spawning dates of *M. paradoxus* larvae caught in September 2007 ranged between 17 August and 16 September 2007, whereas larvae caught in September/October 2008 were spawned between 29 August and 2 October 2008 (Figure 4). For *M. capensis*, most larvae caught in 2007 were spawned between 6 and 11 September and in 2008 between 16 and 23 September. *M. paradoxus* juveniles caught in January 2007 were spawned between 31 August and 22 November 2006 (Figure 4). The majority of these juveniles had back-calculated spawning dates between 24 September and 22 October 2006 (Figure 4). As sampling size was restricted to ≤100 mm, individuals spawned between August and mid-September 2006 were possibly underrepresented.

## Growth

The growth patterns of *M. paradoxus* larvae were analysed to detect an inflexion point in growth at the transition from yolk-sac to feeding larvae using size-at-age data in a CDA. The discriminant scores of the CDA showed a separation into two groups with 96.9% (Wilks' λ 0.33) of all cases classified correctly, marking a turning point in growth at a length of ~3.0 mm, corresponding to the age of 12 dph. This inflexion point in growth marks the change from slower growth in length and the development of the head to faster growth in length.

The age–length relationship was analysed by fitting age in days (number of increments) and $TL_c$ (mm) data to the Gompertz equation as best suited for the growth description of early stages of fish. As the slope and the intercept of the linear relationship of log-transformed $TL_c$ and age of larvae of the same species did not differ significantly between the two years (ANCOVA, $p > 0.05$ for *M. capensis* and $p > 0.05$ for *M. paradoxus)*, these data were fitted into a single Gompertz function (1) for each species (Figure 5):

$$L_t = \begin{cases} 1.528\, e^{4.205(1-e^{-0.023\,t})} & \text{for } M.\ paradoxus\ n = 227 \\ 1.474\, e^{4.950(1-e^{-0.012\,t})} & \text{for } M.\ capensis\ n = 17. \end{cases}$$

The extrapolated sizes at hatching of 1.53 mm for *M. paradoxus* and 1.47 mm for *M. capensis* in the Gompertz function correspond to the lower end of hatching size found for European hake, *M. merluccius*, ranging from 1.6 to 2.9 mm (Bjelland and Skiftesvik, 2006).

Dry masses (*DM*) of the early larvae follow an isometric growth in relation to total length ($TL_c$) in both species, whereas in the *M. paradoxus* juveniles, the relation of dry mass to total length becomes allometric with an exponent <3:

*M. paradoxus*

$$DM = 0.00179\, TL_c^{2.962}$$
$$R^2 = 0.6654 \quad sb = 0.1382 \quad n = 233 \text{ larvae}$$
$$TL_c \text{ range} = 2 - 7 \text{ mm}$$

$$DM = 0.00803\, TL_c^{2.606}$$
$$R^2 = 0.9045 \quad sb = 0.1142 \quad n = 57 \text{ juveniles}$$
$$TL_c \text{ range} = 35 - 100 \text{ mm}$$

*M. capensis*

$$DM = 0.00196\, TL_c^{3.016}$$
$$R^2 = 0.7672 \quad sb = 0.3812 \quad n = 21 \text{ larvae}$$
$$TL_c \text{ range} = 2.4 - 5.4 \text{ mm}$$

The relationships between *DM* and $TL_c$ for the two species were not significantly different (ANCOVA, $p > 0.05$).

For the back-calculation of the estimated *TL* ($TL_i$), *TL* was plotted against the otolith radius ($OR_i$). The slope and the intercept of the linear relationship of log-transformed $TL_c$ and $OR_c$

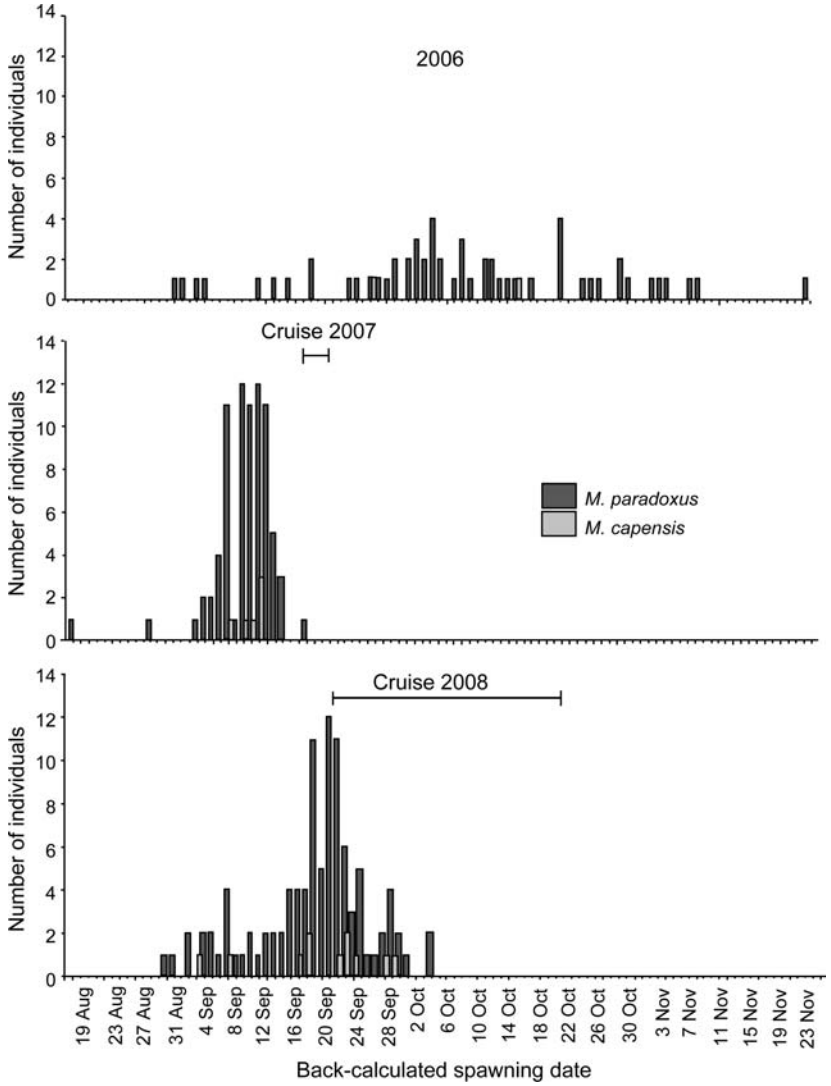


**Figure 4.** Back-calculated spawning dates for *M. paradoxus* and *M. capensis* larvae spawned in 2006 (juveniles caught in 2007), 2007, and 2008. The two plankton sampling campaigns in 2007 and 2008 are indicated as horizontal bars.

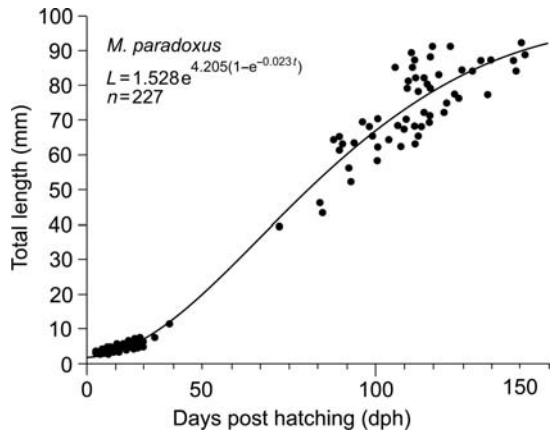


**Figure 5.** Length (mm)–age (dph) relationship for *M. paradoxus* larvae in September 2007 and September 2008 combined and juveniles caught in January 2007 fitted by the Gompertz equation.

of larvae of the same species were not significantly different between the two years (ANCOVA, $p = 0.05$ for *M. capensis* and $p > 0.05$ for *M. paradoxus*) and the data were fitted in single power functions (2) (Figure 6):

*M. paradoxus*

$$TL_i = 0.3941\, OR_i^{\,0.705}$$

$$R^2 = 0.9835 \quad sb = 0.0239 \quad n = 227$$

$TL_c$ range = 2–7 and 35–100 mm

*M. capensis*

$$TL_i = 0.5391\, OR_i^{\,0.5785}$$

$$R^2 = 0.8132 \quad sb = 0.2371 \quad n = 17$$

$TL_c$ range = 2.4–5.4 mm

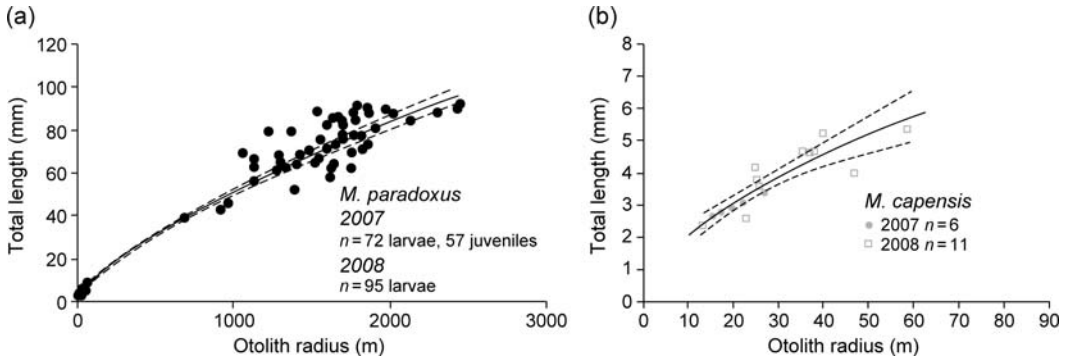


**Figure 6.** Relationship between $TL_c$ (mm) and otolith radius (μm) for *M. paradoxus* (a) larvae and juveniles and for *M. capensis* larvae (b) in 2007 and 2008. Dashed lines indicate the 95% confidence interval for curves.

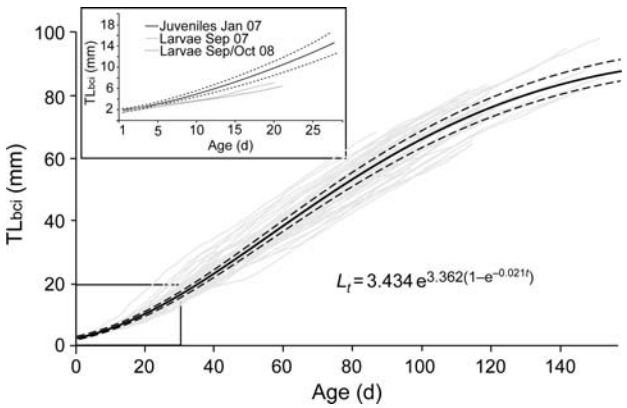


**Figure 7.** Mean back-calculated length-at-age relationship of *M. paradoxus* juveniles hatched in September to November 2006 over the first 150 d. Length as $TL_i$ in millimetres, age in days, dashed lines indicate the 95% confidence interval for the curve. Grey lines indicate the growth of individual fish. (Inset) Mean back-calculated length-at-age relationship of larvae for the first 30 days of life from the September 2007 and September 2008 plankton samples and from juveniles caught in January 2007 that hatched in September–November 2006.

The equations for larvae of the two species were not significantly different (ANCOVA, $p > 0.05$). $TL_i$ was calculated by using Equation (3) for *M. paradoxus*:

$$TL_i = \left(\frac{OR_i}{OR_c}\right)^{0.705} TL_c$$

and used to compare the growth patterns of *M. paradoxus* juveniles from January 2007 and larvae from 2007 and 2008. Back-calculated $TL_i$ of juveniles continuously increased with age (Figure 7). As the juveniles caught in 2007 represent the surviving larvae from spawning events in 2006, $TL_i$ patterns of these juveniles were compared with those of larvae to detect the growth patterns of the survivors. However, no larval sampling was conducted in austral spring 2006. Back-calculated $TL_i$ of *M. paradoxus* juveniles and larvae was similar until the age of 8 d (Figure 7b). From the age of 9 d, mean $TL_i$ of *M. paradoxus* juveniles were higher than those of larvae at the same age in 2007 and 2008.

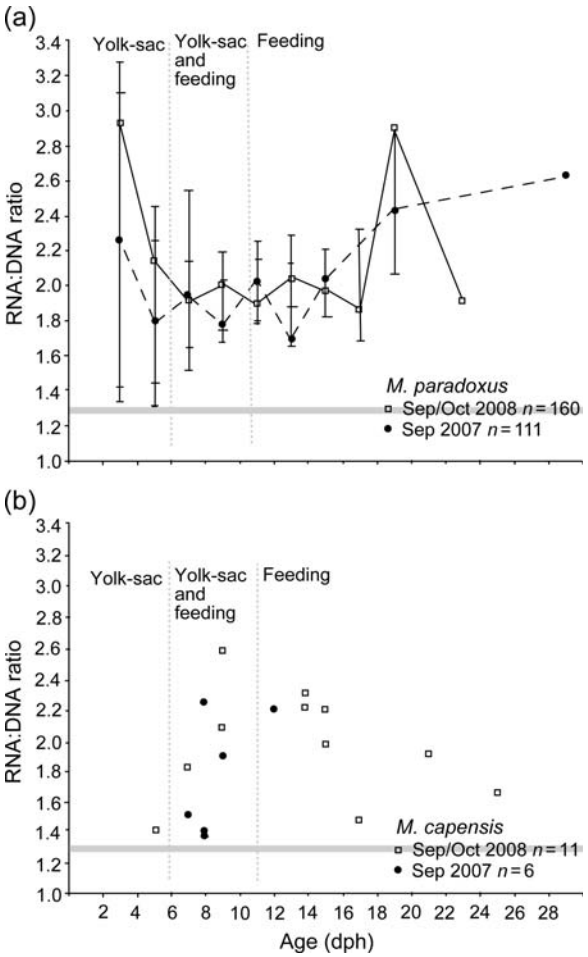


**Figure 8.** Median R:D vs. age (dph) for *M. paradoxus* (a) and *M. capensis* (b) larvae. Whiskers indicate the 25–75 percentiles; grey area indicates the threshold R:D level for growth at a temperature of 10°C.

Interannual differences in the growth trajectory of the first 15 d of life of *M. paradoxus* were found to be significantly different between the survivors from 2006 and larvae of 2007 as well as 2008 (repeated-measures MANOVA, $p < 0.001$). The growth of *M. paradoxus* larvae in 2007 and 2008 was not significantly different (repeated-measures MANOVA, $p > 0.05$). No significant

differences were found between the growth rates of larvae of the two species for both years (repeated-measures MANOVA, $p > 0.05$).

### RNA:DNA ratio

Larval condition and growth potential were estimated by R:D. The R:D threshold level for growth was calculated from instantaneous growth rates [$G_i$; Equation (5)], using CTD-measured ambient temperatures experienced by the larvae. This resulted in R:D threshold levels for hake larvae of 1.3 at 10–11°C, 1.2 at 12–14°C, and 1.0 at 15–17°C. R:Ds of larvae of both species were above the relevant threshold levels in both investigated years (Figure 8a and b), indicating the growth of all larvae. The median R:D of *M. paradoxus* larvae changed with developmental stage, decreasing during the yolk-sac stage, being relatively stable for first-feeding larvae and increasing in feeding larvae (Figure 8a). For *M. capensis*, R:D were low in the yolk-sac stage, increased during first feeding and decreased in more advanced larvae (Figure 8b). The difference in the R:D pattern during the larval development of *M. capensis* compared with *M. paradoxus* is probably related to the small sample size of the former and cannot be interpreted further.

### Growth and condition

To find the indication of a possible coupling between growth and condition in wild-caught larvae, R:Ds, mean growth rates (*MGR* in mm $d^{-1}$) calculated from the last three otolith increments, and medial *in situ* water temperatures by Equation (4) were analysed in conjunction by a PCA for larvae of *M. paradoxus* (Figure 9). PC1 (38% of variance) was characterized by positive loadings of *MGR* (0.8) and temperature (0.7). Contribution of R:D to PC1 was not significant (0.3), whereas PC2 (33% of variance) was almost exclusively explained by the positive loading of R:D (0.9). The contribution of *MGR* (0.004) and temperature (−0.4) to PC2 was minor or negative. This indicates that *MGR* and temperature are more closely coupled factors, whereas R:D seems not to be closely related to *MGR*. Most data occurred in one big cluster, and only a few larval data were placed outside this cluster (Figure 9). High *MGR* (>0.2 mm $d^{-1}$) were never found when larvae

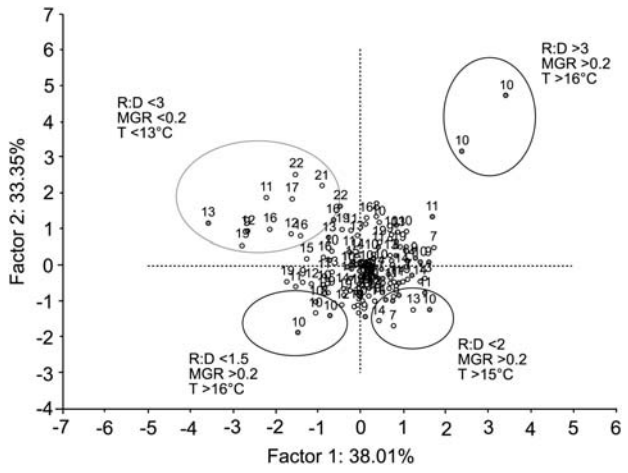


**Figure 9.** PCA of R:D, mean growth rate calculated from the last three otolith increments (*MGR* in mm $d^{-1}$), and temperature (*T* in °C) of feeding larvae of *M. paradoxus* from September 2007 (dark circles) and 2008 (light circles). Numbers indicate the larval age (dph).

experienced temperatures below 15°C, supporting the coupling between temperature and growth rates in hake larvae.

## Discussion

For the first time, the analyses of growth rate and R:D were combined in the same individual larva of *M. paradoxus* and *M. capensis*, presenting the opportunity to directly relate findings from two different growth indices of the same specimen instead of statistical relationships based on analyses of different individuals.

The analysis of otolith daily increments was used to compare the growth of *M. capensis* and *M. paradoxus*. Validations of daily otolith increment deposition have been conducted for *M. merluccius* and *M. productus* (Bailey, 1982; Arneri and Morales-Nin, 2000) and it was assumed that this could be transferred to the South African species. Growth patterns of *M. capensis* and *M. paradoxus* were almost identical during the early life stages in both years. Mean growth rates measured for *M. capensis* and *M. paradoxus* larvae were higher [0.21 mm $d^{-1}$ (s.d. ± 0.06 mm $d^{-1}$ at age 10 d)] than those published for *M. hubbsi* and *M. productus* (0.16 mm $d^{-1}$; Bailey, 1982; Brown *et al.*, 2004). For *M. merluccius* larvae, growth rates ranged from 0.15 to 0.19 mm $d^{-1}$ at a temperature range from 10 to 15°C (Palomera *et al.*, 2005). The findings show that the related hake species have similar growth rates during their early stages, and small differences in the published growth rates are likely related to differences in temperature or in the methodological procedure, e.g. no correction for shrinkage.

The inflection point in the growth of *M. paradoxus* larvae estimated by CDA at the transition from yolk-sac to feeding was around 3.0 mm *TL*, corresponding to 12 dph. The stronger growth of the anterior part in the larvae up to 3 mm is possibly related to the development of the head for feeding. A similar pattern was found in *M. merluccius*, where growth within the first 12 dph was greater in thickness than in length due to the development of the anterior body part (Palomera *et al.*, 2005). Only after complete yolk-sac depletion, which seems to occur shortly after 11 dph (Bjelland and Skiftesvik, 2006), the *M. merluccius* larvae started growing more in length than in height (Palomera *et al.*, 2005).

Similar to the growth pattern, the R:D as a proxy for the condition of hake larvae was found to change with early ontogenetic development. The declining ratio of the yolk-sac larvae seemed to be stabilized by first feeding and appeared to be slightly increasing by external feeding. A similar pattern in the R:D can only be assumed in *M. capensis*, as the small sample size resulted in a low significance. Clemmesen (1994) described an analogical pattern of the R:D during ontogeny for herring larvae. R:Ds of larvae of both *Merluccius* species were well above the relevant threshold levels for growth, indicating that larvae caught were in good condition, with no indication of starvation. However, the calculation of the threshold level was not species-specific and might have been slightly over- or underestimated. On the other hand, starving larvae are more vulnerable to predation and thus may not have been present in the wild catches.

The combined analysis of condition and growth showed that a small number of *M. paradoxus* larvae had high R:Ds and high *MGR*s and, therefore, the highest survival potential. No strong coupling between R:Ds and *MGR*s or temperature was detected, as it has been described, for example, for laboratory-reared cod larvae (Clemmesen and Doan, 1996). For wild-caught larvae, the relationship between temperature, R:D and *MGR* seems to be more complex than results from laboratory experiments suggest. As R:Ds and temperatures do not fully explain the variability of growth rates and

hence survival and mortality patterns, additional factors may influence the growth of the early stages, such as maternal effects (Field *et al.*, 2008) or food quality (St. John *et al.*, 2001), which can be detectable in short-latency parameters like fatty acid composition or amount of essential fatty acids (e.g. Grote *et al.*, 2011).

The difference in the estimated growth rates during the larval life of *M. paradoxus* juveniles and larvae of the two following years possibly indicates the presence of a strong selection for fast-growing larvae in 2006. Similar results were found by Butler and Nishimoto (1997) based on otolith size-at-age data, which showed the evidence of low survival rates for slow-growing Pacific hake, *M. productus* larvae. Threshold levels of growth for survival may vary between cohorts, depending on the intensity of selection pressure and on environmental conditions, and thus these factors may influence year-class strength. To which extent environmental conditions might have influenced growth rates of larvae in 2006 is not known. Therefore, the growth of different developmental stages of hake of the same cohort should be investigated together with environmental parameters in at least two consecutive years in more detail.

The variation in selection pressure on growth between years and ecosystems may explain variances in growth rates of cohorts and species. Low pressure will lead to an overall lower mean growth rate in the cohort, whereas high pressure for fast growth will lead to overall higher growth rates, as the slow-growing individuals are eliminated from the cohort (Robert *et al.*, 2007). Belloc (1935) established the fast growth theory for 0-group hake *M. merluccius* (12–25 mm month$^{-1}$), suggesting a better survivorship for those specimens that pass through the early stages more quickly due to reduced predation pressure. The slow growth theory (7–12 mm month$^{-1}$; Hickling, 1933) was advocated by some growth studies of *M. merluccius* and *M. hubbsi* (Morales-Nin and Aldebert, 1997; Santos and Renzi, 2006). Recent tagging experiments on hake endorsed the fast growth theory (Mellon-Duval *et al.*, 2010 and references therein). In our study, the mean growth rate of *M. paradoxus* juveniles of 19 mm month$^{-1}$ (s.d. $\pm$2 mm month$^{-1}$, age 90–120 d) supports the fast growth theory for a year of assumed high growth-selective mortality. The observed differences in growth rates might be a result of variations in selection pressure.

An indirect reason for high growth-selective mortality during the early life can be the tight aggregating behaviour of hake larvae and juveniles in specific nursery areas (Field *et al.*, 2008) and the cannibalism on younger or slower growing individuals (Payne *et al.*, 1987). In the gadoid species Atlantic cod (*Gadus morhua*), cannibalism occurred in the metamorphosis stage at 12–30 mm length (Folkvord, 1997), which starts in *M. paradoxus* and *M. capensis* at a length of ~12 mm (Olivar and Fortuño, 1991). Cannibalism was observed in older specimens of Cape hake (Macpherson and Gordoa, 1994) and reported for the early stages of European hake, *M. merluccius*, from a semi-intensive culture system (Bjelland and Skiftesvik, 2006). Therefore, size-selective predation by intracohort cannibalism could be a selection pressure on slow-growing individuals in Cape hake.

In conclusion, growth rates of hake larvae in the southern Benguela ecosystem seem to be influenced not only by condition and temperature, but by a more complex interplay of different factors. In years with high selection pressure, fast growth is a key characteristic of survivors, as they speed through the vulnerable early life stages and avoid predation, including cannibalism. The findings indicate that early selection for fast-growth can affect larval survival and possibly recruitment strength of hake.

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