SOME FACTORS INFLUENCING THE TRANSPORT OF CHOKKA SQUID (*LOLIGO REYNAUDII* D'ORBIGNY, 1839) PARALARVAE OFF THE EASTERN CAPE, SOUTH AFRICA

RODRIGO SILVESTRE MARTINS

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Supervisors:
DR MICHAEL J. ROBERTS, Marine and Coastal Management, DEAT
DR ÉRICA A. G. VIDAL, Universidade Federal do Paraná, Brazil
DR COLEEN L. MOLONEY, Zoology Departments and Marine Research Institute, UCT

This thesis is dedicated to my mother, Maria Silvestre Martins, my father, Francisco Martins Filho (in loving memory)*, my brother, Daniel Silvestre Martins, my sister Gabriela Silvestre Martins and my nephews Lucas and Luísa, whose infinite love and support have been essential for my growth in my career and character.*

“SQUID ARE CONSPICUOUS[1]”

Loligo reynaudii d’Orbigny, 1839 paralarva (~ natural size).

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[1] Roberts, M. J., Rodhouse, P., O’Dor, R. K. and Sakurai, Y. 1998. A global perspective of environmental research on squid. ICES CM 1998/M: 27, 20p.

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Declaration

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This work has not been submitted for any other degree at any other university.

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Abstract

Recruitment of chokka squid (*Loligo reynaudii* d'Orbigny, 1839) off South Africa is thought to be related to the successful transport of paralarvae from the spawning grounds located on the Eastern Agulhas Bank to the nursery ground (the 'cold ridge') located on the Central Agulhas Bank. This mismatch of ~100–200 km is thought to be overcome by passive westward transport of paralarvae by shelf currents. This concept has become known as the 'Westward Transport Hypothesis' (WTH) (Roberts, 2005) and was initially tested using a coupled Individual Based-Model (IBM)–Regional Ocean Modelling System (ROMS) model by Roberts and Mullon (in press). The ROMS configuration Results showed that the simulated current patterns on the Agulhas Bank caused large losses of neutrally buoyant particles (representing paralarvae) to the open ocean. Concern was expressed that perhaps the model resolution was too course and moreover that neutrally buoyant particles were not representative of real squid paralarvae.

This thesis focuses on these raised concerns and attempts to refine the modelling approach used to test the WTH. This is achieved by investigating squid paralarval survival and their specific gravity using aquarium experiments together with an improved ocean model and IBM.

The influence of temperature on yolk utilization was assessed through experimentation. Tank experiments found that yolk utilization rates in chokka squid paralarvae are directly affected by temperature with faster rates at high temperature and slower rates at low temperature. Paralarvae incubated at low temperature were found to be larger and had more yolk than their siblings incubated at high temperature. Survival time (with 80% mortality) ranged from 6 d at 12 °C to 5 d at 19 °C. From an energetic perspective, predicted survival time using exponential and linear yolk utilization rates was found to approach the observed survival time only at 12 °C. Overall, the results tended to support the WTH in that even at high temperature (i.e. 19 °C) some paralarvae would be able to reach the cold ridge through passive drift in the shelf current.

The specific gravity properties of early paralarvae were then experimentally estimated and found to range between 1.0373 and 1.0734 g cm^{-3}. The experiments clearly demonstrated that chokka squid paralarvae were negatively buoyant regardless

of yolk content. However, the measured changes in specific gravity were closely correlated with the reduction in the yolk content as a result of its utilization. The observed pattern of change in specific gravity was attributed to selective utilization of yolk components during consumption. The specific gravity data were incorporated into an improved ROMS–IBM model which used outputs from the *South African Experiment* (SAfE) developed by Penven et al. (2006) than the one used by Roberts and Mullon (in press). Paralarvae (i.e. particles) in the model were tracked for seven days during the month of November (i.e. peak spawning). Simulations showed that nearly all particles with specific gravity as a parameter were transported westwards and remained on the shelf. Neutrally buoyant particles, in contrast experienced losses at the southern tip of the Agulhas Bank. The results of this modelling exercise emphasised the importance of paralarval density during the "passive" drift phase of the early life cycle and strongly supported the WTH.

A more complex ROMS–IBM approach was used to address the importance of biologically-mediated vertical position on paralarval transport. Paralarvae (particles) were released every month from the spawning grounds and had their trajectories tracked for 40 d during seven model years. Five variables (*Release area, Year, Month, Specific gravity* and *Diel Vertical Migration* (*DVM*)) were used in the ROMS–IBM experiments. Six release sites were identified spanning the inshore and mid shelf spawning grounds with recruitment success being measured by the number of particles older than 3 days which reached the cold ridge. Overall annual average transport success ranged between 7–51%. A sensitivity analysis using a linear model identified the *release area* to be the most important factor influencing recruitment followed by *specific gravity* and *DVM*. The latter two parameters played an important role in optimizing early transport and retaining paralarvae on the shelf respectively.

It is concluded that the approach of using laboratory based experiments to better define the parameters used in the ROMS–IBM has been a very useful means of testing the WTH. The results obtained here demonstrate quite clearly that the large scale losses observed by Roberts and Mullon (in press) were not realistic. It appears that four factors mainly accounted for this — (1) the domain of the ROMS was extended further east to include Algoa Bay, (2) an improved ROMS and IBM were used with greater spatial and temporal resolution, (3) paralarval density was incorporated into the IBM, and (4) the position of particles (paralarvae) was considered.

Chapter 1 — Introduction and aims

Squids of the family Loliginidae are typical inhabitants of shelf and coastal waters around the world, excluding the polar latitudes (Roper et al., 1984). They constitute an important prey component of the pelagic trophic chain as well as acting as sub-dominant predators (Santos and Haimovici, 1998). Many species have sustained commercial fisheries worldwide due to their relatively high, albeit very variable, biomass (Boyle and Rodhouse, 2005).

In South Africa a single species, *Loligo reynaudii* d'Orbigny, 1839, locally known as 'chokka', sustains an important semi-artisanal fishery, which has been intensively studied over the past 20 years (Augustyn et al., 1994). Importantly, efforts towards understanding the relationships between the species and the complex oceanography off the South African coast (synthesized in Roberts and Sauer, 1994 and Roberts, 2005) have shown that their distribution and life cycle are largely environmentally driven. Whereas the environmental influences on adult squid are relatively well studied, there is a scarcity of similar information on the planktonic early life stages (i.e., paralarvae; Young and Harman, 1988). Despite this, it is hypothesized that paralarval transport from the spawning to the nursery grounds shortly after hatching can play a major role in recruitment and thus abundance of adult squid seasonally available to the fishery (Roberts, 2005).

Background information on the chokka squid species follows.

1.1. The reproductive habitat of chokka squid

The Agulhas Bank (Fig. 1.1), regarded as both an important fishing ground and nursery area for a number of warm temperate demersal and pelagic fish species, including chokka squid (Hutchings et al., 2002; Roberts, 2005), is the broadest extension of the South African shelf. It is triangular in shape, with an area of 116 000 km^2 and a mean depth of approximately 100 m. The oceanography is complex and varies seasonally. The main driving forces of this are the fast-flowing Agulhas Current and wind-driven coastal upwelling (Roberts and Sauer, 1994; Hutchings et al., 2002; Roberts, 2005). Few chokka squid paralarvae have been found (Fig. 1.1) in the field.

Roberts and Sauer (1994) and Roberts (2005) confirmed chokka squid to be quite specific with regard to spawning habitat selection with spawning being restricted to sandy and low profile reef bottoms located in narrow areas between Plettenberg Bay and Port Alfred (Fig. 1.1). Here environmental conditions such as temperature, dissolved oxygen, turbidity and bottom type are considered suitable for both mating, egg deposition (Roberts, 1998), embryonic development and hatching success (Oosthuizen et al., 2002a). The extent of the spawning grounds has been recently investigated, and found to extend from the nearshore (< 40 m) to the mid shelf (150 m) (Oosthuizen and Roberts, 2009). This means that spawning occurs between two very different environments.

1.1.1. Oceanography

Several factors influence the oceanography on the Agulhas Bank. These include the Agulhas Current, the prevailing wind field, coastal trapped waves, internal tides and seasonal insulation (Boyd and Shillington, 1994). The Western Agulhas Bank (WAB) is particularly different from the rest of the bank because it is influenced by both the Benguela Current and the Agulhas Current. Also inshore, intense upwelling is induced by the local wind field during summer. The extent of coastal upwelling on the Western Agulhas Bank often means it is considered an extension of the Benguela upwelling system (Largier et al., 1992). The shelf edge is often strongly influenced by the end of the Agulhas Current. The interaction between the coastal upwelling region and the shelf-edge maintains a strong thermal stratification on the mid-shelf — i.e. intrusions of warm Agulhas Current water at the surface and cold upwelling central water beneath the thermocline. The flow in the region is mostly westward, with narrow and strong currents along the mid shelf upwelling front and the shelf-edge. These currents merge at Cape Point due to a narrowing of the shelf, and result in the formation of Benguela Jet (Largier et al., 1992).

The Central and Eastern Agulhas Bank are characterized by a strong interaction between the Agulhas Current and the shelf topography (Fig. 1.1). The coastal domain is influenced by wind-driven intermittent upwelling at prominent capes, with the cold upwelled water often flooding the adjacent embayments particularly during autumn–summer when upwelling favourable easterly winds predominate (Schumann et al., 1982). Strong thermal stratification on the shelf occurs during summer as a result of solar insulation. In winter, a uniformly well mixed, deep, upper layer occurs on the shelf due to the erosion of the thermocline by storms and wave action. Shelf edge upwelling is also a strong feature in the region caused by the divergence of the Agulhas Current from the coast near Port Elizabeth. This causes cold water to surface between Algoa Bay and Port Alfred where the shelf narrows, and is often leads to cold water moving westwards onto the Eastern Agulhas Bank.

A subsurface tongue of cool upwelled water, known as the ‘cold ridge’, is a quasi-permanent dominant feature on the Eastern/Central Agulhas Bank. This seems to originate from upwelling filaments along the Tsitsikamma coast and extends obliquely offshore roughly over the 100-m isobath (Roberts, 2005) (Fig. 1.1). Circulation on the Central and Eastern Agulhas Bank is thought to be related to the cold ridge. Data collected from ships indicates that the water column on the Eastern Agulhas Bank (EAB) flows westward and offshore around the ridge while inshore surface waters flow eastward, collectively establishing a cyclonic flow around the feature (Boyd and Shillington, 1994).

1.1.2. Primary and secondary production

Although not as productive as the Benguela upwelling system on the west coast, the Agulhas Bank is thought to sustain levels of productivity that support the region as a nursery ground for a number of commercially important fish species and chokka squid (Hutchings et al., 2002). Phytoplankton biomass (measured by chlorophyll levels) has been shown to be closely related to the thermocline (Probyn et al., 1994). Largest mean concentrations (> 3 mg m^{-3}) are found in the coastal upwelling areas of the WAB. In the east, chlorophyll concentrations of > 2 mg m^{-3} are found in Algoa Bay and seem to be associated with shelf edge upwelling caused by the divergence of the Agulhas Current. Inshore and offshore chlorophyll maximum zones exist throughout the Bank. The former is associated with coastal upwelling and may reach peaks of 6–12 mg m^{-3} between Plettenberg Bay and Algoa Bay (Shannon et al., 1984). The chlorophyll maximum on the cold ridge (> 2 mg m^{-3}) occurs at depths of 10–20 m and is associated with the doming of the thermocline. Phytoplankton concentrations vary seasonally with mean chlorophyll concentrations ranging from 2–4 mg m^{-3} in autumn and spring and considerably smaller concentrations (< 1–2 mg m^{-3}) during winter and summer (Probyn et al., 1994).

Secondary production is not as high as that found on the west coast. Zooplankton biomass seems to be limited by primary production and pelagic fish predation, and is dominated by copepods (Verheye et al., 1994). Zooplankton species composition is found to vary seasonally inshore whereas the shelf waters are dominated by the calanoid copepod *Calanus agulhensis* throughout the year. This species alone accounts for up to 82% of mean standing stock of all copepods across the Bank (Verheye et al., 1994). The centre of distribution of *C. agulhensis* is associated with the cold ridge and its biomass appears to respond to the intensity of this oceanographic feature as well as with the cyclonic circulation around the ridge (Largier et al., 1992). *C. agulhensis* is apparently responsible for the moderate levels of chlorophyll in the ridge because of selective grazing of large phytoplankton cells (Huggett and Richardson, 2000). Various life stages are thought to be exported to the WAB where they replenish local populations and feed adult anchovy (Largier et al., 1992). The zooplankton concentration maximum is often associated with the chlorophyll maximum in the thermocline and ranges from 100–350 particles L^{-1} mostly microcrustaceans (Verheye et al., 1994). This means that food availability is greatest between the surface and the thermocline depth (20–50 m, Swart and Largier, 1987).

1.2. Characteristics of early loliginid paralarvae

Similar to some gastropods, loliginid squid lay yolk-rich eggs sheathed in a gelatinous capsule which is attached (glued) to the seabed. These finger-like egg capsules are thought to maximize embryonic survival because they provide (1) a physical and chemical buffer against unsuitable environmental conditions, (2) protect against microbial attack and (3) are unpalatable to predators (Boletzky, 2003). Embryo mortality is consequently minimized and hatching success maximized. Observations indicated that most paralarvae hatch in darkness and this is thought to reduce predation by visual predators (Paulij et al., 1990; Vidal et al., 2005). Early paralarvae have an internal yolk reserve that ensures survival while they improve their predatory skills (Vidal et al., 2005). However, this reserve is ephemeral because of the fast metabolism of cephalopods and the need for paralarvae to quickly find sufficient food to avoid starvation (Vidal et al., 2002). Paralarvae generally become competent planktonic predators within hours (Boyle and Rodhouse, 2005). This very early life stage characterised by limited food and high, vulnerability is considered to be the cephalopod equivalent of the 'critical period' of fish larvae (Hjort, 1914; Vidal et al., 2005). Paralarval natural mortality, however, is considerably lower than in their fish larvae competitors (O'Dor, 1998) and it appears that large, fast-growing, paralarvae are more likely to survive than their smaller, slow-growing, siblings (Steer et al., 2003; Vidal et al., 2006).

Not surprisingly, highest paralarval concentrations have been found in the immediate vicinity of the spawning grounds and in upwelling zones (Rocha et al., 1999; Zeidberg and Hamner, 2002; González et al., 2005; Martins and Perez, 2006; Moreno et al., 2009). Paralarvae also exhibit Type-I diel vertical migration behaviour, remaining deep during the day and surfacing at night (Zeidberg and Hamner, 2002; Moreno et al., 2009). Although paralarvae are already very active and free-swimming on hatching, their swimming velocities are generally slower than shelf ocean currents and therefore dispersal must also be caused by flow patterns (Boletzky, 2003; Boyle and Rodhouse, 2005).

For chokka squid there is considerably less information on the paralarvae than for the adults. The embryonic development and morphology of wild-caught paralarvae have been described in detail by Vecchione and Lipiński (1995), Blackburn et al. (1998) and Oosthuizen et al. (2002a). Food and feeding of chokka paralarvae have been studied using immunoassay analysis which showed that copepods and euphausiids are important prey items (Venter et al., 1999; Hoving et al., 2005). Field survey collections showed most paralarvae are found on the spawning grounds near the surface (Fig. 1.1) (Sauer, 1995; Roberts and van den Berg, 2002), Little is known about their vertical distribution (Roberts and van den Berg, 2002).

More recently, emphasis has been placed on the influence of the physical environment on paralarval ecology. In particular, the importance of the oceanography on larval transport and implications for recruitment prompted field studies with measurements using Acoustic Doppler Current Profilers (ADCPs), satellite-tracked drifters and model simulations using a coupled Regional Ocean Modelling System (ROMS)−Individual-Based Model (IBM) (Roberts and van den Berg, 2002; 2005; Roberts et al., in prep.; Roberts and Mullon, in press). Also, the influence of temperature on embryonic development and hatching success has been studied, both in the laboratory and the field (Oosthuizen et al., 2002a,b; Oosthuizen and Roberts, 2009). Growth and yolk utilization for paralarvae reared at 16 °C were described by Vidal et al. (2005).

1.3 The Westward Transport Hypothesis

These ecological studies (Roberts and van den Berg, 2002; 2005) have led to the development of a working hypothesis which attempts to explain the mechanisms underpinning recruitment of chokka squid. The “Westward Transport Hypothesis” or WTH as it has become known as, states that paralarvae hatched on the main spawning grounds located on the EAB are expected to be transported westward by currents on the shelf towards the cold ridge where enhanced productivity would provide a suitable food environment for paralarvae (Roberts, 2005). The WTH is based on Bakun’s (1996) suggested requirements for successful recruitment — enrichment, concentration and retention in the ecosystem, For chokka squid this implies that recruitment depends on the survival of paralarvae in terms of food availability, feeding success (copepods biomass, density distribution and patchiness) and retention in the ecosystem. The westward transport of paralarvae to the cold ridge is clearly a key component of the WTH.

1.4. Aims and hypotheses

Chokka squid spawning appears to be restricted to sandy and low profile reef bottoms located off the Eastern Cape, both inshore and on the shelf. While environmental conditions are clearly conducive to mating and embryonic development, there appears to be a 100–200 km mismatch between the location of optimal food concentrations near the cold ridge and the place of hatching (see Fig. 1.1). The WTH proposes that this mismatch between the spawning and nursery grounds is overcome by the net westward passive transport of paralarvae in the mid shelf currents (Roberts, 2005). The primary aim of this thesis is to test this hypothesis.

Zooplankton (food) concentration on the Agulhas Bank is greatest in the region of the cold ridge because of the upward doming of the local thermocline which brings nutrients to the photic zone. This enhances both primary and secondary productivity in the area. It is thought that early stage chokka paralarvae will need to survive a food-limited period of several days during transport from the spawning to the nursery ground, relying mostly on their internal yolk reserves. But experiments at 16 °C indicate that yolk utilization is temperature-dependent for chokka squid (Vidal et al., 2005). To extend this understanding, starvation experiments needed to be conducted for a range of temperatures that chokka paralarvae would be exposed in the wild. Hypothesis 1 (*H1*):

Null hypothesis (H_0): Environmental temperature does not affect yolk utilization rates and thus has no influence on early survival

Alternative hypothesis (H_A): Environmental temperature affects yolk utilization rates and thus influences early survival

Passive transportation from the spawning to the nursery ground should be affected by the specific gravity (density) of the paralarvae. Whereas positive buoyancy can translate into faster transport in the surface currents, negative buoyancy could result in a longer transport time in the sluggish deep currents or shorter distances for a given period. In early paralarvae, specific gravity can be affected by the yolk content and its rate of utilization. Changes in specific gravity can therefore influence dispersal. Hypothesis 2 (*H2*):

H_0: *Specific gravity is not influenced by the yolk content and has no effect on dispersal*

H_A: *Specific gravity is influenced by the yolk content and affects dispersal*

Biologically-mediated vertical positioning in the water column can provide profound ecological challenges to planktonic organisms, resulting in trade-offs between feeding and avoidance of predators. Also, varying velocities in the vertically-stratified current field can affect transport and retention within the nursery grounds. The question arises: Can chokka squid paralarvae influence their vertical positions and consequently their transport to the cold ridge? Hypothesis 3 (*H3*):

H_0: Paralarval transport is not affected by biologically-mediated vertical position in the water column

H_A: Paralarval transport is affected by their biologically-mediated vertical position in the water column

1.5. Structure of the thesis

This thesis comprises of six chapters. Chapter 2 describes the rearing aquarium system designed, manufactured and used to perform the starvation experiments. Chapter 3 focuses on *H1* and examines the influence of temperature on yolk utilization rates and affects survival of starved early paralarvae. Chapter 4 describes the specific gravity characteristics of paralarvae and how yolk utilization rates affect this variable. This information is then incorporated into a coupled ROMS–IBM, which is run during the seven days post-hatching period to address *H2*. Chapter 5 focuses on *H3* and investigates how biologically-mediated vertical positioning in the water column can influence paralarval transport using a more complex ROMS–IBM experiment than that in Chapter 3. The final chapter synthesizes the results of chapters 3, 4 and 5 and revisits the validity of the WTH. Chapters 2, 3, 4 and 5 are structured as stand-alone sections of research each with an abstract, introduction, methods, results and discussion. Some repetitions occur in the introductions of these chapters.

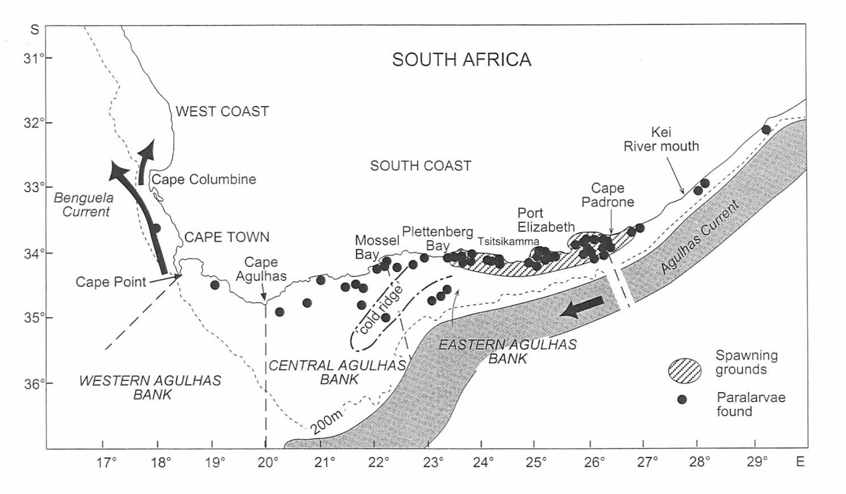


Fig. 1.1. Map depicting the main chokka squid spawning grounds and the positions where paralarvae have been found off the South African coast (from Roberts and van den Berg, 2002 after Augustyn et al., 1994, with permission).

Chapter 2 — Design and manufacture of a temperature-controlled, open, rearing system for chokka squid (*Loligo reynaudii* d'Orbigny, 1839) paralarvae

Abstract

A flow-through, temperature-controlled seawater system was designed and manufactured for the rearing of chokka squid (*Loligo reynaudii* d'Orbigny, 1839) paralarvae. This was based on experience gained from a closed system designed for a similar purpose in Brazil. The system comprised of two sets of three 1 m diameter tanks, with each set being independently temperature-controlled. A separated single tank (tank 7), also temperature controlled, was used for egg development. Water temperature in the system was able to be maintained within the range of 9 to 21 °C using a heat exchanger and point-monitoring software. The system was capable of maintaining water temperature within 0.1 °C of the target temperature over long periods (> 30 days). Make up water was filtered down to 10 µm, skimmed and UV-sterilized before being introduced into the system. Dissolved O_2, salinity and nitrite levels were monitored and found to be similar to natural levels in the surrounding ocean.

2.1 Introduction

Loligo reynaudii d'Orbigny, 1839, locally known as 'chokka', can be characterized as one of the best studied squid species in the world (Augustyn et al., 1994). In the past 20 years, comprehensive studies were undertaken on its systematics, population biology, ecology, reproduction, fishery and behaviour (Roberts, 2005). These focused not only on the general biology, but also provided important elements for the management of this valuable fishery resource for South Africa (Augustyn et al., 1992).

Whereas the biology and ecology of adult squid are relatively well studied, little research has been directed at the early stages. Work has been done on embryonic development and hatching success (Blackburn et al., 1998; Oosthuizen et al., 2002a,b), early growth and yolk utilization (Vidal et al., 2005) and, indirectly, on larval transport (Roberts and van den Berg, 2002; 2005). Despite those efforts, however, little information is yet available on paralarval biology. Nonetheless, this situation has begun to change as the first successful chokka squid rearing experiment was recently conducted in Brazil (Vidal et al., 2005). This yielded a survival time of 42 days, and aspects such as yolk utilization, growth and survival were studied under fed and starved conditions at 16 °C in a recirculating (closed) rearing system.

Loliginid squid rearing studies have undergone important advances in recent years, mostly because of improvements in tank designs, feeding and water quality (see Vidal et al., 2002 and references therein). Most of the effort has concentrated on using closed seawater systems with temperature-control (Hanlon et al., 1987; 1989; Yang et al., 1989; Lee et al., 1994; 1998; Vidal et al., 2002a; 2005). Maintenance of the water quality can be difficult and laborious, and depends upon the mechanical and biological filter system used. Often periodic intervention is necessary for maintaining salinity, pH and trace elements (Yang et al., 1989; Hanlon, 1990; Lee et al., 1998; Huguenin and Colt, 2002). A few studies have been performed using flow-through (open) seawater systems (e.g. LaRoe, 1971; Chen et al., 1996; Preuss et al., 1997; Villanueva, 1994, 2000) which offers good water quality, but presents challenges with the control of temperature (Hanlon, 1990).

Temperature has been hypothesized to influence many aspects of the squid life cycle. But the ability to accurately control and stabilize temperature in tank systems when undertaking experiments on growth and metabolism has been a major challenge. For instance, it is hypothesized that temperature differences as low as 1 °C may affect growth rates (the so-called 'Forsythe Effect'; Jackson, 2004). To test this, however, has not been possible thus far. Furthermore, in the case of chokka squid, temporal analysis of satellite sea surface temperature (SST) data and squid jig catches has demonstrated that good years of recruitment to the fishery were statistically correlated with abnormally low SST in El Niño years (Roberts, 2005). Laboratory studies to test this, as well as the ramifications of the temperatures on aspects such as paralarval growth, survival and recruitment, are clearly desirable.

In view of this, a chokka squid paralarvae rearing experiment commenced in South Africa in 2003, with the aim of providing a reliable source of live specimens of known age, in good condition, and at many developmental stages for studies on growth and yolk utilization rates.

The objective of this chapter was to adapt the methodology proposed by Vidal et al. (2002a), to the South African system. The method given in Vidal et al. (2002a) has been the most successful for rearing medium-sized Loliginid paralarvae and has been used for rearing chokka squid in Brazil (Vidal et al., 2005). In contrast with the closed (recirculating) system used by those authors, however, we developed a flow-through (open) system for South Africa. In addition, temperature-control was developed and integrated for this open seawater rearing system. Special care was taken to reproduce, as best as possible, the environmental conditions on the spawning grounds located on the eastern Agulhas Bank (Roberts, 2005).

2.1.1. Tank design system and operation

The rearing system was built at the Marine and Coastal Management (MCM) Marine Research Aquarium in Cape Town. This is a coastal research facility which receives fresh seawater directly from the adjacent ocean, renewing the whole volume of its reservoirs approximately four times per day (D. Horstman, Marine and Coastal Management, pers. comm., 2009). The Aquarium has four reservoirs, two of which store sea water filtered with a 18 µm filter (300 000 L), one reservoir with a10 µm filter (10 000 L) and the fourth with a 1 µm filter (1 000 L). The air temperature inside the facility is constantly maintained, year round, at ~ 20 °C. The photoperiod (light:dark) at the rearing system was set at 11.5 h:12.5 h in spring and 10.5 h:11.5 h in summer, when the majority of experiments were conducted.

A schematic view of the whole rearing system is depicted in Figure 2.1. Experimental tanks were manufactured according to the original design of Vidal et al. (2002a). These consisted of seven 220 L rearing cylindrical tanks, made of black High-Density Polyethylene (HDP) plastic (0.95 m diameter × 0.40 m height; working volume: 212.6 L), three buffer cylindrical tanks made of white Linear Low-Density Polyethylene (LLDP) plastic (0.46 m diameter × 1 m height), each holding a working volume of ~ 153 L and two 1 500 L reservoir tanks made of green LLDP plastic (1.07 m diameter × 1.50 m height; working volume: 1 079 L). The two reservoirs were used to store cold water (~ 5–9 °C) refrigerated by a heat exchanger (see below). Reservoirs, experimental tanks, buffer tanks and cold water pipes were coated with Expanded Polyethylene (EP) foam (1.0 cm thick) for thermal insulation. The entire system held a volume of approximately 4 157 L.

The experimental tanks were divided into two rearing lines with three tanks each. This allowed experiments to be done with three replicates. A dedicated egg incubation/hatching tank (hereafter referred to just as 'hatching tank') was also included (Fig. 2.2a). A rectangular (18 × 9 cm) acrylic translucent window was installed in the sidewall of each tank for visual observation. A black HDP lid was used to cover the window during the no-observation periods. The outlet pipe in the centre of each tank was suspended 7 cm above the bottom. To prevent the paralarvae and food organisms from being removed from the tank, these outlet pipes were fitted with a plastic core (15 cm diameter) covered with Nytex mesh netting (150 µm) (Vidal et al., 2002a). Illumination was provided by ambient light (i.e., daylight coming through the windows plus the lights of the Aquarium) and three fluorescent bulbs (Degenbac© Super Red T8 BR-HD 40 watt) suspended at a height of one meter above the tanks (Fig. 2.2a). The light levels were 60 lx at the water surface.

The make up water was initially supplied to the system from the main reservoir of the MCM Research Aquarium (18 µm). The water first passed through a 10 µm pore size (18.6 m^2 surface area) modular particle filter (Clean & Clear Cartridge Filters, Pentair Pool Products™, Stanford, NC, USA), then was pumped to a protein skimmer and finally through a 30-W UV sterilizer (Aquamarine Water Treatment®, Cape Town, South Africa) before entering the first of the reservoirs at a rate of ~ 3 L min^{-1}. The first reservoir was connected to a second by a balance line. A second modular particle filter was attached to the second reservoir to ensure filtration of up 90% of its volume. Both filters were manually cleaned once a week. From the fist reservoir the water was pumped to the buffer and experimental tanks as well as to the heat exchanger where it was cooled. From the heat exchanger, the cooled water was pumped back to the second reservoir. The excess water was continuously drained by outlets placed in the first reservoir, in the buffer tanks and in both rearing lines and the hatching tank (Fig. 2.1).

Filtered cold water was fed to the buffer tanks via PCL-controlled solenoid valves (Brights Richdel 2400-MT) and then pumped to the rearing tanks. Water was supplied into the experimental tanks by 0.40 m long, 2.5 cm diameter jet bars evenly perforated on one side (2.0 mm holes, spaced 15 mm apart). The perforated side of the jet bars was tilted at 45–50° and suspended ~ 2 cm above the tank water surface to generate a gentle counter clockwise current. Unlike the design described by Vidal et al. (2002a), the inflow water pipe was connected by a T piece to the middle of the jet bar instead of at one of the sides. This allowed an even distribution of the water pressure within the bar and ensured a smooth water flow through the jet bar holes. The inflow rate to the jet-bar was controlled by a magnetic flow control valve (TIP®, Italy, range: 75–750 L h^{-1}).

2.1.2. Temperature-control system

Heat exchanger

A heat exchanger was used to cool the sea water. This was achieved by way of counter flow by passing the water through a titanium honeycomb-like heat transfer panel. The working fluid used was monoethyleneglycol [$C_2H_4(OH)_2$] solution, in the proportion of 2 parts glycol to 3 parts of tap water. The condensers were cooled with seawater provided by the Aquarium reservoir. The placement of safety sensors in the inlet and outlet pipes allowed the cooling system to automatically close down in the case of disrupted flow of seawater, and so avoid damage to the components. The temperature range for the chiller was 0–10 °C.

PLC control and temperature control software

A Programmable Logic Controller (PLC) was incorporated in the rearing system for the automation of the temperature control. Input data were received from the temperature sensors attached to the reservoirs, rearing lines and the hatching tank. These data were fed to a PC where specifically designed software managed the temperature throughout the tank system. The software was Windows-based and controlled the opening and closing of the solenoid valves, calculating from the input temperature data the number of times and intervals that cold water should be delivered to the buffer tanks of the rearing lines and the hatching tank to reach the desired temperature set point (Fig. 2.2a).

The desired tank temperatures could be achieved within 24 h, depending on the experiment, and if enough cold water was available, within one hour in some cases.. This allowed the carrying out of experiments using two different temperatures (one at each rearing line) simultaneously (see Fig. 2.2a, b). The time intervals between which the solenoids were open (breakpoints) were a function of the temperature difference between the desired set point and the actual readings (i.e., the greater the difference, the longer the solenoid remained open).

The design of this system allows water to be cooled to a minimum of ~ 11–12 °C. Higher temperatures were the result of the heat collected by the pumps, the friction between water and the pipe’s inner surface, friction generated by the water passing through the jet bar holes and by heat transfer at the air/water interface at the surface of the tanks. The program managed heating by decreasing the amount of water delivered by the solenoid valves. The software also presented real time temperature trend lines (Fig. 2.2b), logged temperatures and the number of times and the time intervals in which each solenoid valve opened.

2.1.3. Water quality, current velocity and illumination

Monitoring

The pH (range: 2–20, accuracy: ± 0.2 units, resolution: 0.01 units), dissolved O_2 (range: 0–50 mg L^{-1}, accuracy: ± 0.2 mg L^{-1}, resolution: 0.01 mg L^{-1}), salinity (range: 0–50 psu, accuracy: 0.5% of reading, resolution: 0.01 psu) and temperature (range: −5 to 50 °C, accuracy: 0.08 °C, resolution: 0.01 °C) were measured with a multi-variable sensor system (Eureka, Eureka Environmental, Texas, USA). Temperature was also continuously monitored by the sensors of the PCL control system. Ammonia and nitrite were measured using pre-packaged reagents kits (AquaMerck®, Merck, Germany; precision of 0.5–10.0 mg L^{-1} for NH_4^+ and 0.05–1.0 mg L^{-1} for NO_2^-).

Preliminary current measurements were done along the tank radius, using the jet bar as a reference, and vertically every 5 cm by using a ultrasonic flow meter (FlowTraker® Handheld ADV, SonTek, San Diego, CA, USA; range ± 0.001–4 m s^{-1}; accuracy: ± 1% of reading; resolution: 0.0001 m s^{-1}).

The volume of water exchanged daily was estimated by multiplying the number of times and intervals that the solenoids were open (as logged by the PLC control) by the average volume delivered by the solenoid valves. The latter was estimated by using a measuring cylinder and the time intervals logged by the PLC control ($n = 60$) for both rearing lines and the hatching tank. Measurements were done for each water inlet at each breaking point time interval for both rearing lines and the hatching tank. The turnover rate was then estimated by dividing this value by their holding volumes.

Performance and water quality

The performance of the system was assessed during all the experiments. For brevity, only data from Experiment 2 (see Chapter 3), where experimental temperatures were 12 and 19 °C respectively, are shown. Measurements of water quality parameters were done once a day in each rearing line in order to evaluate the consistence of conditions among the rearing tanks, which were then compared (excluding temperature) using Students *t* test.

Long term monitoring was assessed during all the experiments conducted producing time series of water quality parameters encompassing the whole duration of the experiments. Again, for simplicity, only data from Chapter 4 is shown in the present report.

Water quality

A comparative summary of water quality conditions surveyed in this study are show in Table 2.1. In general, the water quality of the seawater in the tanks was the same as that found in the ocean and supported the standards recommended for squid rearing (Yang et al., 1989; Hanlon, 1990; Lee et al., 1998). This includes the all the parameters of the chokka squid rearing experiment conducted by Vidal et al. (2005), except for pH.

Apart from experimental temperatures, there was virtually no difference in water quality parameters between rearing lines at the set up temperatures. Comparisons made between rearing lines showed that only the dissolved O_2 levels differed significantly (Students $t = -7.85$, $p < 0.00001$) which was clearly a result of the temperature-related solubility of this gas in seawater (Huguenin and Colt, 2002). Temperature showed no statistical differences within each rearing line, and the maximum difference found was 0.3 °C.

Measurements showed that average current speed was 1.3 cm s^{-1} for an inflow rate of 1.25 L min^{-1}. This falls within the range of 1.0−1.4 cm s^{-1} recommended by Vidal et al. (2002).

Long term stability

The system was fully operational during the experiments. Variations in all water quality variables analyzed were negligible for each treatment (Fig. 2.3). Temperature was also stable during the experiments (see Fig. 3.1, Chapter 3). The turnover rate of the experiments monitored ranged between 40–44 × tank volume d^{-1} (hatching tank) and 13–44 × rearing line volume d^{-1} (rearing tanks).

2.2. Discussion

Water quality standards for squid rearing were established empirically from three decades of squid laboratory experiments, the majority of which used recirculating systems. A large amount of this work was based on the Californian market squid (*Doryteuthis opalescens* Berry 1911) (e.g. Hurley, 1976; Hanlon et al., 1979; Yang et al., 1980; Yang et al., 1983a,b; Yang et al., 1986; Yang et al., 1989; Vidal et al., 2002) and more recently, on the Bigfin reef squid (*Sepioteuthis lessoniana* Ferussac, 1830) (Hanlon, 1990; Lee, 1994; Lee et al., 1994, 1998). However, the environmental conditions of the squid's natural habitat have not been studied in detail. The aim of the squid rearing system described here was to mimic the specific environmental conditions experienced by the chokka squid (*Loligo reynaudii*) on their spawning grounds.

This was done to mitigate the stress associated with placing squid paralarvae in laboratory experimental tanks, and thus reduce the mortality during experiments. Fortunately, the environmental conditions on the spawning grounds of the species are relatively well known (Roberts and Sauer, 1994; Roberts, 2005). Also of importance was the similarity of conditions between experimental tanks.. Uniformity of conditions among the replicate tanks is highly desirable, but often overlooked or even unreported, when one conducts experiments with replicate tanks (Underwood, 1997).

A second and important aim of this project was to develop a high resolution, computerized, temperature controlled system for an open seawater system. Moreover, it was important to concurrently run two different temperatures using hatchlings of the same origin. This would ensure a direct and reliable comparison of results. In the case of chokka squid, this is particularly important as the species spawns in two very contrasting thermal environments. The inshore spawning grounds are highly variable with an average temperature of 15.3 °C. But eggs are also exposed to extreme temperatures of 9 °C during upwelling events and 21 °C when the water column is mixed (Roberts and Sauer, 1994). In contrast the mid-shelf deep spawning grounds are more stable with temperature in a narrow range (8.2–13.7 °C) (Roberts and Oosthuizen, 2009). The rearing system developed here allows these temperature regimes to be reproduced. The open seawater system has the advantage of eliminating concerns regarding accumulation of toxic nitrogenous compounds (Hanlon, 1990; Huguenin and Colt, 2002). Also, there is no removal of trace elements often caused by biological filtration (particularly strontium). Lack of these elements has been shown to cause abnormal statolith formation in squid paralarvae (Hanlon, 1990).

However, while the tank system functioned well, several defects came to light. First, despite the fact that the MCM Research Aquarium is located near the ocean and continuously receives fresh seawater through its seawater collecting system, the pH was found to be below the levels measured in the adjacent ocean (see Table 2.1). The causes of low pH are elusive, but perhaps may be related to heterotrophic production of CO_2 due to the accumulation of organic matter in the Aquarium's filters or even somewhere else inside the piping system. This indicates that a correction system is required in the tank system if the natural spawning environment is to be reproduced.

Secondly, several attempts were made to grow chokka squid paralarvae beyond the seven day critical period. However, survival in our rearing system was often poor, with the longest survival time being 13 days, but 6–8 days on average. While an incorrect food regime is probably the cause, high mortalities could also be attributed to paralarval abnormalities caused by improper egg incubation as well as the presence of micro-bubbles in the rearing water. This latter fact only came to our notice late in this study. The bubbles were not easily seen by the naked eye, but their presence was noted by the accumulation of bubbles on the tank's walls and inside the mantle of paralarvae. It is thought that mantle trapped bubbles caused mortality by restricting paralarval swimming which retained them near the surface and lead to starvation.

There also is the possibility that the low pH was an additional source of mortality, as oxygen affinity of the cephalopod haemocyanin-containing blood is very dependent on the pH (Pörtner, 1990).

2.3. Conclusions

In summary, an open seawater system was chosen because of the advantages of attaining high quality water with the least labour and because the ocean was in close proximity. The system was developed specially for the rearing of chokka squid and allows the reproduction of conditions found on the spawning grounds. These conditions were reproducible, albeit with some minor treatment, using seawater from the South Atlantic Ocean. The design maintained the quality of (i.e., temperature, salinity and pH) over long periods and is comparable to the recommendation for squid paralarvae rearing experiments (Hanlon, 1990; Vidal et al., 2002a).

Another challenge introduced here was the development of a fine-scale temperature controlling device for an open seawater system, usually regarded as a difficult and laborious task because of (1) the high turnover rate and (2) the oxidative properties of seawater (which may damage the cooling devices) (Huguenin and Colt, 2002). The configuration of the system permits simultaneous studies with different experimental temperatures, allowing usage of the same egg batch paralarvae. This is a big advantage over other systems, and makes the results comparable.

2.4 Future work

Before further experiments are carried out, the problem of low pH and micro-bubbles need to be addressed.

Table 2.1. Comparative water quality parameters (excluding temperature) of seawater either experienced by chokka squid paralarvae in the wild or used for rearing experiments with the species. Salinity in psu. Dissolved O_2, NH_4-N, NO_3-N and NO_2-N values in mg L^{-1}. - no data.

Locality	Dissolved O_2	pH	Salinity	NH_4-N	NO_3-N	NO_2-N
Kromme Bay	8.57	8.03	34.60	-	-	-
Sunset Beach[1]	7.78	8.01	34.60	0.00	-	5×10^{-3}
Aquarium (main reservoir)	7.60	7.92	34.60	0.00	-	5×10^{-3}
Rearing system	6.90–7.80	7.60–7.93	34.60	0.00	-	5×10^{-3}
FURG (Brazil)[2]	6.77–7.49	8.1–8.3	> 33.00	0–0.04	-	$0–3 \times 10^{-2}$
Recommended[3]	saturated	> 8.00	> 30.00	< 0.10	< 20.00	< 0.10

[1] The beach in front of the Aquarium from where the seawater is pumped.

[2] É. A. Vidal, unpublished data.

[3] Yang et al., 1989; Hanlon, 1990; Lee et al., 1998; Vidal et al., 2000.

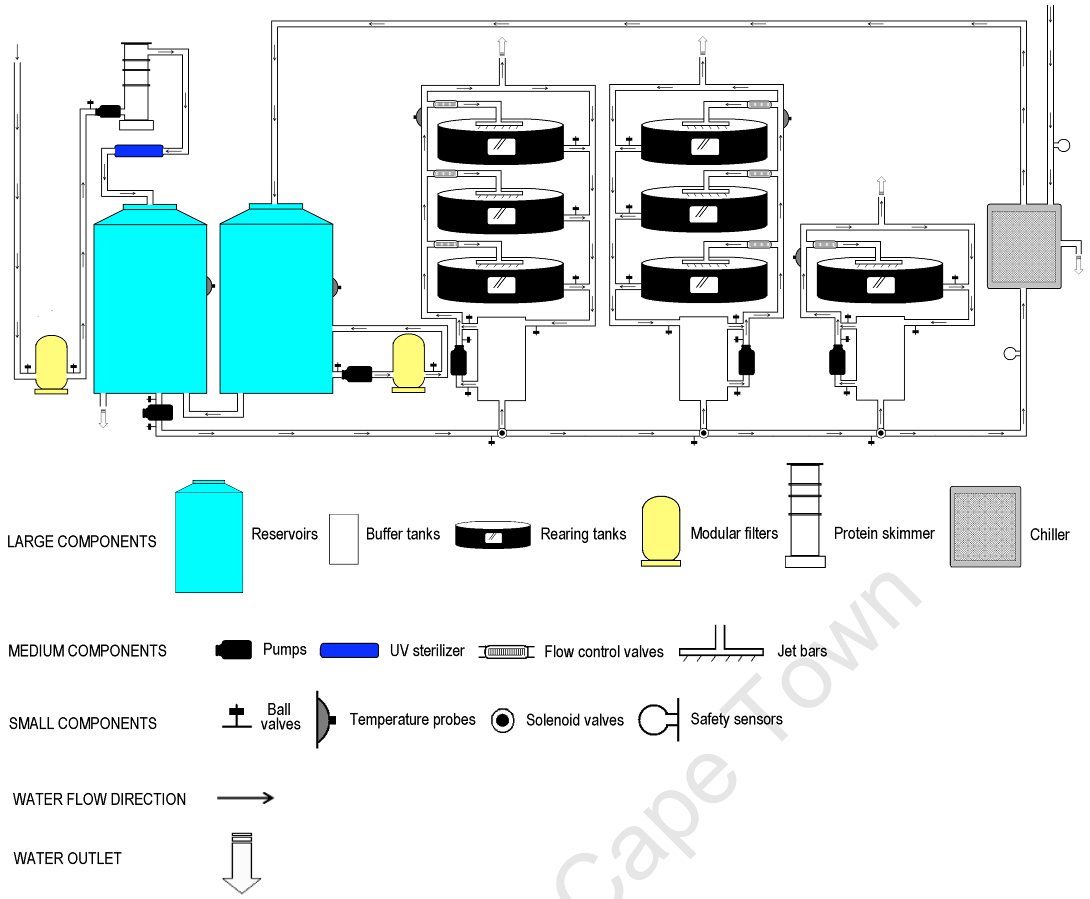
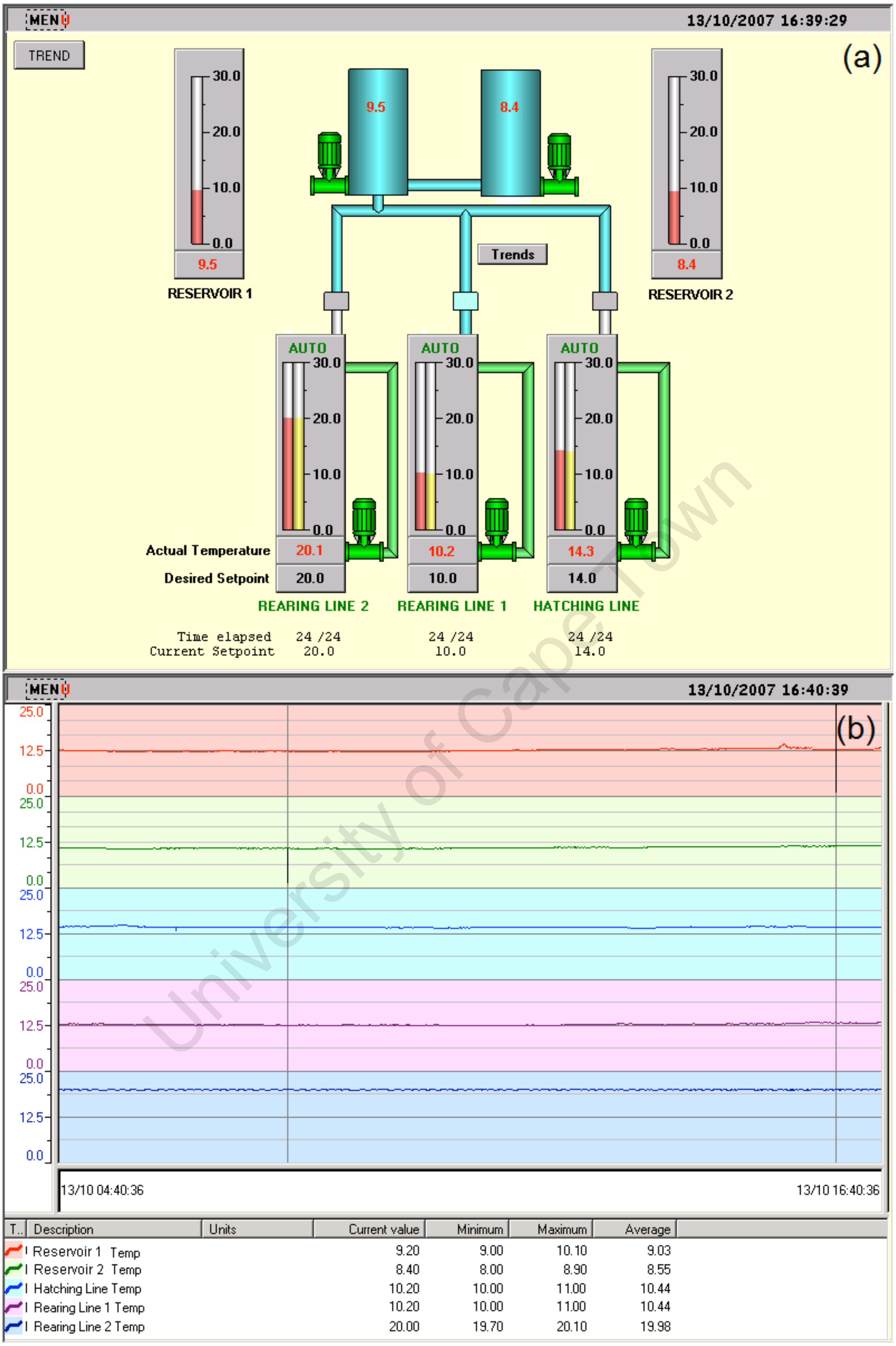


Fig. 2.1. Schematic view of the squid paralarvae rearing system (not to scale).



T..	Description	Units	Current value	Minimum	Maximum	Average
🟥	Reservoir 1 Temp		9.20	9.00	10.10	9.03
🟩	Reservoir 2 Temp		8.40	8.00	8.90	8.55
🟦	Hatching Line Temp		10.20	10.00	11.00	10.44
🟪	Rearing Line 1 Temp		10.20	10.00	11.00	10.44
🟦	Rearing Line 2 Temp		20.00	19.70	20.10	19.98

Fig. 2.2. Screen views of the temperature control software which regulates water temperature in the rearing tanks. (a) shows the monitor screen displaying the actual and desired (set point) temperatures in each compartment of the rearing system. (b) shows the monitor screen with real temperature time trend lines.

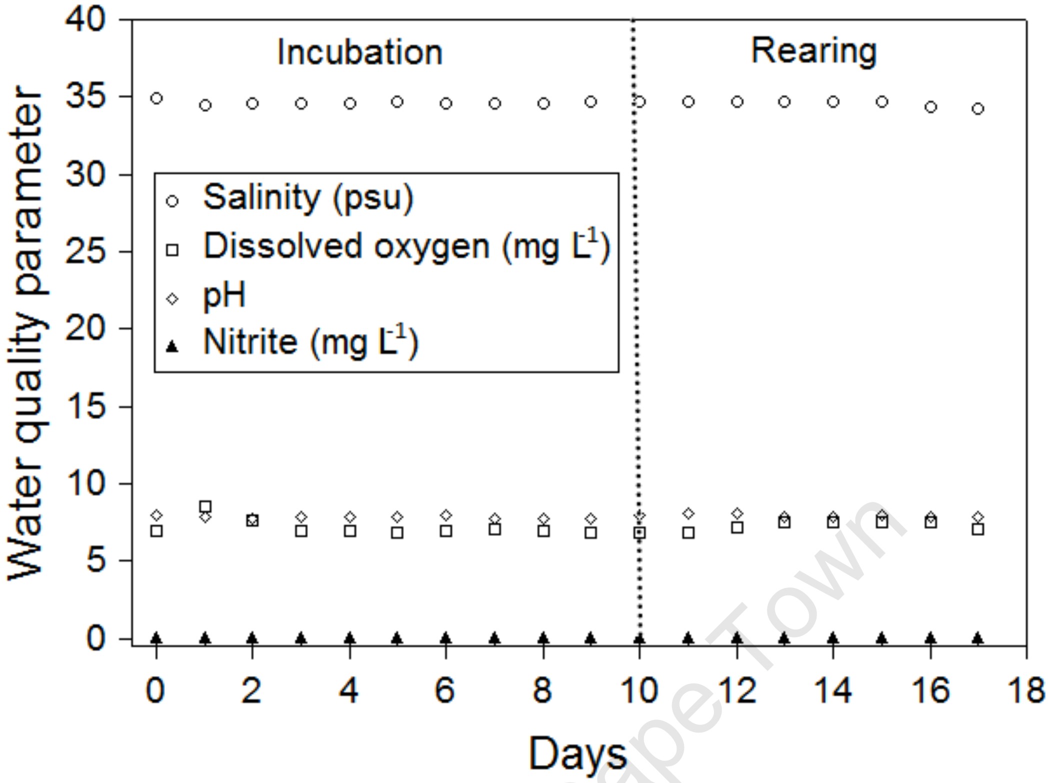


Fig. 2.3. Time series of water quality parameters measured in the rearing tanks during the incubation and rearing of specific gravity experiments (Chapter 4).

Chapter 3 — Effects of temperature on yolk utilization in chokka squid (*Loligo reynaudii* d'Orbigny, 1839) paralarvae

Abstract

The temperature effects on the utilization of yolk by chokka squid (*Loligo reynaudii* d'Orbigny, 1839) paralarvae were studied for starved animals at 12, 16, 18 and 19 °C. This temperature range encompasses those temperatures which are naturally found on inshore (warm) and mid-shelf (cold) chokka squid spawning grounds. Eggs were collected by SCUBA divers in St Francis Bay and incubated in the laboratory at 15.6 °C and 14.4 °C in January–February 2007 and January 2008, respectively. Mean mantle length (*ML*), wet weight (*WW*) and yolk weight (*YW*) for paralarvae incubated at 14.4 °C were 2.58 mm, 2.06 mg and 0.27 mg, respectively. Paralarvae incubated at 15.6 °C had 2.43 mm *ML*, 1.96 mg *WW* and 0.21 mg *YW*. Mean dry weights (*DW*) were 0.47 mg at 15.6 °C and 0.38 mg at 14.4 °C. The yolk was exponentially utilized, and paralarvae maintained at warmer temperatures utilized their yolk faster and had lower survival than their counterparts at colder temperatures. From the energetic perspective, the survival times predicted by using exponential and linear yolk utilization rates approached the observed survival time (with 80% mortality) only at 12 °C. The implications for paralarval dispersal, recruitment and for inshore and mid-shelf spawning grounds are discussed.

3.1. Introduction

Early in their planktonic existence, cephalopod paralarvae rely on both endogenous (yolk) and exogenous (prey) food sources, thus combining lecithotrophy with planktotrophy (Vidal et al., 2002a; Boletzky, 2003). This differs from most planktonic larval fish and marine invertebrate larvae (Boidron-Métairon, 1995; Yúfera and Darias, 2008). Squid paralarvae also differ from other meroplankters, in that they undergo a period of zero net growth after hatching, the length of which depends on yolk utilization rates, there is no conversion of yolky matter into somatic tissues and, metabolism is mostly fueled by yolk energy (Vidal et al., 2002a). This has been proposed to be the cephalopod equivalent of the 'critical period' phase concept for larval fish (Vidal et al., 2002a; 2006). The exponential growth phase begins once the yolk is exhausted and the paralarvae recover their original weight through feeding (Vidal et al., 2005).

This early-life feeding strategy is unusual within the phylum Mollusca. The mixing of exogenous and endogenous food sources has only been found for some nudibranchs and one bivalve species and is generally thought to be important for dispersal (Kempf and Hadfield, 1985). Planktotrophy in non-cephalopod mollusk lecithotrophic larvae seems to be facultative, as food ingestion only takes place when food is readily available (Alatalo et al., 1984; Kempf and Hadfield, 1985). The same is true for some fish, crustacean, polychaete, and echinoid larvae (Kempf and Hadfield, 1985; Hemming and Buddington, 1988; Boidron-Métairon, 1995; Hart, 1996). Mixing of exogenous and endogenous feeding modes is generally interpreted as an evolutionary transition between planktotrophic and lecithotrophic larval conditions (Hart, 1996).

In addition to food availability and predation, the physical environment has also been found to play a key role in the early life stages of squid (e.g. Dawe et al., 2000; Boyle and Rodhouse, 2005). Ocean temperature has been found to be one of the most important parameters as it directly affects metabolism (O'Dor and Wells, 1987) and thus yolk utilization rates, early post-hatching survival, dispersal and ultimately recruitment (Vidal et al., 2005). For example, Roberts (2005) quantitatively linked good annual chokka squid (*Loligo reynaudii* d'Orbigny, 1839) yields off the South African east coast to low sea temperatures. Vidal et al. (2002b) on the other hand have indicated that abnormally high sea temperatures off the Californian coast could be detrimental for the recruitment of Californian market squid (*Doryteuthis* (formerly *Loligo*) *opalescens* Berry, 1911) due to the high yolk utilization rates observed in paralarvae. Cephalopods are not unique in this regard. Similar impacts of sea temperature on recruitment have been found for a number of fish and shellfish species (see Drinkwater and Myers, 1987 and Leggett and Frank, 2008 for comprehensive reviews and further references on the topic).

In the case of the South African chokka squid, understanding the early life stages and challenges of the paralarvae is becoming increasingly important. The spawning grounds are located on the eastern Agulhas Bank off the east coast of South

Africa (Roberts and Sauer, 1994). Spawning occurs in both the inshore and mid-shelf regions where conditions have been shown to be suitable for mating, egg laying and embryonic development (Roberts, 1998; Oosthuizen et al., 2002a,b; Oosthuizen and Roberts, 2009). However, the greatest concentration of food (copepods) tends to be found further west on the central part of the Bank in an area of quasi-permanent upwelling know as the ‘cold ridge’ (Roberts and van den Berg, 2002). This is an elongated oceanographic feature that involves the vertical movement of the thermocline causing cold, nutrient-rich, bottom water to move into the photic zone. The cold ridge is commonly associated with enhanced levels of primary and secondary productivity (Roberts and van den Berg, 2005).

It has been hypothesized that this apparent mismatch between the spawning and paralarval feeding grounds is overcome by the passive westward transport of hatchlings by ocean currents. This has become known as the ‘Westward Transport Hypothesis’ (WTH) (Roberts, 2005). Of critical importance in the WTH is the time taken for the paralarvae to move between the spawning grounds and the paralarval nursery ground as survival will depend mostly on yolk reserves. Roberts (2005) suggested that starvation of paralarvae would probably occur at 5 days. Vidal et al. (2005) examined this further by investigating the effect of temperature on the yolk utilization, survival and growth of chokka squid paralarvae and found that paralarvae can survive for 6 days at 16 °C on their yolk reserve with a survival rate of 20%. However, paralarvae would consume yolk at a lower rate in colder temperatures commonly found on the Agulhas Bank contributing to increased survival time.

In this study we expand on the research by Vidal et al. (2005) by investigating the effects of a range of temperatures on the yolk utilization and survival rates of chokka squid paralarvae under starvation conditions.

3.2 Methods

3.2.1. Aquarium tank system

A detailed description and performance of the squid rearing system is given in Chapter 2. A brief description follows: the water tank system used in these experiments consisted of a hatching tank and two sets of three tanks referred to as a 'tank line'. The tanks in each line were maintained at the same temperature giving replicates. All tanks were identical and consisted of a 220 L round black tank (0.95 m diameter × 0.40 m height) with low light intensity of 13–54 lx at the water surface (for details of tank design and operation, see Vidal et al., 2002a). Water entered each tank through a radial pipe with small holes on one side. These emitted micro jets of water onto the water surface and created a circular current. The radial pipe was positioned 2 cm above the water surface in the two tank lines (see Fig. 1c in Vidal et al., 2002b) but was submerged in the hatching tank system to generate greater turbulence. Water left each tank through a center-positioned overflow drain encircled by a 150 µm mesh to prevent paralarvae from being lost.

Water supplied to the tank system was first filtered to 10 µm and skimmed, and then UV sterilized and cooled to 5–9 °C using a chiller unit. Water temperatures in the tanks were controlled by a computerized system which utilized *Programmable Logic Controller (PLC)*-controlled solenoid valves to regulate cold water inflow to the hatching and independent tank lines. Feedback to the control software came from temperature sensors situated at the inlets of the three subsystems. This enabled different temperatures in each tank line as well as the hatching tank with the variability in temperature being < 0.1 °C. Water turnover rates in the tanks were calculated from the number of times each solenoid valve opened and the flow rate.

During incubation and subsequent experiments, water temperature was recorded at 10 min intervals using *Starmon mini* temperature recorders. These were placed in the hatching tank during incubation and in one of the experimental tanks during each temperature treatment experiment. Temperature was measured once a day in each of the replicate experimental tanks, in order to assess temperature differences among the tanks.

Water quality in terms of salinity, pH, dissolved O_2 and nitrogenous wastes (ammonia, NH_4^+ and nitrite, NO_2^-) was monitored daily. Ammonia and nitrite were measured with pre-packaged reagents (AquaMerck®, Merck, Germany).

3.2.2. Egg collection and incubation

Squid eggs were collected by SCUBA divers on the inshore spawning grounds (20–40 m) near St Francis Bay (South Africa) on 10 January 2007 and 12 January 2008. The eggs were placed in sealed plastic bags with the original seawater, filled with pure O_2, and air freighted in a cooler box with frozen ice packs to the Marine and Coastal Management (MCM) Research Aquarium in Cape Town some 600 km away. Total transport time was 8.5 and 7 hours respectively for each batch. Temperature during transport was monitored (10 min intervals) using a *Starmon mini* temperature recorder placed in one of the bags. Average transport temperatures recorded were (± SD) 12.21 ± 2.12 °C for the January 2007 batch and 18.82 ± 1.12 °C for the January 2008 batch.

On arrival at the aquarium the plastic bags were placed in the hatching tank for ~1 hour to allow the water to equilibrate with the tank. Embryos were found to be at the Arnold's (1965) stage A12 and A28 respectively. Each batch was divided into 50 clusters of 10 egg pods each and then hung in the hatching tank using string tied to the apical tips. The pods were submerged ~20 cm below the water surface using a 17 g weight attached to each cluster. The incubation periods were 25 d in 2007 and 12 d in 2008 at average temperatures of (± SD) 15.60 ± 1.30 °C and 14.39 ± 1.99 °C respectively.

Egg pods in the hatching tank were maintained in constant motion to ensure good oxygenation using a high inflow rate of 13.3 L min^{-1} until developmental stage A29 (Vidal et al., 2002b). Thereafter until hatching, the rate was 12.5 L min^{-1}, to reduce the possibility of mechanical stimuli causing premature hatching (Vidal et al., 2002b). The water turnover rate during incubation ranged between 41–44 × tank volume d^{-1}.

3.2.3. Experimental design

The yolk utilization experiments were conducted as soon as the eggs hatched i.e. between 06–11 February 2007 and 23–30 January 2008, hereafter referred as Experiment 1 and 2 respectively. In both cases, the uniformity of age was ensured by discarding paralarvae that hatched before the main hatching day and removal of the egg pods from the tank after that. On the main hatching day 1 000 same-aged paralarvae were individually transferred to each of the temperature replicate tanks via a small glass beaker. This day was defined as Day 0 as less than 24 h had elapsed since hatching.

The experiments were conducted in the absence of any food and, as allowed by the tank design, simultaneously at two different temperatures. This meant that each temperature had three replicates (three tanks in a 'tank line'). Experiment 1 used 16 and 18 °C while Experiment 2 used 12 and 19 °C. A water inflow rate of 1.25 L min^{-1} was used in all experimental tanks. Estimated turnover rates ranged from 40 × tank line volume d^{-1} at 12 °C to 5 × tank line volume d^{-1} at 19 °C. The photoperiod was 12 h light vs. 12 h dark. Luminosity at the water surface over the tanks ranged from 20–140 lx.

3.2.4. Mantle length, wet- and dry- body weights, yolk weights and survival

Seventy five randomly chosen paralarvae were sampled from each of the temperature treatments with the exception of Day 0 when 150 paralarvae were sampled from the hatching tank before transfer to the rearing tanks. From the sampled paralarvae, 30 were selected for determining mantle length and yolk content. All 75 paralarvae of each treatment (i.e. 150 paralarvae d^{-1}) were subsequently used for estimation of wet and dry body weights.

Sampled paralarvae were anesthetised with magnesium chloride (Oestmann et al., 1997) and two digital images (dorsal and ventral views) were obtained of each paralarva at 20 × magnifications. Measurements (in mm) of the dorsal mantle length (*ML*) and the dimensions of the internal anterior and posterior yolk sac were obtained for each individual using the *AuxioVision LE*$^{©}$ (4.1 version) image analysis software. Volume of the yolk sac was estimated by superimposing standard geometric forms (cylinder, cone and rotational ellipsoid) onto the shape of the yolk sac (Vidal et al. 2002a, 2005). Yolk volumes determined for each paralarva were converted to yolk weights (*YW*) using the yolk specific gravity of 1.036 mg mm^{-3} obtained for *Illex illecebrosus* (O'Dor et al., 1986).

Fifteen samples of five paralarvae each were weighed daily with a *Sartorius A120S* analytical balance (precision of 0.0001 g). Before weighing, the paralarvae were individually rinsed with Milli-Q water to wash out residual chloride (Omori and Ikeda, 1984) and then blotted on absorbent tissue to drain the excess water trapped in the mantle (Vidal et al., 2002a). The wet weight (*WW*) was estimated by averaging the mass obtained by the number of squid in each group. Dry weights (*DW*) were obtained similarly after drying the groups for 20 h in a drying oven at 60 °C until a constant weight was reached and then holding them in a vacuum glass desiccator for 4h before weighing. *ML*, *WW*, *DW* and *YW* of newly hatched paralarvae were compared using the Student *t* test at a significance level of 0.05 (Zar, 1996). Because the weight of paralarvae was estimated using averages, it was not possible to calculate the ratios of yolk weight to wet- and dry-weight at hatching (Vidal et al., 2002a).

Mortality was determined daily by siphoning dead individuals from the bottom of the tanks. Survival at each temperature treatment was estimated as the percentage of live paralarvae left relative to the initial number of paralarvae (1 000), excluding those sampled for data collection.

3.2.5. Yolk utilization and growth rates

Both yolk utilization and growth rates (daily instantaneous) were estimated by fitting a standard exponential model to yolk- and wet weight-at-age data. For yolk utilization rates the formula

$$YW = YW_0 e^{bd}$$

was used where YW is the yolk wet weight (mg), YW_0 is the yolk wet weight at hatching and b is the instantaneous yolk utilization rate constant (% d^{-1}), and d is age (days after hatching) (Vidal et al., 2002a, 2005).

For growth rates the formula

$$WW = WW_0 e^{b'd}$$

was used where WW is the body wet weight (mg), WW_0 is the body WW at hatching, and b' is the instantaneous growth constant (% d^{-1}) (Forsythe and van Heukelen, 1987). In both cases, differences in the curves and their parameters between each temperature treatment were tested by a likelihood-ratio test at a 0.05 significance level (Kimura, 1980).

3.2.6. Energetics

The mean caloric content of the yolk was estimated by multiplying the yolk weights by the yolk caloric value of 1.71 kcal g^{-1} obtained for *Doryteuthis opalescens* (Giese, 1965). Standard metabolic rates were calculated based on the daily mean wet weight of paralarvae at each experimental temperature (O'Dor and Wells, 1987) using

$$MR = aW^b c^T$$

where MR is metabolic rate (kcal d^{-1}), W is the daily mean wet weight of the paralarvae (g), T is temperature (°C) and a, b, and c are constants derived from a multiple linear regression, where $a = 0.0043$, $b = 0.96$ and $c = 1.187$ (O'Dor and Wells, 1987).

With the procedures described above, it was possible to predict the survival times of the paralarvae using two methods: (1) linear yolk utilization rates, where the caloric content of the yolk at hatching is divided by the standard metabolic rate and (2) exponential yolk utilization rates using the instantaneous yolk utilization rate constants obtained at each temperature treatment.

3.3 Results

3.3.1. Water quality

No deterioration in the quality of the tank water was observed during the incubation of eggs or the starvation experiments. Salinity remained constant at 34.6

psu, pH ranged from 7.7–7.9 and dissolved oxygen was always > 7 mg L^{-1}. The latter was close to the saturation point for all experimental temperatures. Nitrogen waste levels were very low with the concentration of nitrite ranging between 0–5 × 10^{-5} mg L^{-1}. Ammonia was not detectable in the water during the experiments.

In general, the control system managed to maintain reasonable stability in the water temperature for the course of the experiments i.e., ± 0.6 °C SD for 12 °C, ± 0.3 °C SD for 16 °C, ± 0.2 °C SD for 18 °C and ± 0.1 °C SD for 19 °C (Fig. 3.1). The initial departures of 4.9 °C and 2.3 °C from the set temperatures of 19 °C and 12 °C during the first day in the second experiment were fortunately minimised once the system settled. The daily recorded data in each tank line showed a maximum temperature difference between tanks of 0.3 °C. Most of the time, however, the difference was smaller than 0.1 °C.

3.3.2. Comparison of ML, WW, DW and YW between temperatures

Significant differences were observed in *ML*, *WW*, *DW* and *YW* of newly hatched paralarvae incubated at the two experimental temperatures (Table 3.1). These variables tended to be larger for squid incubated at 14 °C compared to those exposed to 15 °C. Interestingly, *DW* was found to be smaller, but is most likely the result of error during the drying and/or the weighing process.

Yolk reserves of the paralarvae were utilized exponentially at all experimental temperatures at an instantaneous rate of 28–37% d^{-1} at 12–19 °C and 22–38% d^{-1} at 16–18 °C respectively. Paralarvae exposed to low temperatures utilized yolk slower than their counterparts at high temperatures. Interestingly, some *YW*s were observed to increase from Day 4 onwards (data not show). This could be due to (1) better survival of paralarvae that utilized their yolk at slower rates and/or hatched with more yolk than the average, (2) data variability, and (3) inaccuracy of the method used to estimate small amounts of yolk (Vidal et al., 2005). Nonetheless, it is clear that most of the yolk was utilized between Day 0 and Day 2.

When the sample groups were compared for Days 0 to 3 only (Fig. 3.2), the yolk utilization rates were observed to increase and better regression fits to the data obtained compared with the complete data set i.e. $r^2 > 0.80$. Significant differences were found between the rate constants of the curves for all temperature treatments (Table 3.2).

Daily loss in *WW* occurred at instantaneous rates of 4–5% d^{-1} and 7% d^{-1} respectively at the cool temperatures of 12 and 16 °C and warm temperatures of 18 and 19 °C (data not show). When data were compared only for the first four and five days (Fig. 3.3) the coefficients remained unchanged. As in the case of the yolk weight-at-age data plots, the *WW* rate loss constant differed significantly at all temperature treatments (Table 3.2).

3.3.3. Predicted and estimated survival times of paralarvae

An observed mortality rate of 80% was reached by Day 4 for the temperatures of 16, 18 and 19 °C, and Day 6 for 12 °C. Predicted survival times using a linear yolk utilization rate and the caloric content of the yolk at hatching were 5.6 d at 12 °C, 2.3 d at 16 °C and 1.7 d at 18 °C and 19 °C (Table 3.3). When using instantaneous exponential rates in conjunction with estimated standard metabolic rates for each temperature, predicted survival periods were 4, 2, 1 and 1 d respectively (Table 3.3). The similarity in survival times obtained for 19 °C and 18 °C (i.e. 1.7 d) appears to be not only due to similar yolk consumption rates for these temperatures (i.e., 56% d^{-1} at 19 °C and 59% d^{-1} at 18 °C respectively) but importantly, the initial yolk energetic content of paralarvae used in the 19 °C experiment (0.462×10^{-3} kcal) was determined to be higher than that of the paralarvae used in the 16–18 °C experiments (0.359×10^{-3} kcal) (Table 3.3).

3.3.4. Comparing results

It is instructive to compare these results with those of Vidal et al. (2005). This is possible because the temperatures and methods used in the two studies are very similar. The experimental mean temperatures deferred by about 0.60 ºC with the Vidal et al.’s (2005) temperature being slightly cooler (Table 3.4), but given the large standard deviation reported by Vidal et al. (2005), i.e. ± 1 °C, it can be concluded that the temperature regimes in both studies are similar and therefore the results comparable.

The experimental temperatures, sizes and weights at hatching, energetics and survival times for the two studies show that the paralarvae hatched slightly larger and heavier in the present study with *DW* and *YW* being virtually identical (Table 3.4). The energetic content was the same in both studies, but standard metabolism was higher in the present study reflecting the heavier *WW* at hatching. Survival time (defined by 80% paralarval mortality) was shorter in this study compared with Vidal et al. (2005) i.e. 4 d vs. 6 d.

Yolk utilization rates obtained in the two studies were also compared using exponential curves fitted to data for Days 0–3 (Fig. 3.4). The comparison shows that overall the initial yolk weights were on average 31.6% higher than the values found by Vidal et al. (2005) — although significant differences occurred only at Days 2 and 3 (Student *t* test, $p = 0.002$ and $p < 0.0000001$, respectively). This may have contributed to the slower utilization rates observed in the present study (52% d^{-1} vs. 96% d^{-1}). When compared using the likelihood-ratio test, only the instantaneous rates of the two curves were found to be significantly different ($\chi^2 = 5.19$, $p = 0.022$).

3.4. Discussion

3.4.1. Yolk utilization and dispersal

Results obtained in this study clearly demonstrate that yolk utilization rates in chokka squid paralarvae are affected by environmental temperature. This not only corroborates the findings of Vidal et at. (2002a) for *Doryteuthis opalecens*, but also reinforces their concept of squid having a critical period in the early life history as indeed is the case with fish (Hjort, 1914; Marr, 1956; May, 1974). Importantly, evidence from this study and that of Vidal et al. (2002a) suggests there is no apparent physiological compensation in yolk utilization in loliginid paralarvae (at least in the experimental temperatures where data are available, ranging from 11.4–19.0 °C), as found for some fish larvae (Johns and Howell, 1980; Hemming and Buddington, 1988). The critical period in squid paralarvae therefore seems to be related to the temperature dependent rate at which their yolk is utilized rather than energy reserves contained in their yolk sac. In fact, the amount of yolk at hatching seems to be independent of incubation temperature and has been shown to be proportional to the body mass in *D. opalecens* (from 10–15% of *WW* and from 40–50% of *DW*, Vidal et al. (2002a)) and *Sepia officinalis* Linnaeus, 1758 (Bouchaud, 1991).

In contrast to other feeding lecitotrophic larvae, the paralarval yolk reserves alone are insufficient to allow survival over long periods of time to maximize dispersal distances. Dispersal seems instead to be maximized by planktivorous foraging behavior already present at hatching (Villanueva, 1994) as in true planktotrophic larvae (Boidron-Métairon, 1995). However, the possibility of this condition being a byproduct of the typically high metabolic rates of cephalopod mollusks, which are even higher at paralarval stages (O'Dor and Wells, 1987), cannot be ruled out.

3.4.2. Predicted vs. experimental survival rates

Predicted (modelled) survival was found to differ greatly between the two methods used in this study (i.e. linear vs. exponential rates) with predicted survival generally less than the observed survival time. At 12 °C the survival time predicted using linear rates derived from the standard metabolism formula of O'Dor and Wells (1987) (5.6 d), approached the observed survival time (6 d). Results of Vidal et al. (2002a) for *Doryteuthis opalescens* paralarvae using the same methodology yielded a survival time of 9.1 d at the same temperature. When employing exponential rates, paralarvae were found to enter into an energy deficit in 1–4 d post-hatching, depending on the rearing temperature, in line with the findings of Vidal et al. (2002a, 2005).

3.4.3. Yolk consumption on the mid-shelf vs. inshore spawning grounds

It is known that chokka squid spawn close to the coast at depths of 20–40 m and on the mid-shelf of the eastern Agulhas Bank between 60 and 200 m (Roberts et al., 2002; Oosthuizen and Roberts, 2009). Inshore temperatures, as a result of coastal upwelling, fluctuate dramatically around an average of 15.3 °C, while in sharp contrast temperature on the mid-shelf is more stable around ~10 °C (Oosthuizen and Roberts, 2009).

From the yolk utilization–paralarval survival perspective, such a split of spawning environments may reduce the risk of recruitment failure by taking advantage of an inherent phenotypic physiological flexibility which is directly affected by temperature (Pechenick, 1987). In theory, as temperature has a major effect on size-at-hatching and yolk utilization rates (Boidron-Métairon, 1995; Vidal et al., 2002a; Pecl et al., 2004), recruitment would be strengthened by a differential contribution of paralarvae with different phenotypical attributes hatched across these two different environments. Inshore paralarvae may experience a faster exhaustion of the yolk reserve that may be counterbalanced by the rapid increment in body size/mass, which possibly would reduce mortality by predation. In contrast larger paralarvae would hatch on the mid-shelf and survive longer on their yolk reserves thus having more time for the improvement of predatory skills.

Spawning in different thermal environments is not restricted to chokka squid and is known to occur with other loliginid species. For example egg pods of *Loligo forbesii* Steenstrup, 1856 have been recovered in a depth range of 135–730 m (Lordan and Casey, 1999; Salman and Laptikhovsky, 2002) and *Doryteuthis gahi* d'Orbigny, 1835 was recently found to spawn in waters deeper (68–71 m) than previously thought for this species (i.e., < 20 m, on kelp stalks/leaves) (Laptikhovsky, 2007). These species too may be taking advantage of the different thermal environments to reduce paralarval mortality during the critical period to enhance recruitment.

3.4.4. Implications for the WTH and recruitment

The experimental survival rates obtained in this study are in agreement with the WTH which estimates that newly hatched paralarvae take 3–7 d of passive drift to reach the feeding ground near the cold ridge on the central Agulhas Bank (Roberts, 2005). Even when we consider water temperatures warmer (18–19 °C) than those commonly found on the central Agulhas Bank, these experiments have shown that paralarvae can survive for 4–5 d in the absence of food. At colder temperatures survival is extended to 6 d.

It is important to recognise that, irrespective of the temperature, paralarvae in their very early life must obtain sufficient food to cover the energy deficit within the yolk utilization period, otherwise the ‘point-of-no-return’ (*sensu* Blaxter and Hempel, 1963) will be reached leading to mortality (Vidal el al. 2002a). This was confirmed in a study using the RNA/DNA ratio as a nutritional condition index for *D. opalescens* (Vidal et al., 2006). For chokka squid in the WTH context, the theoretically assumed low food scenario during the transport period should at least supply sufficient food to ensure the survival of the most fit individuals until paralarvae reach the food maximum area. As has been shown for *D. opalescens* by Vidal et al. (2006), starvation may promote the selection of faster growing and more starvation-resistant paralarvae. These selected paralarvae may comprise the basis (or at least the bulk) of recruitment in the following year. This agrees with the general consensus that total interannual recruitment variability cannot be attributed to a single mechanism acting on the early life history (Leggett and Frank, 2008).

Table 3.1. Mantle length, wet- and dry- weights and yolk weight of *Loligo reynaudii* paralarvae at hatching for two experimental incubation temperatures. Values are mean ± SD.

Incubation temperature	Experiment 1 (15.60 ±1.30 °C)	n	Experiment 2 (14.39 ± 1.99 °C)	n	*t*	p
Mantle length (mm)	2.43 ± 0.08	59	2.58 ± 0.07	60	− 8.78	0.000001
Wet weight (mg)	1.96 ± 0.10	30	2.06 ± 0.08	30	− 2.89	0.005393
Dry weight (mg)	0.47 ± 0.10	30	0.38 ± 0.04	30	4.89	0.000008
Yolk weight (mg)	0.21 ± 0.08	59	0.27 ± 0.09	60	− 3.82	0.000214

Table 3.2. Likelihood-ratio test for yolk weight-at-age and body wet weight-at-age curves fitted for chokka squid (*Loligo reynaudii*) paralarvae reared at different temperatures for the first four days of rearing. Base case: where the two separate curves are fitted independently. RSS: total residual sum of squares. YW_0: yolk weight at hatching (mg), WW_0: body wet weight at hatching (mg), b: yolk utilization rate constant (% d^{-1}), b': weight loss rate constant (% d^{-1}).

Yolk weight					
Temperature pair		Base case	Coincident curves	$= YW_0$	$= b$
	RSS	0.0015	0.0031	0.0046	0.0028
Experiment 1	χ^2		5.7663	8.8788	5.0754
(16 and 18 °C)	df		2	1	1
	p		0.0560	0.0029	0.0243
	RSS	0.0011	0.0082	0.0101	0.0116
Experiment 2	χ^2		16.3228	17.9786	19.0911
(12 and 19 °C)	df		2	1	1
	p		0.0003	< 0.00001	< 0.00001
Wet weight					
Temperature pair		Base case	Coincident curves	$= WW_0$	$= b'$
	RSS	0.0182	0.0495	0.0227	0.0408
Experiment 1	χ^2		9.9779	2.1778	8.0420
(16 and 18 °C)	df		2	1	1
	p		0.0068	0.1400	0.0046
	RSS	0.0078	0.0094	0.0147	0.0987
Experiment 2	χ^2		2.2269	7.5005	30.3895
(12 and 19 °C)	df		2	1	1
	p		0.3284	0.0062	< 0.00001

Table 3.3. Energetics of starved *Loligo reynaudii* paralarvae incubated and reared at different temperatures, based on experimental results and linear and exponential yolk utilization rates. Experiment 1, incubation temperature: 15.60 ± 1.30 °C SD. Experiment 2, incubation temperature: 14.39 ± 1.99 °C SD.

Temperature pairs	Experiment 1		Experiment 2	
	16 °C	18 °C	12 °C	19 °C
Energy content of yolk at hatching (kcal)	0.359×10^{-3}		0.462×10^{-3}	
Standard metabolic rate (kcal d^{-1})	0.153×10^{-3}	0.213×10^{-3}	0.082×10^{-3}	0.270×10^{-3}
Observed survival time (maximum) (d)	6.0	5.0	8.0	6.0
Observed survival time (with 80% mortality) (d)	4.0	4.0	6.0	5.0
Predicted survival time (linear metabolic rate) (d)	2.3	1.7	5.6	1.7
Predicted survival time (exponential metabolic rate) (d)	2.0	1.0	4.0	1.0

Table 3.4. Comparative data summary of incubation and rearing experimental temperatures, size, dry and wet weight and yolk weight at hatching, survival time and energetics for starved *Loligo reynaudii* paralarvae of the present and Vidal et al. (2005) study. Values are means ± 1 SD.

Parameter	Present study	n	Vidal et al. (2005)	n	*t*	p
Incubation temperature (°C)	15.60 ± 1.30	-	16.00 ± 1.00	-	-	-
Rearing temperature (°C)	16.59 ± 0.49	-		-	-	-
Egg stage at arrival	A12	-	-	-	-	-
Incubation time (d)	25	-	17	-	-	-
Mantle length (mm)	2.43 ± 0.08	59	2.30 ± 0.05	43	− 6.05	0.000001
Wet weight (mg)	1.96 ± 0.10	30	1.86 ± 0.16	45	− 2.32	0.024418
Dry weight (mg)	0.47 ± 0.10	30	0.45 ± 0.05	53	− 0.20	0.840369
Yolk weight (mg)	0.21 ± 0.08	59	0.21 ± 0.04	94	− 0.51	0.610530
Yolk content (kcal)	0.359×10^{-3}	-	0.359×10^{-3}	-	-	-
Standard metabolism (kcal d^{-1})	0.153×10^{-3}	-	0.121×10^{-3}	-	-	-
Observed survival time (with 80% mortality) (d)	4.0	-	6.0	-	-	-
Predicted survival time linear (d)	2.3	-	3.0	-	-	-
Predicted survival time exponential (d)	3.0	-	1.0	-	-	-

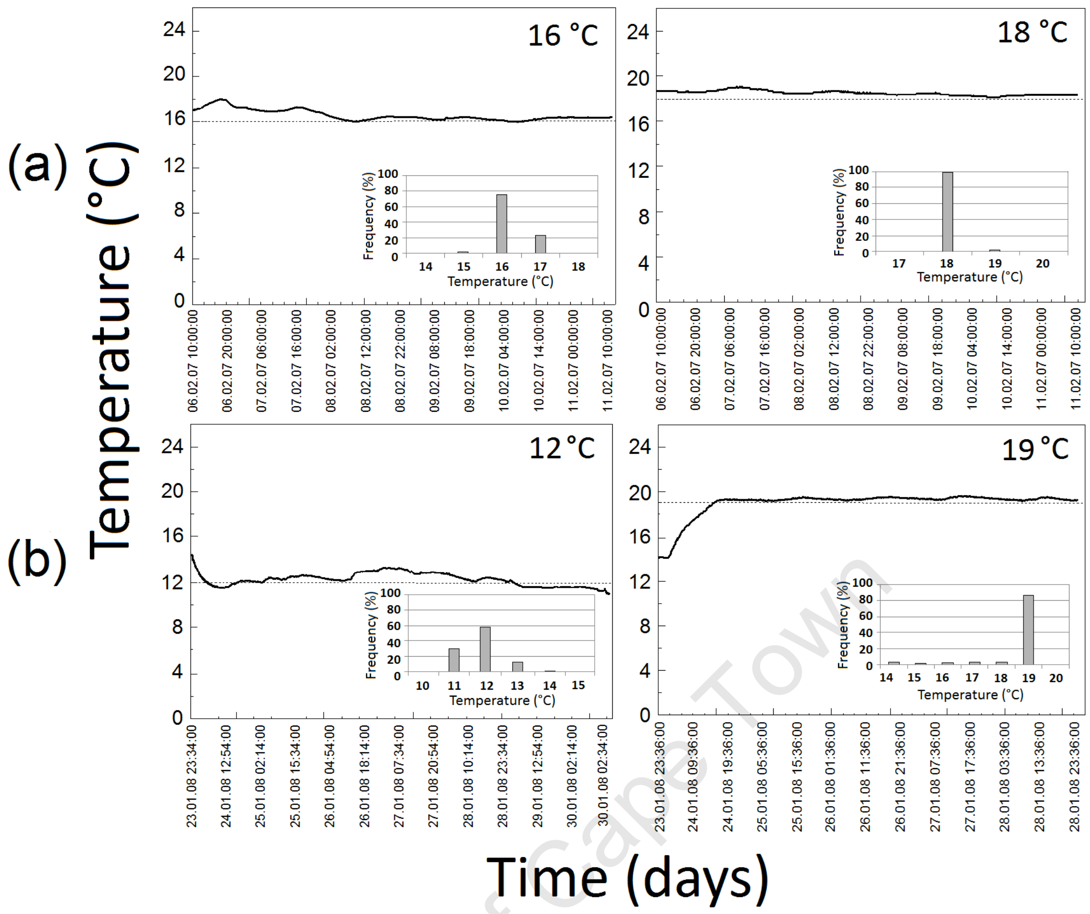


Fig. 3.1. Temperature time-series recorded during the experiments. Experiment 1 (a) conducted between 06–11 February 2007. Experiment 2 (b) conducted between 12–20 January 2008. The inserted histograms depict the frequency of each temperature. The dotted lines represent the mean temperature of each treatment.

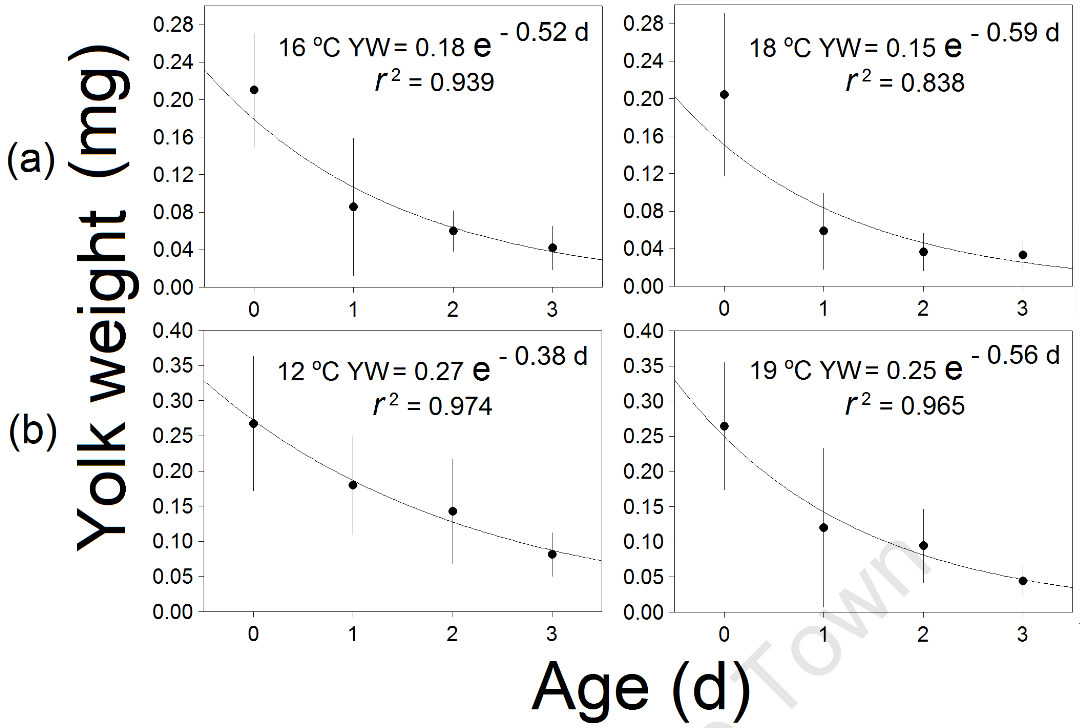


Fig. 3.2. *Loligo reynaudii*. Yolk weights at age at (a) 16 and 18 °C and (b) 12 and 19 °C during the first four days. Age is expressed as days post-hatching and yolk weights are means ± 1 SD of 30 paralarvae.

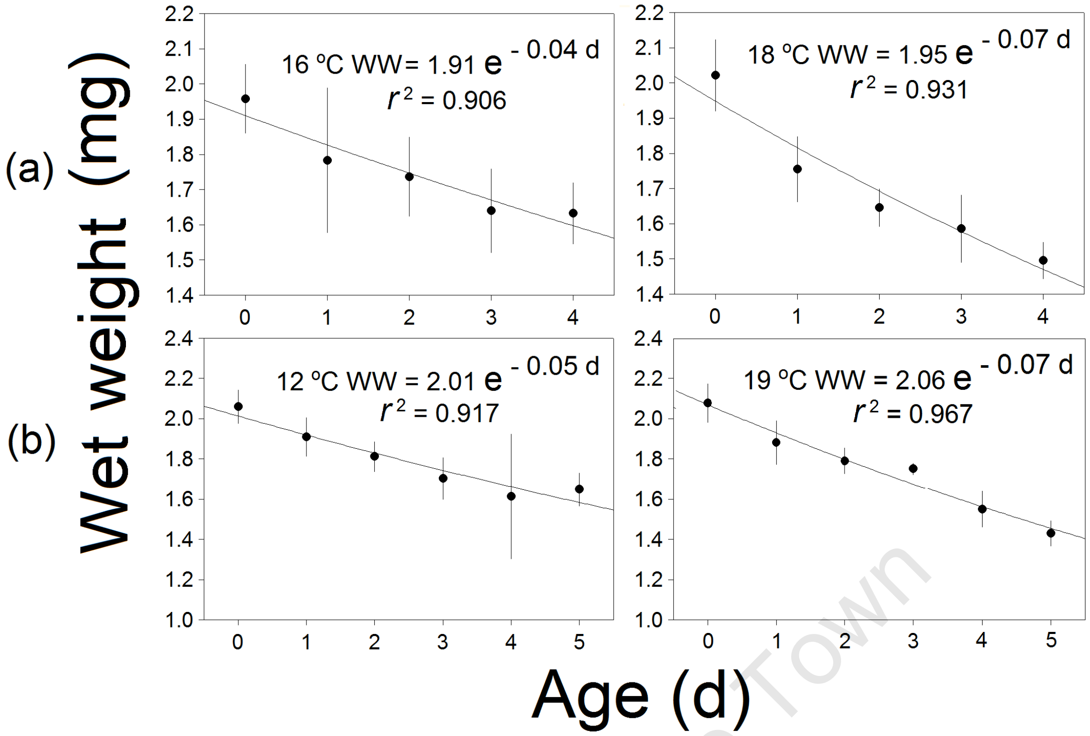


Fig. 3.3. *Loligo reynaudii*. Weight loss of starved paralarvae at (a) 16 and 18 °C for the first five days and (b) 12 and 19 °C for the first six days, expressed as wet weight versus age. Age is expressed as days post-hatching. Values are means of 7–15 groups of 4–7 paralarvae each.

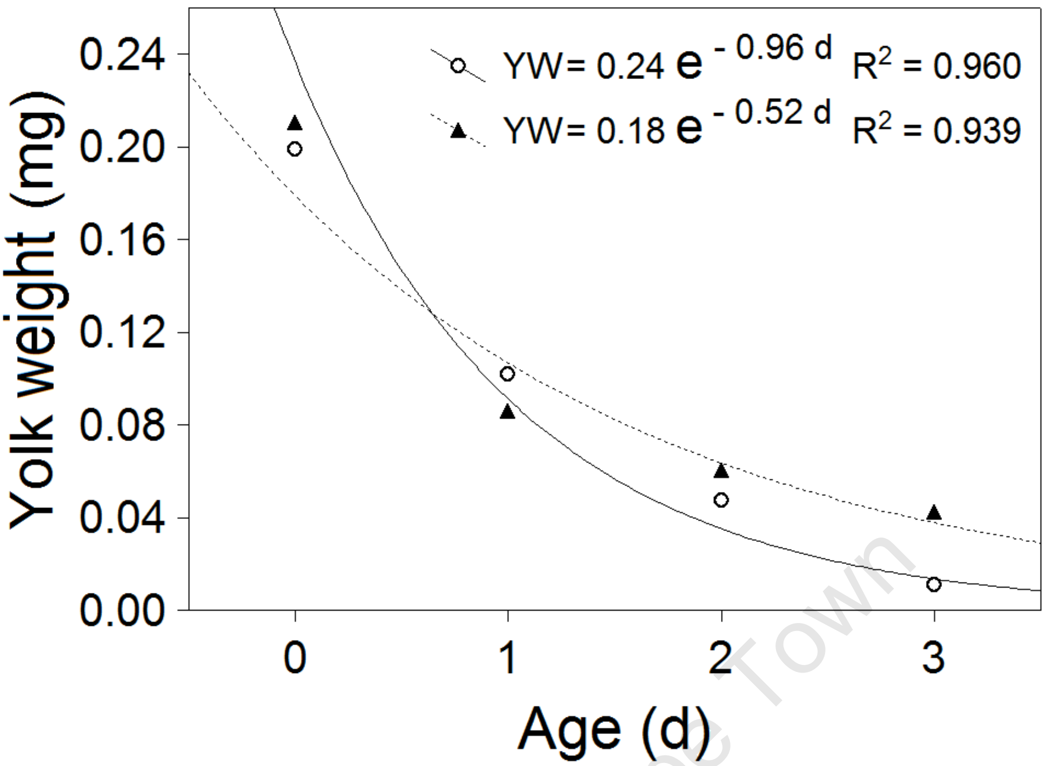


Fig. 3.4. *Loligo reynaudii*. Comparison of yolk utilization rates between present (filled triangles, dotted line) and Vidal et al. (2005) (empty circles, continuous line) study for starved paralarvae reared at 16 °C. Age is expressed as days post-hatching. Values are means of 30 paralarvae (present study) and 17–46 paralarvae (Vidal et al., 2005).

Chapter 4 — Effects of yolk utilization on the specific gravity of chokka squid (*Loligo reynaudii* d'Orbigny, 1839) paralarvae: implications for dispersal and vertical distribution

Abstract

Specific gravity is an important parameter in the dispersal of many marine zooplankters. In yolk-sac larvae, the specific gravity changes during the yolk utilization phase and impacts their vertical distribution in the water column. This in turn determines the degree of transport by currents as velocity is usually great near the surface. In the case of the commercially important 'chokka' squid (*Loligo reynaudii* d'Orbigny, 1839), recruitment is thought to be influenced by the successful transport of paralarvae from the spawning grounds to the cold ridge some 100–200 km away known as the 'Westward Transport Hypothesis'. The cold ridge is known to be a place of high zooplankton abundance. To assess the change in specific gravity of paralarvae during the yolk utilization phase, experiments conducted were on the 7-day post-hatching phase. Specific gravity was found to decrease between hatching and yolk exhaustion ranging from 1.0373–1.0734 g cm^{-3}. This implies that paralarvae are always negatively buoyant regardless of their yolk content. These results were incorporated into a coupled Individual Based-Model (IBM)–Regional Ocean Modelling System (ROMS) model. Simulation outputs showed that, at least during the peak of the spawning activity (November), dispersal was mainly westwards towards the nursery grounds and close to the shore. The vertical distribution of paralarvae was similar to the published data on loliginid vertical distribution only in shallow spawning grounds. These results indicate that the increased specific gravity of early chokka squid paralarvae appear to enhance survival by maintaining the paralarvae in productive shelf waters and moreover prevent advective losses to the open ocean.

4.1 Introduction

Virtually all teuthoid squid and several octopus species are known to spend at least ⅓ of their short life cycle as meroplankters (Sweeney et al., 1992; Villanueva, 2000; Arkhipin and Roa-Ureta, 2005). Because of the lack of a true metamorphosis, the term ‘paralarvae’ is used for cephalopod hatchlings during the planktonic phase, and differences in relation to adults are basically determined by their morphology, ecology and behaviour (Young and Harman, 1988; Boletzky, 2003). Due to their small size, cephalopod paralarvae are subject, to some extent, to water viscosity (Vecchione, 1987; Thompson and Kier, 2001; Zeidberg, 2004; Bartol et al., 2008, 2009), which has some influence on their movements. Data on swimming behaviour and paralarval dispersal are scarce but there is sufficient evidence to suggest that their dispersal is related to water movements (Boletzky, 2003) such as currents (O’Dor and Dawe, 1998; Roberts, 2005) or upwelling events (Rocha et al, 1999; González et al., 2005; Martins and Perez, 2006).

In addition to currents and swimming capabilities of squid paralarvae, specific gravity should also influence their distribution, as has been demonstrated for marine fish and invertebrate larvae (Chia et al., 1984; Sclafani et al., 1997; Kelman and Emlet, 1999; Trotter et al., 2005). Ultimately, the specific gravity of any planktonic organism will influence vertical distribution, not only assisting in position maintenance but also differential transport patterns resulting from vertically-stratified currents (Bradbury and Snelgrove, 2001; Campbell and Dower, 2003). Specific gravity, either negative or positive, can allow some protection against visual predators whenever neutral-buoyancy is achieved (Chia et al., 1984). This can also affect energy conservation or expenditure in situations (Chia et al., 1984; Campbell and Dower, 2003) by reinforcing or counteracting vertical swimming (Young, 1995). This would be advantageous during the planktonic phase, as hydromechanical costs of movement can be relatively high in the viscous fluid environment in which paralarvae are entrained, increasing the energetic cost of locomotion (Thompson and Kier, 2001). Furthermore, even small changes in specific gravity have been proven to be important in affecting the distribution and transport of both fish eggs and larvae (e.g., Sclafani et al., 1997; Sundby, 1997; Parada et al., 2003), and thus the same would be expected for squid paralarvae.

As in other cephalopods, loliginid embryos develop from yolk-rich, telolecithal eggs (Boletzky, 2003), and the paralarvae hatch with a relatively high amount of internal yolk reserve that may represent up to 50% of the body dry weight

(Vidal et al., 2002). The utilization of yolk is temperature-dependent (Chapter 3), and there is little, if any, conversion of yolky matter into somatic tissues (Vidal et al., 2002, 2005). Therefore, the specific gravity of paralarvae should be affected by changes in the yolk content, influencing dispersal during the yolk utilization phase. For newly hatched chokka squid (*Loligo reynaudii* d'Orbigny, 1839) paralarvae in the Agulhas Bank ecosystem, different dispersal regimes are expected, particularly during the yolk utilization phase which takes place during the days immediately after hatching. This would be due to different current regimes found across-shelf (near-shore and mid-shelf) and along-shore (between embayments) on the chokka squid spawning grounds off the South African coast (Roberts, 2005).

Of particular concern is the dispersion regime imposed by across-shelf bathymetric gradients on the spawning grounds. According to current models of chokka squid early life-cycle, paralarvae hatched on the spawning grounds located along the eastern Agulhas Bank can be transported by currents some 100–200 km westwards to the 'cold ridge' region on the central part of the Bank. In this region oceanographic conditions sustain a relatively high primary and secondary productivity which in turn may furnish a suitable nursery ground for paralarvae (the Westward Transport Hypothesis, see Roberts, 2005). However, due to the fast-flowing Agulhas current (Hutchings et al., 2002), paralarvae hatched in the deeper mid-shelf spawning grounds (Augustyn et al., 1994; Roberts et al., 2002; Oosthuizen and Roberts, 2009) can face a higher risk of advective losses to the adjacent oligotrophic oceanic waters than those hatched in sheltered embayments inshore. On the other hand, even if deep-spawned paralarvae managed to return to more productive inshore areas via other mechanisms, such as shelf-edge upwelling or active migration, mortality rates would be very high. Early paralarvae are extremely sensitive to starvation and the majority would not recover even upon finding food of proper quality and in sufficient densities (Vidal et al., 2006). Understanding whether specific gravity might assist in retaining paralarvae on the shelf is relevant to understanding recruitment dynamics of the species.

The aims of this study were to: (1) describe the specific gravity properties of newly-hatched chokka squid paralarvae and changes during the yolk utilization phase, (2) examine the effect of yolk utilization on specific gravity and (3) address the influence of early specific gravity properties on horizontal transport and vertical distribution of paralarvae.

4.2. Material and Methods

4.2.1. Egg collection, incubation, rearing procedures and experimental design

On 18 November 2007, SCUBA divers collected chokka squid eggs on a spawning ground 25 m deep in St Francis Bay. These were air-freighted to the Marine and Coastal Management (MCM) Research Aquarium in Cape Town. The journey was ~ 11 hours and mean temperature (± SD) was 18.88 ± 3.90 °C. On arrival, embryos were found to be at Arnold's (1965) stage 25. Eggs were incubated for 12 d in a flow-through hatching tank. Incubation, rearing procedures and the rearing system were identical to those described in Chapter 3. Estimated water turnover during incubation was 42 × tank volume d^{-1}. A low luminosity was maintained at the water surface (13–54 lx) and incubation temperature averaged (± SD) 13.66 ± 0.55 °C. Dissolved oxygen was > 7 mg L^{-1} and mean salinity and pH were 34.6 psu and 7.85 respectively. Nitrogenous waste levels were 5×10^{-5} mg L^{-1} for nitrite and 0 mg L^{-1} for ammonia.

Hatching occurred on 01 December 2007, defined as experimental Day 0. Paralarvae hatched before this date were discarded to ensure homogeneity of ages. One thousand five hundred paralarvae were individually transferred from the hatching tank to each of three flow-through experimental replicate tanks, i.e. total of 4 500 paralarvae. Rearing temperature (± SD) was 14.44 ± 0.10 °C for the 7 d of the experiment, with a maximum temperature difference of 0.1 °C between tanks. No food was provided. A water inflow of 1.25 L min^{-1} was used in all experimental tanks. Estimated water turnover rate during the experiment was 13 × rearing line volume d^{-1}. Luminosity at the water surface ranged between 37–98 lx. Dissolved oxygen (mg L^{-1}), salinity (psu) and pH averaged (± SD) 7.29 ± 0.26, 34.6 ± 0.17 and 7.92 ± 0.09, respectively. Nitrite level was 5×10^{-5} mg L^{-1} and ammonia was not detectable in the rearing water during the experiment.

4.2.2. Measurement of settling rates and seawater specific gravity

Settling rate experiments were conducted in a temperature-controlled room at 14 °C (the same temperature as incubation/rearing). A group of 75 paralarvae was collected daily, at random, from the rearing tanks and anesthetized with magnesium chloride (Oestmann et al., 1997). From these, 30 were selected for the settling trials and determination of yolk content. All paralarvae from each group were subsequently used for estimation of wet and dry weights (see below).

The settling rate measurements were made in a glass column 50 cm high and 7.5 cm in diameter. The column was filled with the seawater collected in the rearing system and stored in a 60 L plastic bin. This was allowed to equilibrate to the ambient temperature of the room three days in advance. The anesthetized paralarvae were individually collected with a pipette, visually evaluated under a microscope at 20 × magnification for life signals (systemic and/or brancheal heart beat) and checked for tiny air bubbles and/or dirt adhering to the body surface or trapped inside the mantle cavity. Each paralarva was then gently placed in the column and allowed to settle for ~ 10 cm to reach terminal velocity (Zeldis et al., 1995). Settling time was recorded with a stopwatch from 40 cm downward. Velocity was calculated from the slope of the plot of settling distance vs. time. Paralarvae were siphoned from the bottom of the column and placed in a plastic tray with divisions for further measurements (see below). Specific gravity of the water was measured with a Sea Bird SBE CTD placed into the water storage bin for 10 minutes before each experiment.

4.2.3. Body measurements, yolk weights, moisture content, survival, yolk utilization and growth rates

Dorsal and ventral digital images of the paralarvae used in the settling experiments were recorded with a compound microscope at 20 × magnification fitted with a high-resolution video system. Measurements (mm) of the mantle length (ML), total length (TL) and other relevant dimensions (see below) of the paralarvae were taken using *AuxioVision LE*© (4.1 version) image analysis software. Measurements of the anterior and posterior internal yolk sac were taken from the ventral images. Yolk sac volume was estimated by superimposing standard geometric forms on the body shapes (Vidal et al., 2002, 2005) and yolk wet weight was obtained by multiplying the yolk volume obtained by a specific gravity of 1.036 g cm^{-3} (O'Dor et al., 1986).

Wet and dry weights were obtained using a *Sartorius A120S* analytical balance as described in Chapter 3. Fifteen groups (range: 13–15 groups) of five paralarvae each (range: 3–5) were weighed per day. The wet weight was estimated by dividing the measured total mass by the number of squid in each group. Dry weights were obtained as above after drying the paralarvae in a drying oven at 60 °C for 20 h and then holding them in a vacuum glass desiccator for 4 h. Moisture content was expressed as the percentage difference between the wet and dry weights relative to the wet weight.

Mortality was determined daily by siphoning dead individuals from the bottom when cleaning the experimental tanks. Survival was estimated as the percentage of live paralarvae left in each tank in relation to the initial number of paralarvae (1 500), excluding those sampled for data collection. Yolk utilization and growth rates (daily instantaneous, in% d^{-1}) were determined by fitting a standard exponential model to wet weight-at-age and yolk-at-age data (Vidal et al., 2002, 2005; Chapter 3).

4.2.4. Paralarval specific gravity estimation

Due to limitations in accurately estimating the three-dimensional shape of a squid paralarva, some assumptions had to be made:

- The paralarvae were assumed to approximate a rotational ellipsoid in form (Fig. 4.1) and volume (V) was estimated using the formula $V = 0.1667\pi LH^2$ (Heming and Buddington, 1988);
- The major axis (L) was assumed to be the length between the tip of the mantle and the base of the 4th pair of arms, while the height (H) was taken halfway along the major axis (both measures were taken with the animal in ventral position and with the mantle relaxed) (Fig. 4.1);

- As the bulk of the paralarval mass is contained in the head and mantle, the fins and arms were not considered in the calculations, for simplicity;
- The diameter (*d*) of the spherical particle used in subsequent equations was based on the radius of a sphere with the same volume of the ellipsoid (Tanaka, 1992);
- Because the resistance against spheres ranges 95–110% of that of the ellipsoids (McNown and Malaika, 1950), it can be assumed that this sphere represents the ellipsoid motion in the viscous fluid (Tanaka, 1992);

To address the applicability of the Stokes equation for estimating specific gravity of the paralarvae, the Reynolds number (Re), a non-dimensional index which characterizes the relative importance of inertial forces and viscous forces on a body embedded in a fluid environment (Mann and Lazier, 1991), was calculated individually for each transformed spheroid particle using the following formula (Moore and Villareal, 1996):

$$\mathrm{Re} = \frac{wd}{v/\rho_w} \tag{1}$$

where d (cm) is the diameter of the transformed spheroid particle, w (cm s^{-1}) is the settling velocity (corrected, explained below), v is the seawater molecular viscosity (0.01 g s^{-1} cm^{-1}; Tanaka, 1992) and ρ_w is the seawater specific gravity (g cm^{-3}). All transformed spheroid particles had $\mathrm{Re} > 0.5$ (range: 1.93–3.91; average: 2.66 ± 0.39 SD). Therefore the traditional Stokes' equation could not be used (Sundby, 1983), and an adapted equation was employed (see below).

The observed settling velocities (cm s^{-1}) in the glass column were biased because of wall and end effects and thus were corrected using the formula proposed by Cambalik et al. (1998):

$$w_\infty = w\left(1 + 2.4\frac{r}{R}\right)\left(1 + 3.3\frac{r}{h}\right) \tag{2}$$

where w is the observed settling velocity, w_∞ is the corrected settling velocity in a medium of infinite width and height, r and R are the radii of the transformed spheroid particle and the apparatus, respectively, and h is the height of the measured volume.

The specific gravity of the paralarvae (ρ_p) was then estimated from the transformed spheroid particles and settling velocity data with following formula (Sundby, 1983):

$$w_\infty = K_I d_0 \left(\rho_p - \rho_w\right)^{2/3} v^{-1/3} \tag{3}$$

where K_I is a constant equal to ≈ 19, $d_0 = d - D\zeta$, where d is the true diameter of the paralarva, D is the uppermost limit of size where the Stokes equation applies (i.e. 0.6 mm), ζ is a constant equal to 0.4 for spheres.

4.2.5. Effect of paralarval specific gravity on dispersal

The effect of the specific gravity on paralarval dispersal was assessed using the open source *Ichthyop* software (2.1.1 version). This tool tracks passive movements of Lagrangian particles using velocity fields stored from hydrodynamic model simulations (Lett et al., 2008a). The outputs generated by *Ichthyop* record the position (latitude and longitude), depth, temperature and salinity experienced by each tracked particle embedded within the hydrodynamic fields of the model. The program was modified to change particle specific gravity with age, according to the following formula:

$$Sp.\ gr. = 1.0539\ e^{-0.0018\ a}\ (r^2 = 0.84) \tag{4}$$

where *Sp. gr.* is the specific gravity in g cm^{-3} and a is the age in days after hatching. Estimated specific gravity of particles ranged from 1.054 g cm^{-3} at the beginning to 1.043 g cm^{-3} at the end of simulations.

The ROMS hydrodynamic model configuration used in this study [Southern African Experiment (SAfE); Penven et al., 2006], was developed for the region between 2.5°W–54.75°E longitude and 4.8–46.75°S latitude. This is a self-explicit, free-surface, hydrostatic, primitive equation ocean model (Shchepetkin and McWilliams, 2005) and uses stretched terrain-following coordinates in the vertical direction, and orthogonal curvilinear coordinates in the horizontal direction (Song and Haidvogel, 1994). The bottom topography (sigma levels) follows the general bathymetric chart of the world oceans (GEBCO) database. Horizontal resolution ranges from 19 km in the south and 27.6 km in the north. Vertical resolution is high at the surface (0.37 to 5.70 m) and coarse (11 to 981 m) in the bottom layers, with 32 s-coordinate levels.

The model was run using a 10-y time series of averaged monthly temperature and salinity data extracted from WOA 2001 database (Conkright et al., 2002). The model was initially forced from rest conditions at the surface by the WOA temperature and salinity data and had a spin-up time of 2 y to reach statistical equilibrium. Therefore, only outputs from years 3 to 10 are used for simulations. More detailed information on the structure and functioning of the SAfE can be found in Lett et al. (2008b).

Because the area of interest was located on the southeast coast of South Africa (see Fig. 4.6) and a high resolution was needed to reproduce fine scale coastal oceanographic phenomena, a nested modelling approach was used. A child model was extracted from the parent model, encompassing the area from 11.58–27.42°E longitude and 27.73–38.83°E latitude. The child model had a temporal and spatial resolution three times finer than the parent model, with 160 × 190 grid points in the horizontal plane and 32 vertical levels. The boundary conditions of the child grid were supplied by the parent grid.

Particles were seeded in three locations off St Francis Bay, i.e. at ~8 km east from the point where the eggs were collected (34°09'S 24°53'E, 25 m deep) (Fig. 4.2). Release areas were defined as rectangles of approximately 8 km × 19 km (~152 km^2), and were termed *Bay In*, *Bay Off* and *Mid-Shelf*. *Bay In* and *Bay Off* were spaced 2.5 km apart. *Mid-Shelf* was 17 km from *Bay Off* (Fig. 4.2). These areas aimed to be representative of the bathymetric gradient of the chokka squid spawning environment, which ranges from nearshore embayments to the mid-shelf (Oosthuizen and Roberts, 2009). Depth ranged between 35–80 m, 86–124 m and 110–130 m for the three release areas. The release areas encompassed 4–8 grid points of the SAfE model (Fig. 4.2).

In total, 15 000 particles were released during each trial (i.e., 5 000 particles released area^{-1}), following Lett et al. (2008b). Those authors used the same ROMS configuration employed here, but used 30 000 particles over six release areas. No diffusion terms or active behavioural traits were included in the simulations, so movement of particles was entirely passive and relied only on advection i.e. Lagrangian. As chokka squid paralarvae hatch from benthic eggs, particles in the model were released one meter above the shallowest depth inside the release area: 34 m at *Bay In*, 84 m at *Bay Off* and 109 m at *Mid-Shelf*. Particles were released in years 3, 7 and 10 and during November only when spawning activity is usually greatest (Augustyn et al., 1994).

Particles were released once a week for four weeks and tracked for 7 d. Simulations were performed with (1) particles that changed specific gravity and (2) particles that were neutrally buoyant to assess the importance of buoyancy for dispersal. Dispersal was described horizontally and expressed as the average displacement distances from the release areas and their associated variance. Displacement distances between initial and final gep-referenced positions of each non-beached particle were calculated by (Koordinaten.de, 2008):

$$\text{Displacement distance} = \delta \times 6\ 378.137 \text{ km} \qquad (5)$$

where 6 378.137 km is the radius of the Earth at the equator and δ is the distance (in radians) between two geographical locations assuming a spherical shape of the planet, calculated as follows:

$$\delta = \text{ArcCos}[\text{Sin}(-\text{Lat}_1) \times \text{Sin}(-\text{Lat}_2) + \text{Cos}(-\text{Lat}_1) \times \text{Cos}(-\text{Lat}_2) \times \text{Cos}(\text{Long}_2 - \text{Long}_1)]$$
(6)

where ArcCos is the Arc Cosine, Sin is the Sine, Cos is the Cosine, and Lat_1–$Long_1$ and Lat_2–$Long_2$ are radian-transformed coordinates of initial and final positions of each particle.

In addition, numbers of particles retained within each release area and particles beached were recorded. The effect on dispersal distance of the release area, week, year and specific gravity and the interactions among all factors were assessed with a multifactor ANOVA (Engie and Klinger, 2007), allowing several levels of interactions to gain insights in the global model (Leberton et al., 1992).

4.2.6. Effect of paralarval specific gravity on vertical distribution

Vertical profiles of frequencies of particles at 10-m intervals were calculated from model outputs for the seven days of simulations for the three years pooled for each release area. The frequency distributions over the depth classes were compared between changing specific gravity and neutrally buoyant particles using the Kolmogorov-Sminorv test (Zar, 1996). Field data on vertical distribution of *Doryteuthis* (formerly *Loligo*) *opalescens* Berry, 1911 paralarvae off the California coast (Zeidberg and Hamner, 2002) were used for comparison with our results (L.D. Zeidberg, pers. Com., 2008).

4.3. Results

Mean (± SD) paralarval body measurements were 2.54 ± 0.08 mm ML and 4.97 ± 0.11 mm TL at hatching. Wet, dry and yolk weights were 2.02 ± 0.09 mg, 0.40 ± 0.04 mg and 0.26 ± 0.10 mg respectively. Yolk was exponentially utilized at an instantaneous rate of 32% d^{-1} and was exhausted 6–7 d after hatching (Fig. 4.3). Body weight followed the same trend, with a weight loss rate of 10% d^{-1} (data not shown). Eighty percent mortality occurred on Day 5. Daily changes in specific gravity were best modelled by fitting an exponential function to the dataset (Fig. 4.3). Paralarval moisture levels increased while specific gravity decreased with age (Fig. 4.4). Because paralarvae were starved and lost weight, the increase in moisture content appeared to be related to the removal of relatively dry yolk matter as a result of its utilization instead of water uptake by the body tissues.

The relationships between specific gravity, yolk and body weight are shown in Fig. 4.5. Log-linear functions provided the best fit to the dataset. It was clear that the yolk content ($P < 0.05$) could explain much of the variability in the specific gravity measurements, as shown by large coefficients of determination ($r^2 > 0.60$) for the fitted curves (Fig. 4.5 a,b). When the yolk content was removed from the body weight, fit deteriorated ($r^2 = 0.48$) (Fig. 4.5 c), suggesting that the yolk content accounts for some of the variability in specific gravity.

In the simulations of Lagrangian particles, most of the dispersal was westward in both shallow and deep release zones, being similar for the three years, although some particles with changing specific gravity released in the *Bay Off* area were transported eastwards to Algoa Bay (Fig. 4.6). However, particles released at *Mid-Shelf* tended to be transported further west and dispersed over a greater area than those released inshore (Fig. 4.6). Neutrally buoyant particles dispersed over a much wider area than those with changing specific gravity, particularly for those released on the *Mid-Shelf* (Fig. 4.6). A striking difference between the particles with changing specific gravity and those that were neutrally buoyant was that, regardless of the release area, the dispersal envelopes of the former were smaller, located closer to the shore, and spread over shorter distances (Fig. 4.6). Many neutrally buoyant particles were found to follow the shelf break contour, and ended up entrained in a zone positioned south of 35° S and west of 23° E, where they were exported to the open sea (Fig. 4.6).

Dispersal distances increased from inshore to offshore (Table 4.1). Distances covered by changing specific gravity particles averaged shorter than their neutrally buoyant counterparts. This was especially evident for the *Mid-Shelf* release area, where the overall distances travelled by particles with changing specific gravity were 1.7 times shorter and the associated variance nearly half those of the neutrally-buoyant particles (Table 4.1). In contrast with dispersal distances, relative retention and beaching of particles decreased from inshore to offshore. The proportion of particles retained and beached was higher for particles with changing specific gravity than for those that were neutrally buoyant (Table 4.1). Release areas, followed by specific gravity and all the situations where those two variables interacted accounted for the largest portions of the variance explained by the model (Table 4.2). All the remaining variables and their interactions, although statistically significant, were each responsible for less than 1% of the variance (Table 4.2).

Vertical profiles showing percentage concentration of modelled particles at 10-m intervals after seven days are shown in Fig. 4.7. In all release zones, the proportion of particles at the release depths was often disproportionally high, suggesting a conservative, isodepth transport. However, if one ignores the release depth, it was evident that the remaining particles had a tendency to move to shallower depths, except for changing specific gravity particles from the *Bay In* release area. In this area, particles outside the release depth were distributed in the upper and lower parts of the water column (Fig. 4.7). Also worth noting is that, at *Mid-Shelf*, particles with changing specific gravity tended to spread shallower in the water column than their neutrally buoyant counterparts, concentrating at 60–70 m. The vertical distribution of neutrally buoyant and changing specific gravity particles differed significantly in all release areas (Kolmogorov-Sminorv test, $p < 0.0001$). This suggests that the interaction between specific gravity and the hydrodynamic regime is important in determining vertical distribution patterns.

4.4. Discussion

The results showed that chokka paralarvae always had a specific gravity greater than the surrounding seawater in the rearing tanks ($\Delta\rho = 0.0193–0.0310$ g cm^{-3}). This was strongly influenced by the yolk content and changes resulting from its utilization. This was shown by the temporal trend in specific gravity values which decreased with a decrease in yolk and an increase in water content during the experiment. Changes in specific gravity during yolk utilization have been observed in a number of species of fish and invertebrate larvae, both in field and laboratory studies (Henri et al., 1985; Sclafani et al., 1997; Kelman and Emlet, 1999; Trotter et al., 2005; Takashi et al., 2006).

Selective utilization of yolk components has been described for both fish and invertebrate embryos and larvae (Subramoniam, 1991; Hartling and Kunkel, 1999; Sewell, 2005; Kamler, 2008; Martínez et al., 2008). In the case of *Loligo reynaudii*, no data on the biochemical profiles of yolk are currently available, but it is known that cephalopod yolk is composed mostly of phospholipoproteins (Lee, 1991). Protein is preferentially catabolized for energy production over lipids (Nelson and Cox, 2004), and this would be particularly true in cephalopods, which have a vigorous protein-based metabolism (Lee, 1994). The observed changes in specific gravity appeared to deviate from the exponential decay model in a systematic way, with some indication of a discontinuity after four days (Fig. 4.2). It is proposed that this pattern could be related to selective use of yolk components, because buoyancy has been shown to be sensitive to biochemical composition in fish larvae and invertebrate zooplankters (Yin and Craik, 1992; Campbell and Dower, 2003).

In laboratory conditions, newly-hatched chokka paralarvae actively jet upwards and disperse to within a few body lengths of the surface for the first 2–3 days (Vidal et al., 2005). The initial ascent in the water column may be related to positive phototaxis and/or to negative geotaxis (Sidie and Holloway, 1999). In the latter case, the higher specific gravity of the paralarvae over the first few days could act as a cue triggering a strong negative geotactic response. This coincides with the period of maximum paralarval specific gravity. Therefore, to sustain such energetically costly behaviour (O'Dor et al., 1986; Thompson and Kier, 2001), protein could be consumed before the lipid components of the yolk. This would account for most of yolk mass utilization (Vidal et al., 2002, 2005, Chapter 3), reflecting the dramatic decrease in specific gravity between Days 0 and 1. The second sharp decrease observed in the last two days of the yolk utilization period may reflect the complete depletion of the protein components of the yolk and subsequent utilization of the lipid components. This is schematically illustrated in Fig. 4.8.

Changes in specific gravity related to yolk utilization rates are unknown for paralarvae of other cephalopod species. In fish, for example, similar relationships to those observed in this study have been found for a number of species, including the Atlantic salmon (*Salmo salar* Linnaeus, 1758) (Peterson and Metcalfe, 1977), Atlantic cod (*Gadus morhua* Linnaeus, 1758) (Ellertsen et al., 1980), Atlantic herring (*Clupea harengus* Linnaeus, 1758) (Henri et al., 1985), the goby *Sicydium punctatum* Perugia, 1896 (Bell and Brown, 1995) and striped trumpeter (*Latris lineata* (Forster, 1801)) (Trotter et al., 2005). As in chokka squid paralarvae, specific gravity in all those fish larvae is maximum at hatching and decreases to a minimum at yolk exhaustion. A difference, however, is that the yolk utilization-driven changes in the specific gravity in fish larvae seem to be related to utilization of yolk for somatic growth (Heming and Buddington, 1988). Squid paralarvae do not appear to convert yolky matter into somatic tissue (Vidal et al., 2000; 2005; Chapter 3), implying that the observed decrease in specific gravity and concomitant increase in water content is caused by catabolism of high specific gravity, low-moisture internal yolk to fuel metabolism.

A comparison between the specific gravity of chokka squid paralarvae and several fish larvae, some invertebrate larvae and holoplanktonic crustaceans is given in Table 4.3. It is clear that chokka paralarvae are heavier than most of the organisms listed, but lighter than heavy-shelled invertebrate larvae, the ostracod *Conchoecia* sp. and the copepod *Calanus finmarchicus* (Gunner, 1765). The paralarvae have a similar specific gravity range to that of demersal Atlantic salmon (*S. salar*) larvae but their mean specific gravity (1.0483 g cm^{-3}) is close to the mean specific gravity (1.0463 g cm^{-3}) of Pacific bluefin tuna (*Thunnus orientalis* (Temminck and Schlegel, 1844)) larvae of similar size (~ 5 mm total length; Table 4.1), which occupy a planktonic habitat (Takashi et al., 2006). The chokka squid specific gravity range also encompasses the mean specific gravity of slightly larger planktonic paralarvae of oceanic squid (7 mm, 1.0610 g cm^{-3}; Tsukamoto et al., 2009). The greatest concentrations of *Doryteuthis opalescens* paralarvae off the California coast range in depths between 15–30 m suggesting a mid-water, planktonic habitat (Zeidberg and Hamner, 2002). This implies that specific gravity *per se* may not be a diagnostic indicator of vertical distribution of squid paralarvae in the water column, and species-specific swimming and vertical migration behaviour (e.g. Franks, 1992) should be taken into account in interpreting their vertical distribution patterns.

Vertical distribution of *Doryteuthis opalescens* paralarvae off the Southern California Bight (Zeidberg and Hamner, 2002) were extracted and redrawn as frequency distributions in order to make them comparable to our results (Fig. 4.7). Some similarities were noted with the results for the *Bay In* area, where many released particles concentrated at around 30 m deep, particularly in the case of changing specific gravity particles. The same appeared to be the case for the *Bay Off* area but, in this case, neutrally buoyant particles resembled the field distribution more than their counterparts with changing specific gravity (Fig. 4.7). Although this could be interpreted as a near-bottom concentration of particles, this seems unlikely because both release areas had steep bathymetric gradients, with a difference between the shallowest and deepest bottom depths of about 50 and 38 meters for *Bay In* and *Bay Off* release areas respectively.

Vertical distribution patterns of both neutrally buoyant particles and those with changing specific gravity generally did not match observed loliginid vertical distributions, except for the *Bay In* release area (Fig. 4.7). However, the observed data are for a different geographic region and species than considered here, and these differences might explain the difference in patterns observed in the field (Zeidberg and Hamner, 2002). Most of Zeidberg and Hamner's (2002) paralarvae were sampled close (< 8 km) to islands, where a land mass effect known as 'sticky waters' (Wolanski and Spagnol, 2000) could have contributed to retention at the depths reported by those authors (i.e., between 15–30 m). Sticky waters are the result of high turbulence induced by tidal flow through an alignment of islands or high-profile reefs, which increases local water residence time by reducing drift currents, resulting in water stagnation and thus retention of zooplankton (Wolanski and Spagnol, 2000).

High turbulent mixing has been invoked as a factor that assists in maintenance of vertical position for negatively-buoyant squid paralarvae and ichthyoplankton (Zuev, 1964; Sundby, 1991; Zeidberg and Hamner, 2002). It may be possible that inshore boundary effects caused by the resolution of the employed ROMS model produced an effect similar to 'sticky waters' (i.e., high turbulent mixing). As one moves offshore, the coarser model resolution probably does not reproduce satisfactorily the turbulent mixing, which may partially explain the slightly deeper distribution of changing specific gravity particles for the *Bay Off* release area in relation to neutrally buoyant particles, and the observed discrepancies between modelled results of *Mid-Shelf* area and field data.

For the *Mid-Shelf* release area, the modelled distribution of particles was disproportionately higher at the release depth (109 m) than in the rest of the water column. Because the bathymetric gradient of this area was shallow (only 20 m across the width of the area) it suggests a demersal habitat for the paralarvae, as has been proposed elsewhere (Hutchings et al., 2002; Boyle and Rodhouse, 2005; Young et al., 2008). However, it seems unlikely that high metabolism, visual planktonic predators such as chokka paralarvae would survive in deep waters far from the productive euphotic zone. Nonetheless, adult loliginid squid have been reported to spawn in waters as deep as 120–730 m (Lordan and Casey, 1999; Roberts et al., 2002; Salman and Laptikhovsky, 2002; Laptikhovsky, 2007) suggesting that, under particular conditions (such as low temperature, which would slow down paralarval metabolism, and sufficient food), some paralarval survival could be feasible in deep waters. Alternatively, deep-spawned paralarvae could ensure survivorship by migrating to shallower (and thus more productive) waters.

Horizontal dispersal in the simulations was mostly westward and subsurface for both inshore and offshore release areas. This supports the Westward Transport Hypothesis, proposed by Roberts (2005), which postulated that paralarvae hatched on the eastern Agulhas Bank spawning grounds would be passively transported by currents towards the central Agulhas Bank, where there is a suitable nursery ground for the paralarvae in the vicinity of the cold ridge. It is important to remember that no biological attributes were assigned to the particles, and therefore the results reflect only the interactions between the circulation and specific gravity. Also, simulations were designed to only cover the duration of yolk reserves obtained in the present study, and the fate of particles thereafter were not assessed.

Several limitations need to be considered. First, independent of the yolk content, specific gravity may vary with a circadian cycle reflecting the physiological condition of paralarvae (Vidal et al., 2006), as observed in fish larvae (Sclafani et al., 1997; Hare et al., 2006). Second, fed paralarvae may show different changes in specific gravity compared to starved paralarvae during the yolk utilization phase. This is due to slower utilization of yolk in fed paralarvae (Vidal et al., 2002, 2005) and/or temporary influence of the specific gravity of the type of food in their guts. Finally, the whole body specific gravity could be affected by temperature-induced changes in the specific gravity of the aqueous component of paralarval tissues, as in fish eggs and larvae (Peterson and Metcalfe, 1987; Zeldis et al., 1995).

The results show that release area is an important factor influencing dispersal, with particles seeded on the mid-shelf spreading wider and travelling farther than those released inshore. Particle buoyancy was similarly important, with negative buoyancy causing particles to be retained closer to the shore with no leakage to offshore waters, in contrast to the neutrally buoyant particles. Within the context of the Westward Transport Hypothesis and early life cycle of chokka squid, large specific gravity can help to maintain the paralarvae inshore, where feeding conditions are favourable during passive transport to the nursery ground on the central Agulhas Bank. This may impact recruitment success because the highest susceptibility to starvation apparently takes place in the first few days post-hatching, during the so-called 'no net growth' phase (Vidal et al., 2006).

In summary, the specific gravity data for paralarvae and the Lagrangian dispersal simulations indicate a general westward dispersal for both the inshore and offshore spawning grounds. This supports the Westward Transport Hypothesis proposed by Roberts (2005), at least during the peak of spawning activity in November. However, most of particles were found subsurface, implying that transport to the cold ridge in the deep currents will be slower. Only at inshore release areas did vertical distribution of particles show some similarity with field data. This could be caused by greater turbulent mixing inshore. If swimming behaviour is ignored, specific gravity of chokka squid paralarvae would help retain paralarvae on the shelf, and therefore mitigate the high offshore advective losses proposed by Hutchings et al. (2002) for most meroplankters on the Agulhas Bank. In fact, there is a tendency for loliginid squid species to produce large (and thus heavier) paralarvae over narrow shelf environments (i.e., *Loligo reynaudii*, *Doryteuthis opalecens*) and small (lighter) paralarvae over broad shelf environments (i.e., *D. pealei* Lesueur, 1821, *D. Plei* Blainville, 1823). This seems to be an evolutionary strategy to maximize the retention of paralarvae in productive continental shelf areas, and to prevent advective losses to oligotrophic oceanic waters (Vecchione, 1987).

Table 4.1. Mean dispersal distance (and variance in km^2) for the Lagrangian experiment by site and year. The overall proportions of particles retained on the release areas and beached are also given.

Release areas	Dispersal distances (km)			Dispersal distances across all years	% retained	% beached
	Year 3	Year 7	Year 10			
	Neutrally buoyant particles					
Bay In	17.1 (461.48)	17.5 (372.4)	17.7 (444.3)	17.4 (422.8)	5.2	16.8
Bay Off	15.5 (243.9)	14.4 (202.4)	15.6 (236.7)	15.1(228.1)	5.1	0.7
Mid-Shelf	51.8 (2188.3)	42.0 (1406.9)	68.6 (3675.2)	53.9 (2526.9)	4.4	0.02
	Changing specific gravity particles					
Bay In	11.8 (89.1)	11.8 (81.4)	12.2 (97.2)	11.9 (89.0)	6.0	18.5
Bay Off	11.1 (93.1)	10.3 (76.8)	11.3 (97.0)	10.9 (89.3)	5.8	1.2
Mid-Shelf	37.7 (1828.1)	36.7 (1244.6)	21.4 (392.5)	31.9 (1209.9)	5.4	0.04

Table 4.2. Multifactor ANOVA results for the Lagrangian experiment showing the contributions of the different variables to determining modelled passive dispersal. * : Significant (P < 0.01).% variance explained: 100×SSeffect/SStotal.

	Sum of Squares	Degrees of Freedom	Mean Square	*F*	p	% variance explained
Intercept	112631253	1	112631253	136427.5	<0.01*	
Single variable						
Specific gravity	5770409	1	5770409	6989.6	<0.01*	1.85
Release area	58525540	2	29262770	35445.3	<0.01*	18.75
Year	229632	2	114816	139.1	<0.01*	0.07
Week	466882	3	155627	188.5	<0.01*	0.15
Interaction terms						
Specific gravity × Release area	5240520	2	2620260	3173.9	<0.01*	1.68
Specific gravity × Year	1871507	2	935753	1133.5	<0.01*	0.60
Specific gravity × Week	841159	3	280386	339.6	<0.01*	0.27
Release area × Year	353010	4	88253	106.9	<0.01*	0.11
Release area × Week	1141113	6	190185	230.4	<0.01*	0.37
Year × Week	450573	6	75096	91.0	<0.01*	0.14
Specific gravity × Release area × Year	5453815	4	1363454	1651.5	<0.01*	1.75
Specific gravity × Release area × Week	1265772	6	210962	255.5	<0.01*	0.41
Specific gravity × Year × Week	2428710	6	404785	490.3	<0.01*	0.78
Release area × Year × Week	1440686	12	120057	145.4	<0.01*	0.46
Specific gravity × Release area × Year × Week	3665217	12	305435	370.0	<0.01*	1.17
Error	223055709	270182	826			71.45
Total	312200254					

Table 4.3. Comparison of the estimated specific gravity of *Loligo reynaudii* paralarvae and some fish and invertebrate larvae, an ostracod and some calanoid copepods. TL: total length. Sp. gr.: specific gravity. Values of TL and Sp. gr. are averages and, where data are available, ranges. - : no data.

Species	TL (mm)	Sp. gr. (g cm^{-3})	Seawater Sp. gr. (g cm^{-3})	Mean temperature (°C)	Habitat
Chokka squid (*Loligo reynaudii*)	4.45–5.29	1.0373–1.0734	1.0258	14.3	Planktonic?
Ostheichthyes					
Atlantic cod (*Gadus morhua*)[1]	5.75	1.0275*	1.0265	5	Planktonic
Atlantic salmon (*Salmo salar*)[2]	15.00–30.00	1.0460–1.0675	-	15	Demersal
Blue whiting (*Micromesistius poutassou*)[3]	< 2.00	1.0282**	-	10	Planktonic
Halibut (*Hippoglosus hippoglosus*)[4]	6.00–7.00[5]	1.0206**	1.0240	5	Neustonic
Japanese eel leptocephali (*Anguilla japonica*)[6]	3.40–56.60	1.019–1.025	1.0240	22	Neustonic
Leptocephali larvae (six species)[6]	21.70–185.10	1.028–1.043	1.0240	22	Neustonic
Pacific blue tuna (*Thunnus orientalis*)[7]	3.00–3.80	1.0278	-	-	Planktonic
Pacific blue tuna (*Thunnus orientalis*)[7]	5.60	1.0463***			
Striped trumpeter (*Latris lineata*)[8]	< 5.30	1.0265–1.0290****	1.0265	16.4	Neustonic
Echinodermata					
Cushion star (*Pteraster tesselatus*)[9]	-	1.0220–1.0240	1.0220	12.2	Planktonic
Sand dollar (*Dendraster excentricus*)[10]	-	1.2500	-	-	Planktonic
Polychaeta					
Terebellid polychaete (*Eupolymnia bebulosa*)[11]	-	1.0080–1.0120	-	16	Planktonic
Tomopterid polychaete[6]	3.80	1.0470	1.0240	22	Planktonic
Mollusca					
Giant scallop (*Placopecten magellanicus*)[12]	-	1.2600–1.3400*****	-	-	Planktonic
Gould shipworm (*Bankia gould*)[13]	-	1.1920*****	-	20	Planktonic
Unidentified oceanic squid paralarvae[6]	7.00******	1.0610	1.0240	22	Planktonic
Crustacea					
Calanoid copepod (*Calanus finmarchicus*)[14]	> 3.00	1.5060	-	20	Planktonic
Halociprid ostracod *Conchoecia* sp.[6]	2.20	1.2400	1.0240	22	Planktonic
Unidentified calanoid copepods[6]	2.00–4.90	1.0580–1.0610	1.0240	22	Planktonic

*at yolk sac exhaustion, **newly hatched larvae, ***post yolk larvae, ****before feeding/swim bladder inflation, *****pediveliger, ******total length

[1]Sclafani et al. (1993), [2]Peterson and Metcalfe (1977), [3]Ådlandsvik et al. (2001), [4]Magnor-Jensen and Huse (1991), [5]Blaxter et al. (1983), [6]Tsukamoto et al. (2009), [7]Takashi et al. (2006), [8]Trotter et al. (2005), [9]Kelman and Emlet (1999), [10]Pennington and Emlet (1986), [11]Nozais and Duchêne (1996), [12]Gallager et al. (1996), [13]Gallager (1985), [14]Gross and Raymond (1942).

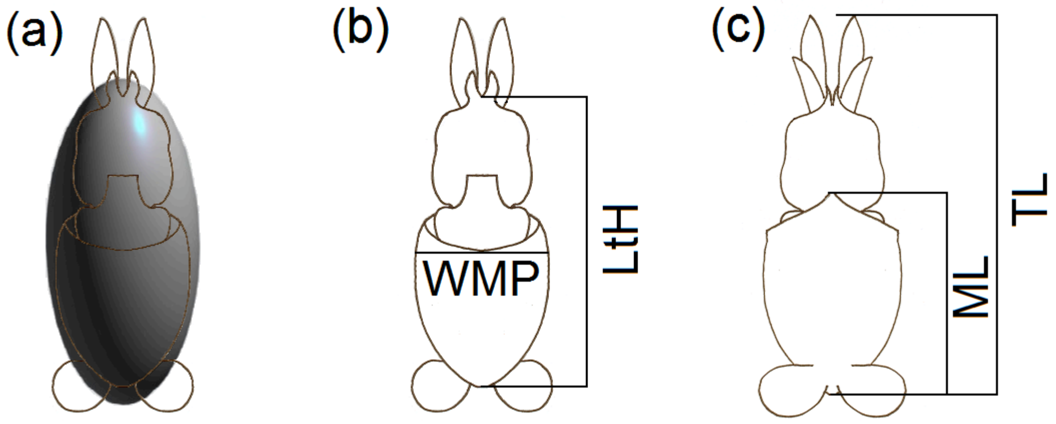


Fig. 4.1. *Loligo reynaudii*. The approximate ellipsoid form concept and measurements. (a) Schematic drawing of the approximate ellipsoid concept. (b) Measures taken on ventral side for the ellipsoid volume calculation. (c) Measures taken on the dorsal side. LtH: length between the tip of the mantle and the base of the 4th arm pair (= ellipsoid major axis *L*). WMP: width of the middle point (= ellipsoid height *H*). ML: dorsal mantle length. TL: total length.

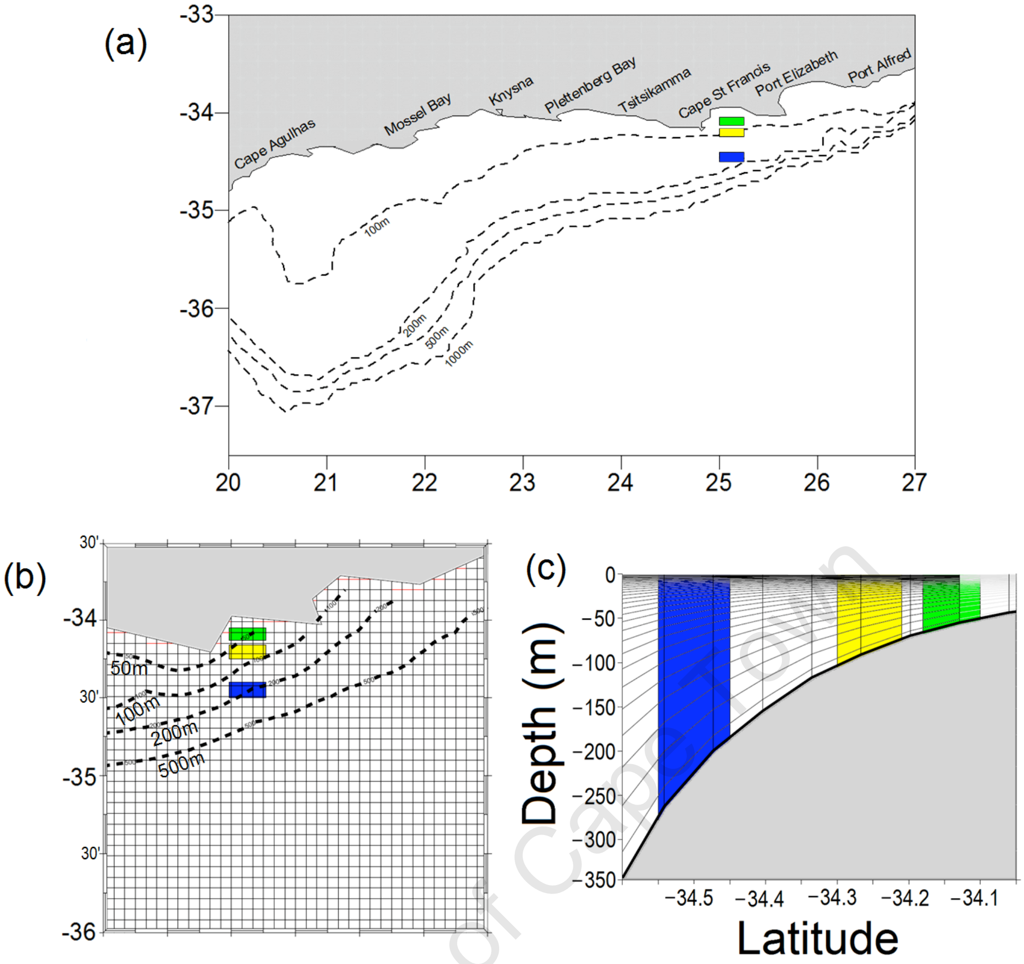


Fig. 4.2. Particle release areas. (a) Geographic position of Bay In (green), Bay Out (yellow) and Mid-shelf (blue). (b) Horizontal (close-up, coloured rectangles) and (c) vertical grids (meridional section across 25.1667°) of the regional domain of SAfE ROMS model, showing release areas (coloured columns).

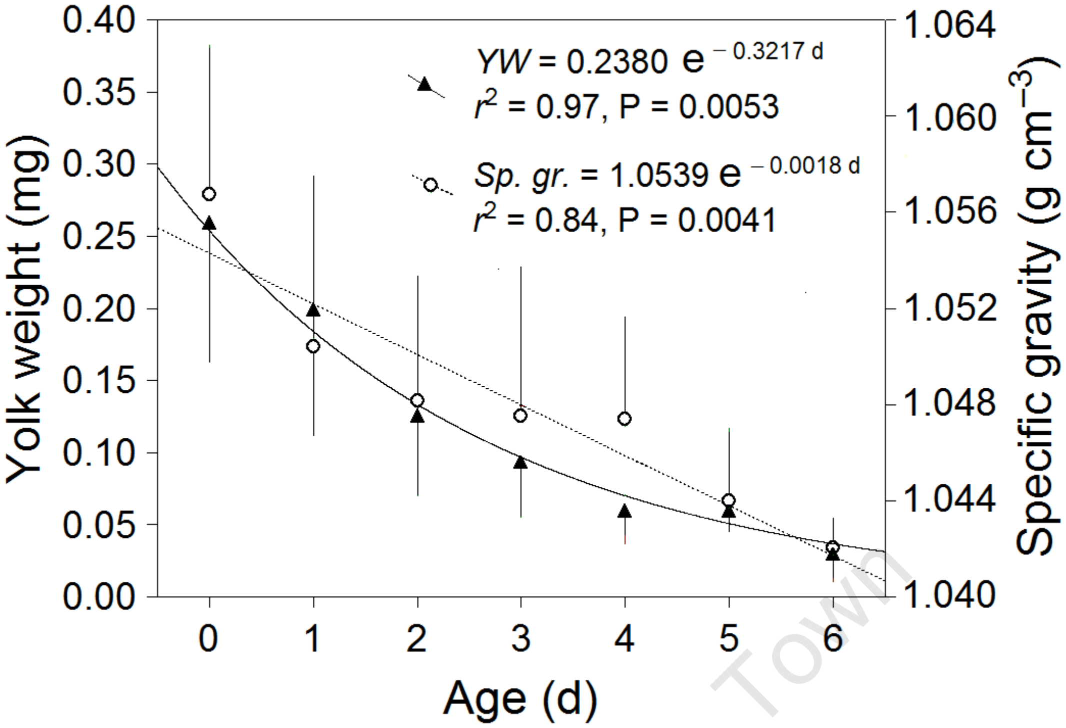


Fig. 4.3. *Loligo reynaudii*. Yolk utilization (empty circles, dotted line) and changes in specific gravity (filled triangles, continuous line) at 14.44 ± 0.10 °C (SD). Values are means of 29–30 paralarvae ± SD. Age is expressed as days post-hatching.

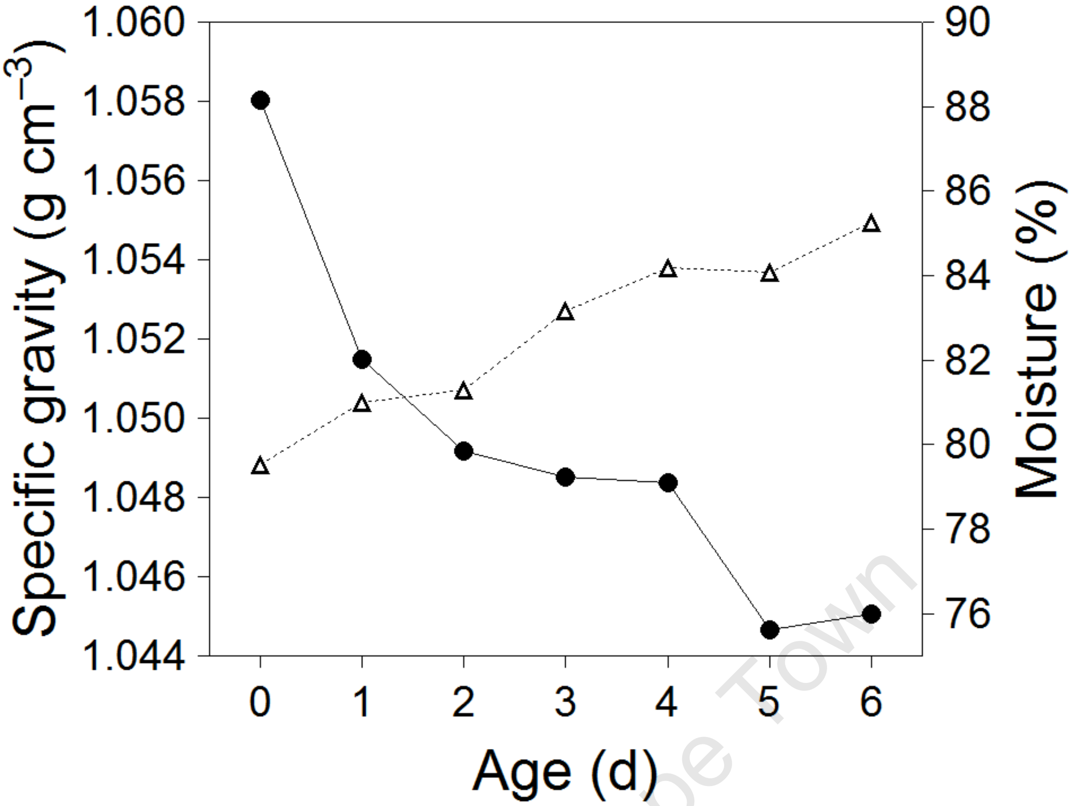


Fig. 4.4. *Loligo reynaudii*. Specific gravity (filled circles) and percentage moisture content (empty triangles) plotted against age. Specific gravity values are means of 29–30 paralarvae. Moisture values are means of 13–15 groups of 3–5 paralarvae each. Age is expressed as days post-hatching.

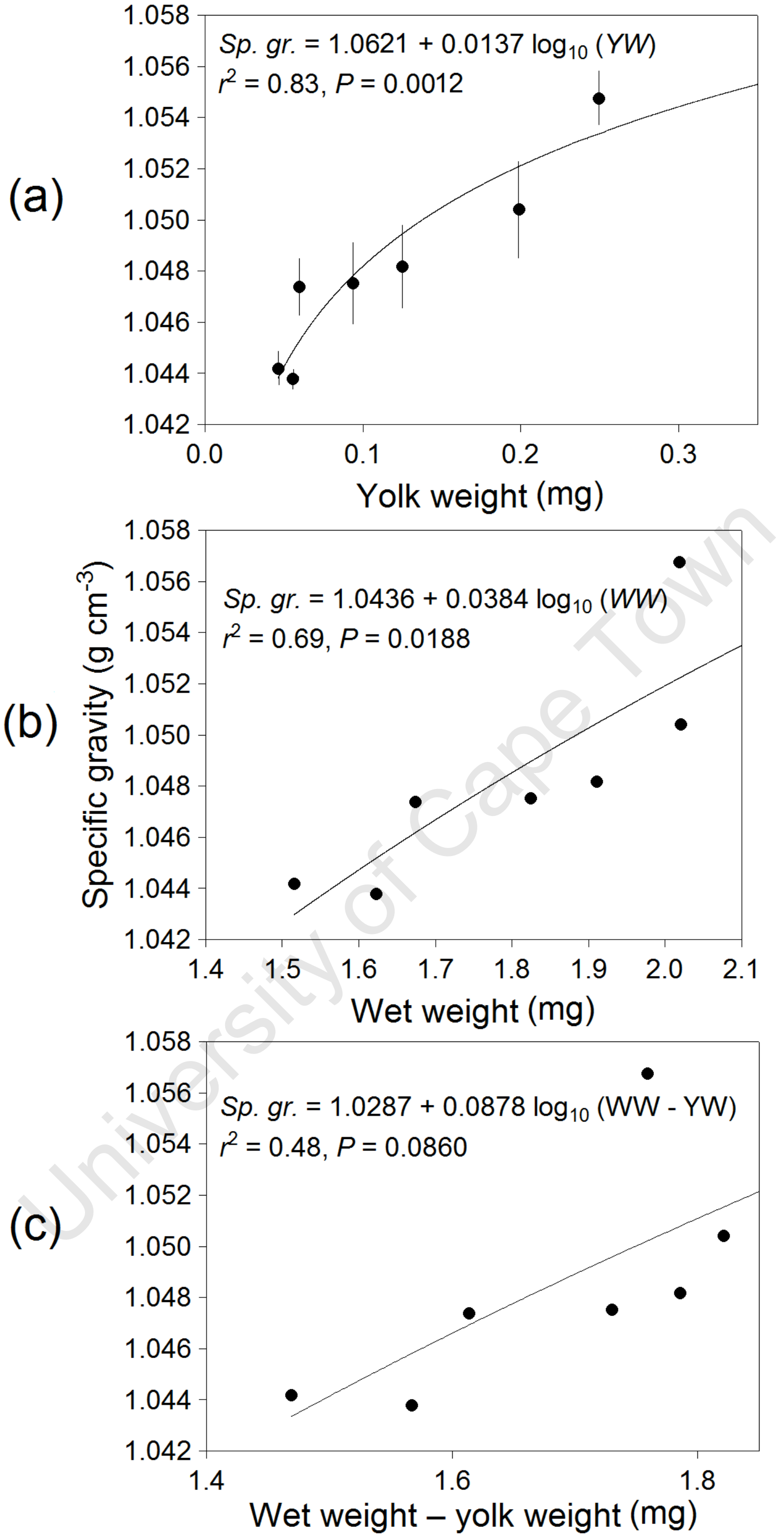


Fig. 4.5. *Loligo reynaudii*. Specific gravity plotted against the (a) yolk weight (b) the wet weight and (c) the wet weight minus the yolk weight. Specific gravity and yolk weight values are means of 29–30 paralarvae. Wet weight values are means of 13–15 groups of 3–5 paralarvae each.

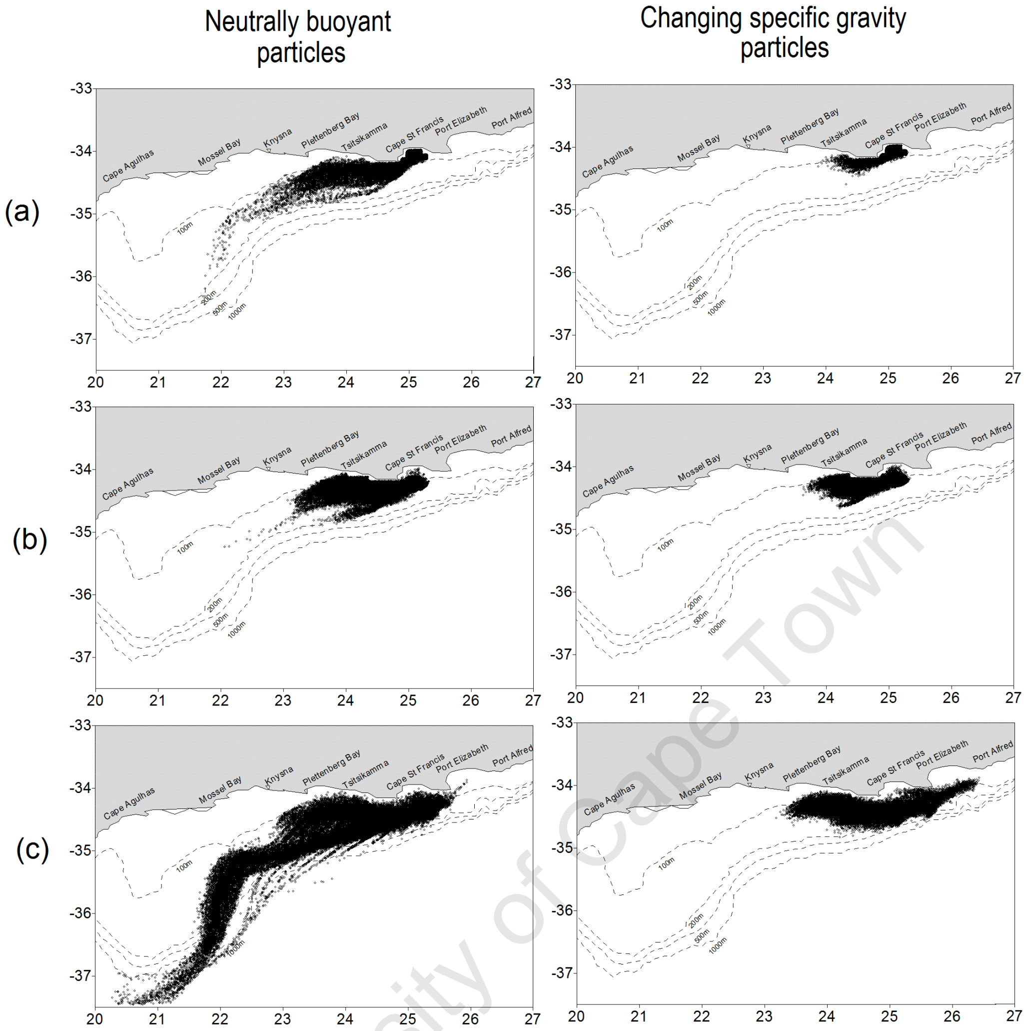


Fig. 4.6. Dispersal envelopes for 7-d simulations, combined across the three years. (a) *Bay In* release area. (b) *Bay Off* release area. (c) *Mid-Shelf* release area. Latitude and longitude are decimal transformed.

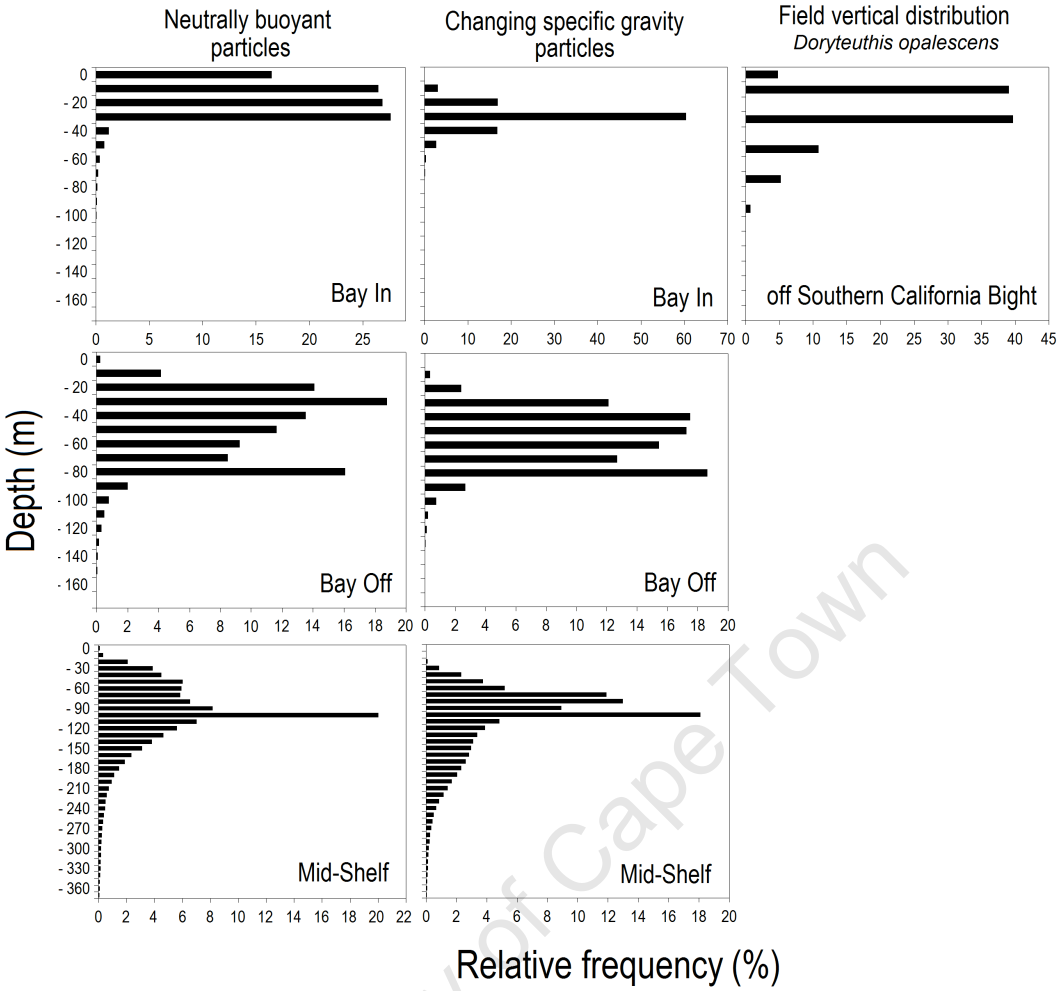


Fig. 4.7. The modelled vertical distributions of particles from Ichthyop showing outputs after seven days for two different buoyancy conditions and the three release areas (all years pooled). Field data for *Doryteuthis opalescens* paralarvae were obtained from Zeidberg and Hamner (2002) and redrawn here.

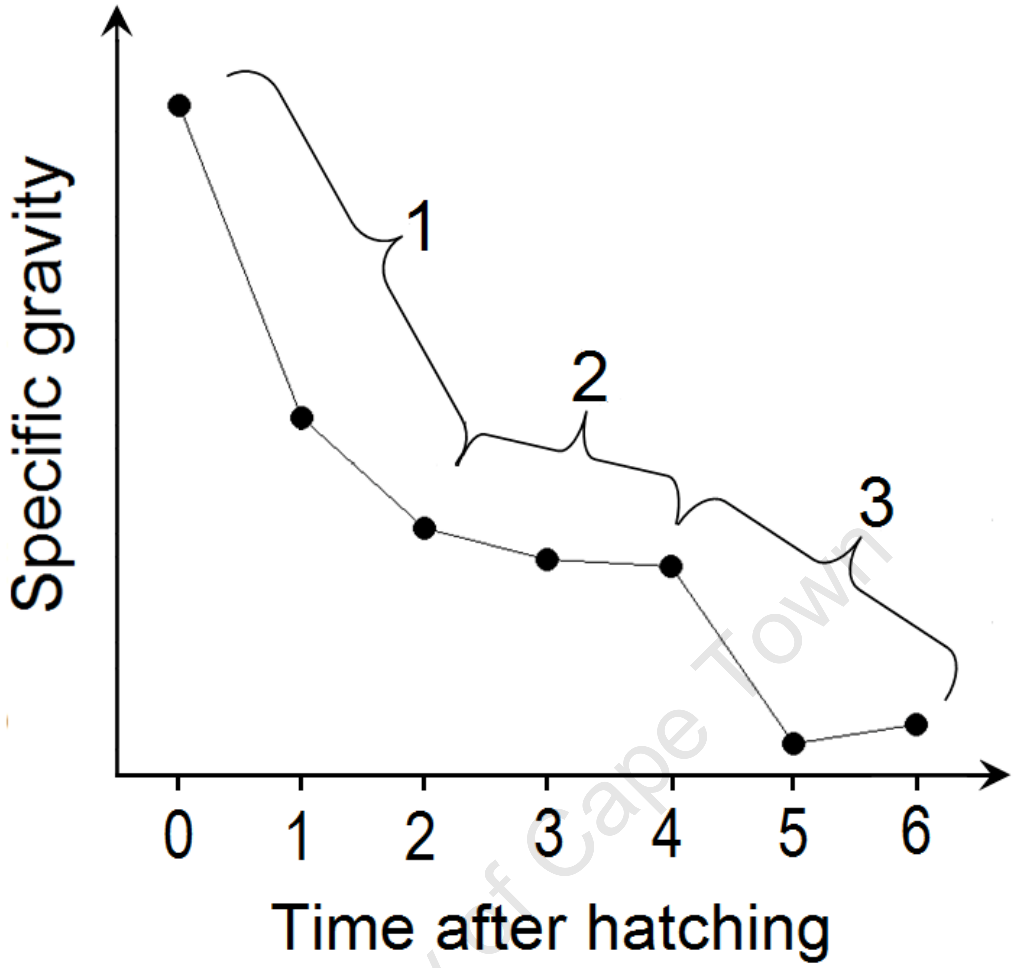


Fig. 4.8. Conceptual hypothetical schematic phases in the changes of the body specific gravity in *Loligo reynaudii* paralarvae, based on the data shown in Figs. 1 and 2. (1) Initial phase: high energy expenditure due to active swimming to the surface, the protein components of the yolk are catabolized quickly, dramatic changes in specific gravity. (2) Intermediate phase: lipid and protein components became evenly distributed, slow down in the change in specific gravity. (3) Final phase: protein components exhausted, yolk becomes mostly lipidic in composition, dramatic changes in the specific gravity.

Chapter 5 — Modelling transport of chokka squid (*Loligo reynaudii* d'Orbigny, 1839) paralarvae off South Africa: reviewing, testing and extending the 'Westward Transport Hypothesis'

Abstract

Chokka squid (*Loligo reynaudii* d'Orbigny, 1839) annual landings fluctuate greatly. In part this is attributed to the success of recruitment, which has been proposed to be linked to successful transport of newly-hatched paralarvae from the spawning grounds to the cold ridge some 100–200 km to the west where food is abundant (referred to as the Westward Transport Hypothesis). Moreover, the first ROMS–IBM using neutrally buoyant particles demonstrated that at times 90% of squid paralarvae on the eastern Agulhas Bank are advected off the shelf into the Agulhas Current similarly causing recruitment failures. In this study an improved ROMS (SAfE) and IBM (*Ichthyop* version 2.1) are coupled and used to test the WTH and the apparent high advective paralarvae losses. Five variables (*Release area, Year, Month, Specific gravity* and *Diel Vertical Migration* (*DVM*)) were used in the ROMS–IBM experiments. Six release sites were identified spanning the inshore and mid-shelf spawning grounds. Recruitment success was measured by the number of particles older than 3 days reaching the cold ridge. Overall annual average transport success ranged between 7–51%. A sensitivity analysis using a linear model identified the *release area* to be the most important factor influencing recruitment, followed by *specific gravity* and *DVM*. The latter two parameters played an important role in optimizing early transport and retaining paralarvae on the shelf respectively.

5.1. Introduction

The South African *Loligo* fishery is based on a single species, locally known as 'chokka' squid (*Loligo reynaudii* d'Orbigny, 1839) (Augustyn et al., 1992). Directed exploitation began in 1984, when hand-jigging fishing methods were introduced, establishing the chokka squid as an important target species and replacing their previous 'minor by-catch' status in the demersal trawl fishery (Augustyn, 1990, 1991; Sauer et al., 1992). Because of the improvement in fishing methods and resulting profitable catches, a specialized squid boat fleet flourished, basing their efforts mostly off the Eastern Cape coast. The fishery targets squid that aggregate and spawn on sandy and low profile reef bottoms located inshore between Plettenberg Bay and Port Alfred (Fig. 5.1; Sauer et al., 1992; Roberts and Sauer, 1994). Although landings show great variability, historical annual landings reported for the species average at ~7 000 mt (Augustyn et al., 1994).

Adult chokka squid distribution over the species' habitat range and inshore spawning aggregations are relatively well studied and thought to be largely influenced by environmental variables, such as dissolved bottom oxygen, temperature and turbidity (Sauer et al., 1991; Roberts and Sauer, 1994; Roberts, 1998). However, the causes of the annual variability in the squid fishing yields are poorly understood. It has been proposed this variability is linked to recruitment and influenced by environmental conditions over the Agulhas Bank, which affects both embryonic development and paralarval nourishment (see Roberts, 2005).

The impact of environmental conditions on the Agulhas Bank on recruitment of chokka squid has been the subject of directed research. Roberts (2005) carried out a comprehensive review of the environmental conditions believed to be important for recruitment. Also studied in detail is the influence of environmental temperatures on embryonic development and hatching success (Oosthuizen et al., 2002a,b; Oosthuizen and Roberts, 2009), temperature influences on yolk utilization and paralarval growth (Vidal et al., 2005; Chapter 3) and the role of paralarval transport in recruitment. Studies have been carried out using acoustic Doppler current profiler (ADCP) measurements (Roberts and van den Berg, 2002, 2005), satellite-tracked drifters (Roberts et al., in prep.) and models, linking an application of the Regional Ocean Modelling System (ROMS) to an individual-based model (IBM) (Roberts and Mullon, in press). More recently, the role of post-hatching paralarval specific gravity on dispersal has been assessed (Chapter 4).

All the above cited studies aimed to verify the validity of the so-called 'Westward Transport Hypothesis', which postulates that paralarvae hatched on the spawning grounds on the eastern Agulhas Bank would be transported westward to nursery areas over the central part of the Bank. In this region an oblique cross-shore, quasi-permanent upwelling tongue (the 'cold ridge', Swart and Largier, 1987) boosts local productivity, ensuring a suitable food environment for the paralarvae (Roberts, 2005). In general, the studies tend to support the Westward Transport Hypothesis, by confirming a general net westward transport and retention over the Agulhas Bank (Roberts, 2005; Roberts et al., in prep.; Chapter 4). However, the physical environment was also shown to be complex, with variable current patterns that could potentially cause paralarvae to be advected from the ecosystem or transported to unsuitable areas. Only one of these approaches (Chapter 4) considered the role of biological characteristics of the paralarvae, by including information on paralarval specific gravity. Nonetheless, the studies provide useful insights into the potential fate of paralarvae after hatching and the potential role of currents on larval transport and recruitment.

This study supplemented and extended the results obtained by previous modelling studies of chokka squid paralarvae in the region (Roberts and Mullon, in press) using computer-based Lagrangian transport simulations to test the Westward Transport Hypothesis. The ROMS model output was coupled with an IBM of 'virtual paralarvae' hatched on inshore and offshore spawning grounds off the Eastern Cape. The study aimed to simulate different transport scenarios and link these to biological scenarios that consider changes in specific gravity and diel vertical migration of chokka squid paralarvae. Thus the study aimed to understand how biologically-mediated changes in vertical position in the water column might affect horizontal transport of chokka squid paralarvae in the Agulhas Bank ecosystem.

5.2. Material and Methods

5.2.1. Hydrodynamic model

A configuration of the ROMS hydrodynamic model (Shchepetkin and McWilliams, 2005), called the Southern African Experiment (SAfE), was developed for the African shore between 2.5°W–54.75°E longitude and 4.8–46.75°S latitude (Fig. 5.1, Penven et al., 2006). Horizontal resolution ranges from 19 km in the south to 27.6 km in the north. Vertical resolution is high in the surface layer (0.37 to 5.70 m) and coarser (11 to 981 m) in the bottom layer, with 32 s-coordinate levels. A higher resolution (three times finer) grid was embedded in the SAfE grid, covering the area from 11.58–27.42°E longitude and 27.73–38.83°E latitude (Fig. 5.1).

5.2.2. Individual-based model

Simulations were performed using the public domain software, *Ichthyop* (Version 2.1.1). This IBM tool uses ROMS offline outputs (i.e., 3D velocity fields) to track Lagrangian movement of particles (Lett et al., 2008b), which in the present case represent squid paralarvae hatched over the main (inshore and offshore) spawning grounds. Thirty thousand particles were 'hatched' over six release areas (5 000 particles released area^{-1}) and had their trajectories tracked for 40 d. The initial number of particles was chosen following the study of Lett et al. (2008a), where a similar experimental design was adopted and the same parent ROMS model was employed. The duration of 40 d was chosen as reared *Doryteuthis* (formerly *Loligo*) *opalescens* Berry 1911, with ML of 6.0 to 8.0 mm, first start gathering in schools between 35 to 45 days after hatching (Yang et al., 1986; Hanlon et al., 1989; Vidal et al., 2009). The formation of schools was assumed to represent the end of the planktonic phase for *Loligo reynaudii*. The randomness of initial particle positions and horizontal and vertical dispersal parameters are default properties of the *Ichthyop* tool (Lett et al., 2008b).

Six areas for release of particles were used. Three areas represented the shallow water spawning grounds (20–50 m) and the remaining three represented the deep water spawning grounds (60–120 m) (Oosthuizen and Roberts, 2009) (Fig. 5.1). The areas were selected based on recorded hydroacoustic signals of spawning aggregations, records of chokka squid egg pods from trawls in research surveys and observations from SCUBA divers (Sauer et al., 1992; Sauer, 1995; Roberts et al., 2002). Release areas were named according to the geographical reference on the shore and were: Plettenberg–Tsitsikamma inshore (P-T_in), Plettenberg–Tsitsikamma offshore (P-T_off), St Francis inshore (SF_in), St Francis offshore (SF_off), Algoa Bay inshore (AB_in) and Algoa Bay offshore (AB_off). Because the ROMS bottom layer resolution is coarse and the bathymetric gradient of the release areas is steep, a single release depth was chosen as representative for all release areas according to their location; inshore, particles were released at 35 m whereas offshore they were released at 90 m. These depths were chosen because they are the average depths of the shallow and deep spawning grounds (Oosthuizen and Roberts, 2009).

Simulations were run with particles that were neutrally buoyant and also with particles that had a specific gravity of 1.048 g cm^{-3}, this being the average specific gravity found for chokka paralarvae (Chapter 4). In test runs, particles with changing specific gravity have been used in the simulations, but a comparison of mean dispersal distances covered by particles with changing specific gravity and particles with constant specific gravity showed no significant differences (Students $t = 0.834$, $p = 0.401$, $n = 25\,411$). Therefore this single value of specific gravity was used in the simulations, for the sake of simplicity.

5.2.3. Transport scenarios

Because vertical behaviour of chokka squid paralarvae is poorly known, two simple transport scenarios were used, based on information from the literature. These were:

1. Passive Lagrangian transport

In this scenario, the virtual paralarvae are assumed to behave as passive particles, seeded in each of the release areas and tracked for the duration of the simulation. This scenario represents the null hypothesis of no ability to change vertical position in the water column. Simulations for this scenario were run with (1) neutrally-buoyant particles and (2) particles with a specific gravity of 1.048 g cm^{-3}.

2. Diel vertical migration (DVM)

This scenario is based on the diel vertical migration (DVM) behaviour described for *Doryteuthis opalescens* Berry 1911 off the southern California coast (Zeidberg and Hamner, 2002) and *Loligo vulgaris* Lamarck, 1798 off the western Iberian Peninsula (Moreno et al. 2009). These authors found paralarvae to be concentrated at 30 m in the daytime and 15 m at night (*D. opalescens*) and at ~ 50–75 m during the day and surfacing at night (*L. vulgaris*). Thus, loliginid paralarvae seem to display Type-1 DVM (Forward, 1988). Such behaviour can have important implications for larval transport (Forward, 1988; Forward et al., 1999). In the present study, DVM was mimicked using the *Ichthyop* default vertical migration scheme (Lett et al., 2008b), which transports particles at depth during the day (07h00 to 19h00) and shallow at night (19h00 to 07h00). The particles were all assumed to have neutral buoyancy. For the inshore release areas, there was one depth change between 10 and 30 m, whereas in the offshore release areas there were three depth changes: between 10 and 30 m, 10 and 60 m and 10 and 90 m.

5.2.4. Assumptions

Because there are some current gaps in the knowledge of biological and oceanographic processes involved in paralarval transport, a number of implicit and explicit assumptions were made in applying the models:

- The resolution, forcing and topography employed in the SAfE model resulted in realistic circulation patterns;
- The subdivision of spawning grounds into six areas was adequate to capture the spatial variability of paralarval transport;
- The thermocline had an average depth of 30 m (Largier and Swart, 1987) all year round;
- The cold ridge is a permanent feature;
- Paralarvae have a specific gravity of 1.048 g cm^{-3};
- Diffusion and active horizontal swimming are negligible for paralarvae in the model;
- The planktonic phase of paralarvae ends after 40 d (Yang et al., 1986; Vidal et al., in prep.);
- Particles (‘paralarvae’) are successfully transported if (1) they reach the cold ridge three days (or more) after release and (2) they are located in any one of the recruitment depth ranges (see below), according to the scenario simulated;
- The release of 5 000 particles per release area during each run was sufficient to ensure stability in the outputs of the model.

5.2.5. Modelled transport success and retention

According to the Westward Transport Hypothesis, the cold ridge should be the nursery area for chokka squid paralarvae. Thus, particles (‘paralarvae’) were considered to have successfully transported if (1) they were located in this area at the age of ≥ 3 d and (2) they were located between the surface and 80 m in the passive

Lagrangian transport scenarios and ≤ 30 m in all DVM scenarios. Model outputs were analysed using multifactor ANOVA that related the percentage of particles released from each spawning ground reaching the cold ridge area (Lett et al., 2006) to a number of explanatory variables: release area, year, month, depth of 'recruitment' and depth range of DVM. Because the percentage success data were heteroscedastic, they were transformed using a $\log_{10}(x + 1)$ transformation. Normality of residuals was visually evaluated using quantile-quantile (Q-Q) plots and plots of mean and variance of residuals against observed values for each variable were also evaluated for trends.

5.3. Results

5.3.1. Overall patterns

Univariate analyses were used to investigate the roles of each explanatory variable and transport scenario in determining the success of transport to the cold ridge area, expressed as the percentage of particles reaching the cold ridge area at an age of ≥ 3 d (Table 5.1). Transport success decreased from the release area in the west (Plettenberg-Tsitsikamma) to the east (Algoa Bay). There was little interannual variation in transport success for all transport scenarios and slight seasonality, with transport success generally reduced from April to July. The depth of arrival into the nursery area tended to occur between 10 and 50 m for inshore release of particles and between 20 and 80 m for offshore release. DVM apparently had a massive impact on particles released inshore. For instance, for the P-T_in release area, transport success increased from 24% for passive Lagrangian particles to nearly 89% for DVM-undergoing particles, and this pattern was consistently reflected in all other variables investigated (Table 5.1). DVM transport scenario also had a significant impact for those particles released offshore, with transport success 4–5 times higher than for the passive Lagrangian scenario in the west-east release areas (Table 5.1). However, because the 'recruitment' depth criterion was different between Lagrangian and DVM simulations, these scenarios may not be directly comparable.

5.3.2. Retention after 40 days

Retention was visually assessed for particles remaining on the Agulhas Bank after 40 d of transport during a cold (July) and warm (October) month of Year 3. Results for only Year 3 were shown because year explained ≤ 1% of the variance in all models fitted (Tables 5.2 to 5.7). The final positions of particles after 40 d of transport are shown for all transport scenarios from inshore (Fig. 5.2) and offshore (Fig. 5.3). From a strictly Lagrangian viewpoint, the Agulhas Bank environment appears to be more retentive during July (winter) than during October (spring), regardless of the release depth (Fig. 5.2 a–b and Fig. 5.3 a–b). The different simulations that included DVM behaviour have relatively large advective losses to the west coast. Particles reaching the western Agulhas Bank appear to be entrained by northwest flow on the western Bank, moving into the Benguela jet when they reach Cape Point. The particles located along the shelf break are advected to the South Atlantic through entrainment within meanders and rings of the Agulhas Current (Fig. 5.2 c–d and Fig. 5.3 c–h).

Assigning a constant value of specific gravity to the particles affected their retention for both inshore and offshore release regions on the Agulhas Bank, with the majority of 1.048 g cm^{-3} particles located close inshore after 40 d of transport, although some particles were found slightly farther offshore in July (Fig 5.2 e–f) than October (Fig. 5.3 i–j). In contrast, some of the neutrally-buoyant particles, either behaving as passive Lagrangian drifters or performing DVM behaviour, travelled as far as the Namibian coast (Fig. 5.2 a–d and Fig. 5.3 a–h).

There were discernible differences in retention in relation to release areas for neutrally-buoyant particles, particularly for the Plettenberg-Tsitsikamma area. Particles released inshore tended to be retained on the Bank (Fig. 5.2). In contrast, particles released offshore tended to be exported to the South Atlantic (Fig. 5.3). Particles released in Algoa Bay, either inshore or offshore, tended to be advected to the South Atlantic, mostly because of entrainment into and transport by the Agulhas Current (Figs. 5.2 and 5.3). Those particles released in the St Francis area (inshore and offshore) displayed an intermediate pattern of retention/advective losses in relation to the other areas (Figs. 5.2 and 5.3).

5.3.3. Passive Lagrangian and specific gravity transport scenarios

1. Inshore release areas

Results of analyses of multiple factors affecting transport success of neutrally-buoyant particles are shown in Table 5.2. Of the three significant main effects, recruitment depth and release area together explained 41.8% of the variability, with a significant interaction between these two variables explaining an additional 6.8% of the variability. All the remaining significant factors and interactions explained < 7% each of the variance (Table 5.2). In general, transport success tended to be greater for particles released in the P-T_in area than for SF_in. AB_in particles had smallest transport success (Fig. 5.4a). Most of the particles reached the nursery area between 10 and 50 m depth (Fig. 5.4b), with shallow recruitment depths being more important for eastern than western release areas (Fig. 5.4c). There was a clear seasonality in transport success throughout the year (Fig. 5.4d), with smallest values in winter months.

For transport success of passive particles with a specific gravity of 1.048 g cm^{-3}, release area was the most important factor affecting variability, accounting for 29.8% of the total variance, followed by the month×recruitment depth interaction (13.7% of total variance) (Table 5.3). The remaining factors and interactions were each responsible for < 7% of the variance. Most of the particles successfully transported to the nursery area were the ones released at P-T_in area (Fig. 5.5a), which partly overlaps with the cold ridge (Fig. 5.1). This indicates that there is more retention than transport in this scenario. Similar to the results for neutrally-buoyant particles, most of the successfully transported particles with a constant specific gravity were < 50 m deep (Fig. 5.5b), the recruitment depth×release area interaction decreased from west to east and with depth (Fig. 5.5c) and there was a clear seasonality (Fig. 5.5d).

2. Offshore release areas

The passive Lagrangian transport scenario for neutrally-buoyant particles released offshore showed that release area and recruitment depth were the most important factors, accounting together for 33.6% of the variance (Table 5.4). The interaction between those two factors also was significant, explaining 13.2% of the variance (Table 5.4). In spite of the considerable variability, transport success for particles released in P-T_off and SF_off was similar, followed by AB_off release area. In terms of recruitment depth, most particles arriving in the nursery area were > 40 m deep (Fig 5.6b). Again, the recruitment depth×release area interaction was more important for P-T_off than the remaining areas, increasing with depth (Fig. 5.6c). Overall transport success showed a clear seasonality, with the lowest transport success observed in autumn-winter months, and the highest transport success observed during spring-summer (Table 5.1, Fig. 5.6d).

Different factors affected particles with a specific gravity of 1.048 g cm^{-3} than those that affected neutrally buoyant particles (Table 5.5). Month and recruitment depth were the most important factors, followed by release area (Table 5.5). Transport success for this scenario was higher for all parameters investigated in relation to their neutrally-buoyant counterparts (Table 5.1). Transport success also decreased from west to east. As for their neutrally-buoyant counterparts, transport success was similar for P-T_off and SF_off release areas (Fig. 5.7a). Most of particles arriving in the nursery area were > 40 m deep (Fig. 5.7b). The recruitment depth×release area (Fig. 5.5c) interaction was also similar to the situation found for neutrally-buoyant particles. There was a clear seasonality in transport success (Fig. 5.7d).

5.3.4. Diel vertical migration (DVM) transport scenarios

1. Inshore release areas

Transport success variability (44.5%) for the DVM transport scenario was explained by release area, followed by the month×year interaction (21.1%) (Table 5.6). Accordingly, transport success decreased from west to east (Fig. 5.8a). Transport success was high (~ 50%) throughout the year (Table 5.1) and there was a weak seasonality (Fig. 5.8b).

2. Offshore release areas

Release area and the month×year interaction were the most important factors affecting transport success for the DVM scenario with offshore release, together accounting for 37.3% of the variance. The three different DVM depth ranges explained < 3% of the total variance (Table 5.7). As in all previous scenarios, transport success decreased from west to east (Fig. 5.9a). There was no discernible seasonality in transport success and the 10–90 m DVM scenario yielded a higher transport success than its shallower counterparts (Fig. 5.9d).

5.4. Discussion

No biological characteristics other than specific gravity and DVM were assigned to the particles. The results therefore indicate the interaction of modelled circulation for different transport scenarios with biologically-mediated vertical position. This is a reasonable approach, because swimming velocity of a number of cephalopod paralarvae of several sizes and ages ranges from 0.003 to 0.25 m s^{-1} (Zuev, 1964; Packard, 1969; O'Dor et al., 1986; Villanueva et al., 1995; Zeidberg, 2004; Vidal et al., 2009), which is slower than typical horizontal current velocities (0.2–3.5 m s^{-1}) found on the Agulhas Bank (Roberts, 2005). Accordingly, much of the paralarval dispersal could be the result of passive transport within the current velocity field they are embedded in.

In this study, we simulated a suite of possible transport scenarios interacting with vertical shear in horizontal currents on the Agulhas Bank, in order to address Roberts' (2005) Westward Transport Hypothesis. A common feature among all transport scenarios simulated was that particles were transported mainly westward towards the cold ridge area, thus supporting the Westward Transport Hypothesis. Nonetheless, it is important to recognize the weaknesses in the modelling approach adopted. First, the currently incomplete information on vertical and horizontal distributions of chokka squid paralarvae limits validation of the results. A second important limitation is that entire populations of particles were transported at discrete depths in the DVM scenarios, whereas a real planktonic population would likely be distributed above and below a centre of gravity, introducing additional variability in the transport patterns (Leis, 2007).

The substantial contribution of area of particle release to variability in transport success in all modelled transport scenarios (except for passive Lagrangian scenarios) suggests that the location of the spawning grounds favours successful paralarval transport, particularly for those sites located west of Algoa Bay. This, in addition to appropriate bottom substrata for egg pod attachment, water visibility conducive to mating and suitable environmental conditions for embryonic development (Roberts and Sauer, 1994; Roberts, 1998; Oosthuizen et al., 2002; Oosthuizen and Roberts, 2009), may partially explain why these areas are repeatedly used every year by spawning chokka squid (Sauer et al., 1992). This implies that adult spawning behaviour (i.e., birth site fidelity) may be more important than paralarval behaviour in determining paralarval transport success variability (Edwards et al., 2008).

5.4.1. Importance of upwelling in the modelled paralarval transport

Regardless of the transport scenario or release depth, the Plettenberg-Tsitsikamma area had the greatest proportion of successfully transported particles among all release areas. Whereas this may be explained by the partial overlap of this area with the nursery area, transport success was greatest for particles transported at the release depth. In contrast, the greatest transport success for the two other release areas was for particles transported above the release depth. This was reflected by the elevated importance of the release area×recruitment depth interaction in the passive Lagrangian transport scenarios. This result is consistent with particles moving upwards, which suggests upwelling could be the mechanism underpinning this pattern. In fact, both empirical and modelling studies have shown that coastal upwelling is very common on the eastern Agulhas Bank (Schumann et al. 1982; Lett et al., 2006). This may partially explain why high loliginid paralarval concentrations are often found during upwelling and uplifted thermocline events (Rocha et al. 1999; Zeidberg and Hamner, 2002; González et al. 2005; Martins and Perez, 2006; Moreno et al. 2009).

5.4.2. Specific gravity and the Westward Transport Hypothesis

From a strictly Lagrangian perspective, assigning specific gravity of 1.048 g cm^{-3} to the particles released inshore, making them denser than seawater, introduced a major challenge to the Westward Transport Hypothesis, as the retention in the release areas was elevated, with few particles being transported to the cold ridge (except where release areas merge with the nursery area). However, inshore productivity is high in the study area because of wind-driven intermittent upwelling (Shannon et al. 1984; Probyn et al., 1994; Lett et al., 2006), sustaining an assorted plankton assemblage (Verheye et al., 1994), which may provide food for paralarvae. Indeed, most chokka paralarvae sampled thus far have been caught inshore, overlapping with the spawning grounds (Sauer, 1995; Roberts and van den Berg, 2002). This suggests that the cold ridge might not be used as a nursery ground by at least part of inshore spawned paralarvae.

In the offshore release areas, in contrast, approximately 21.2% of the 1.048 g cm^{-3} particles reached the nursery area between the surface and 30 m (Table 5.1), where the bulk of the zooplankton (food) is present (Verheye et al. 1994). This suggests that the transport success strategies for paralarvae hatched inshore and offshore could be different, with the latter tending to use westward current transport (supporting the Westward Transport Hypothesis) to take advantage of the enhanced productivity of the cold ridge.

5.4.3. Comparison with field data and other IBMs and implications for the life-cycle

Although these ROMS–IBM results support the Westward Transport Hypothesis, across-shore field data have been both conflicting and consistent with the hypothesis. Close inshore (off the Tsitsikamma coast), acoustic Doppler current profiler (ADCP) measurements and release of satellite-tracked drifters revealed a general eastward current flow and leakage of shelf waters into the open sea near the southern apex of the Agulhas Bank (Roberts and van den Berg, 2002, 2005; Roberts et al., in prep.). However, ADCP data also indicate the presence of an inshore counter-current (Roberts and van den Berg, 2002; 2005). In addition, drifters are poor simulacra of paralarvae, as their trajectory can be affected by the roughness of the sea surface and wind conditions (Roberts et al., in prep.). This would be particularly true in inshore areas because of increased wave action induced by shallow bottoms. Trajectories of drifters released offshore, on the other hand, were more similar to these ROMS–IBM results, suggesting that paralarvae hatched on the eastern Bank would likely be transported westwards and retained in the ecosystem near the cold ridge (Roberts et al., in prep.).

A comparison of our data with the results of the first chokka squid ROMS–IBM study carried out by Roberts and Mullon (in press) showed important differences. Whereas the transport was mostly westward in our simulations, their results suggest mostly eastward transport, regardless of the release position, with massive losses of particles to offshore waters. There are two possible reasons for this difference. (1) They used a different ROMS model configuration (the Plume, Penven et al. 2001) with a small domain and therefore influenced by boundary effects, particularly on the eastern Agulhas Bank, where the main spawning grounds are located (Parada et al. 2003; Roberts and Mullon, in press). (2) The previous study modelled the paralarvae as neutrally-buoyant particles only, whereas specific gravity and DVM have been shown here to be important factors determining transport trajectories on the shelf.

Other ROMS–IBM studies have been carried out for anchovy (*Engraulis encrasicolus*) and sardine (*Sardinops sagax*) in the southern Benguela, including the Agulhas Bank. These also suggested the cold ridge area was important for recruitment variability of these fish species (Parada et al., 2003; Lett et al. 2006; Miller et al. 2006). However, the neutral buoyancy of clupeoid fish eggs and early yolk sac larvae can result in huge advective losses of progeny to offshore waters, mainly on the outer eastern Agulhas Bank (Boyd et al. 1992; Roel et al. 1994; Hutchings et al. 2000; Parada et al. 2003). Those losses can be compensated by high fecundity typical of clupeoid fish (Alheit, 1989; Hutchings et al. 2000; Huggett et al. 2003). In contrast, paralarvae are negatively buoyant and this appears to help in their retention in productive inshore areas and mitigates offshore losses (Chapter 4 and present study). This can partially account for the relative success of chokka squid as a species in a dynamic ecosystem such as on the Agulhas Bank, even though their fecundity is considerably lower than most of their fish competitors.

5.4.4. Food availability vs. transport and recruitment depth

Copepod mean abundance over the central and eastern Agulhas Bank is about 3 individuals L^{-1} (several species pooled year round) (J. Huggett, Marine and Coastal Management, pers. comm., 2009). However, traditional plankton abundance estimates derived from net samples are typically < 10% of the actual abundance (Gallienne and Robins, 2001). In addition, up to 100–350 particles L^{-1} (mostly microcrustaceans) can be found in the chlorophyll maximum layer associated with the thermocline (Verheye et al., 1994; Roel et al., 1994), and chokka paralarvae also prey upon zooplankton taxa other than copepods (Venter et al., 1999). Therefore, zooplankton concentration on the Agulhas Bank can approach (or even exceed) the minimum threshold feeding concentration (50 prey L^{-1}; Vidal et al., 2002) that allows loliginid paralarval survival and growth, at least in laboratory conditions.

The criterion for transport success time in the present study was initially based on data of starvation and recovery experiments carried out with 15 d old *Doryteuthis opalescens*, where paralarvae were found to regain the before-starvation condition if fed after 3 d of food deprivation (Vidal et al., 2006). Newly-hatched chokka squid paralarvae, however, can withstand up to 1–4 d on their yolk reserves before entering in irreversible starvation, considering an exponential yolk utilization rate and depending on the temperature (Chapter 3). In addition, complete absence of food is probably not often the case in the wild. This implies that a demographically meaningful number of good condition paralarvae could reach the cold ridge area beyond the minimum ‘3 d deadline’. This would be particularly important for those individuals hatched further east, i.e., between Algoa Bay and Port Alfred.

The depth at which paralarvae reach the cold ridge is especially relevant to the Westward Transport Hypothesis, because only those arriving and maintaining themselves ≤ 30 m (this being the average thermocline depth on the Agulhas Bank; Largier and Swart, 1987) would have access to sufficient food in the wild, as most zooplankton (prey) are found in the top 30 m (Verheye et al., 1994). This is particularly critical, because early paralarvae are prone to starvation, and first-feeding failure can lead to mortality (Vidal et al., 2006). In that sense, paralarvae located at 30 m would be at an advantage compared with those located shallower or deeper, because food concentration (zooplankton) is greatest in the chlorophyll maximum layer, generally at the depth of the thermocline (Verheye et al., 1994; Huggett and Richardson 2000).

5.4.5. DVM vs. Lagrangian transport scenarios

In other ROMS–IBM studies of larvae of different organisms, the value of vertical migration behaviour for larval transport has been (1) particularly important (Cowen et al. 2006; North et al. 2008), (2) only of secondary importance (Edwards et al. 2008; Parada et al. 2008) and (3) not important (Hare et al., 1999) in relation to passive Lagrangian transport. Our results are similar to the second case, as although all DVM transport scenarios improved transport, they explained only a small amount (~ 3%) of the total variance in the models. Nonetheless, transport success of particles undergoing DVM was much greater than for their passive Lagrangian counterparts for all variables investigated. This suggests that DVM could be a strategy adopted in the wild to influence transport to favourable areas.

Although DVM behaviour resulted in enhanced transport success, most DVM-undergoing particles were transported off the Agulhas Bank and lost to the South Atlantic and southern Indian Ocean after 40 days of transport. This implies poor retention potential for paralarvae undergoing DVM behaviour on the Agulhas Bank (at least for the depth shifts simulated in the present study). However, paralarval swimming capability increases with age and size (Zeidberg 2004; Vidal et al., 2009) and thus 40 d old (or even younger) paralarvae might be able to use directed horizontal swimming to remain in the nursery area, as observed in fish larvae (Leis, 2006).

5.4.6. Transport success and recruitment

The overall annual average transport success from the release areas to the nursery area (cold ridge) ranged from 7.4% (worst case scenario, for Lagrangian transport of particles assigned 1.048 g cm^{-3} specific gravity released inshore) to 51% (best case scenario, for DVM-undergoing particles released inshore). Natural mortality of squid paralarvae appears to be much slower than for fish eggs and larvae (O'Dor, 1998), even considering sources of mortality such as advective losses, predation, starvation and inappropriate environmental conditions. Therefore, it is likely that there would be sufficient individuals recruiting to the adult population, even in the worst case scenario. By comparison, a survival rate of only 0.002% of progeny is thought to be sufficient to maintain the equilibrium of the population of high-fecundity anchovy in the southern Benguela (Hutchings et al., 1998).

5.4.7. Conclusions and future directions

This study represents a step towards predicting squid-stock abundance fluctuations by providing insights on some processes affecting the early life stages of chokka squid. Results of the present ROMS–IBM study proved to be a useful tool for investigating paralarval transport in the dynamic environment of the Agulhas Bank. Successful transport to the nursery area (cold ridge) seems to be affected by a number of factors. Although spawning location was found to be the most important, other factors such as upwelling, buoyancy of the paralarvae and DVM behaviour were also found to play a role in the variability of paralarval transport in the model. Upwelling can facilitate transport by moving paralarvae upwards and thus exposing them to faster surface currents. Specific gravity, on the other hand, seems to help retain paralarvae in the productive inshore areas, preventing offshore advective losses. Finally, DVM behaviour appears to optimize early transport by taking advantage of the vertically stratified flow field.

Improved knowledge is required of the horizontal and vertical distributions and the swimming abilities of chokka squid paralarvae to validate the results obtained in the present modelling study. Extensive plankton sampling efforts with bongo nets, WP-2, MOCNESS multiple net and hyperbenthos samplers (Zeidberg and Hamner 2002; Moreno et al. 2009; Bouali et al., 2009) should be carried out in the study area to compare the situation in the wild with the ROMS–IBM results. It is likely that horizontal and vertical distributions of early paralarvae probably coincide with, or are very close to, the egg beds. Our results suggest that this is the first place to search.

Table 5.1. Results of univariate analyses showing the percentage of particles successfully transported from the release areas to the nursery area (cold ridge) for each explanatory variable and transport scenario. Spec. grav.: specific gravity.

Variable	% particles successfully transported					
Release area	Lagrangian inshore	Lagrangian offshore	Spec. grav. inshore	Spec. grav. offshore	DVM inshore	DVM offshore
P-T	23.85	17.08	37.18	19.64	88.89	67.10

SF	12.68	11.47	6.29	20.47	66.76	50.23
AB	5.22	5.68	0.89	5.45	31.75	31.10
Depth (m)						
0–10	5.94	1.13	2.31	1.18		
10–20	15.59	3.93	7.73	8.00		
20 –30	21.17	7.62	10.64	12.05		
30–40	19.00	11.54	12.08	15.89		
40–50	10.43	11.75	10.37	18.16		
50–60	5.35	17.22	7.67	20.14		
60–70	2.49	17.16	4.90	20.13		
70–80	1.22	16.03	3.42	20.62		
Month						
January	11.50	13.38	9.06	15.97	48.37	36.22
February	12.44	14.69	6.41	16.69	50.55	58.03
March	12.38	13.56	8.52	15.28	50.58	43.37
April	7.57	8.97	8.16	14.22	44.74	48.76
May	8.77	8.96	6.86	7.62	42.50	36.51
June	7.62	9.26	6.24	9.16	50.65	53.64
July	7.14	6.30	7.24	7.22	44.16	46.17
August	11.12	12.31	5.55	10.16	50.48	59.79
September	9.32	9.03	7.85	22.52	59.81	56.54
October	10.29	12.43	7.82	15.31	54.75	45.57
November	12.23	12.03	6.91	18.24	60.16	47.51
December	11.42	13.35	8.01	21.89	54.66	55.68
Year						
3	10.37	11.13	8.09	13.95	50.58	54.65
4	10.12	10.96	5.85	14.70	52.05	46.35
5	10.95	10.38	6.94	14.94	51.22	47.42
6	10.36	11.64	8.28	14.67	49.87	51.02
7	9.76	11.35	7.59	11.56	53.29	48.34
8	9.28	11.34	7.70	14.99	48.38	44.05
9	10.22	11.53	7.25	16.85	50.86	49.29
DVM (10–30 m)					50.95	45.84
DVM (10–60 m)						45.84
DVM (10–90 m)						54.52

Table 5.2. Results of a multifactor ANOVA applied to the simulation results of neutrally buoyant passive particles released inshore, indicating the contributions of different explanatory variables to transport success. ns: not significant (p>0.05); ***: p < 0.001;% Var:% variability in the data explained by each variable, calculated as a percentage of SS_{TOTAL}.

Factor	df	SS	MS	*F*-value	p (> *F*)	Significance	% Var.
Release area	2	260.47	130.24	139.950	< 2.2e−16	***	5.72

Month	11	47.00	4.27	4.591	6.48e−07	***	1.03
Year	6	1.23	0.2	0.220	0.9706	ns	0.02
Recruitment depth	7	1645.83	235.12	252.653	< 2.2e−16	***	36.14
Release area × Month	22	59.13	2.69	2.888	8.62e−06	***	1.29
Release area × Year	12	16.43	1.37	1.472	0.1277	ns	0.36
Release area × Recruitment depth	14	310.92	22.21	23.865	< 2.2e−16	***	6.82
Month × Year	66	219.14	3.32	3.568	< 2.2e−16	***	4.81
Month × Recruitment depth	77	314.59	4.09	4.390	< 2.2e−16	***	6.90
Year × Recruitment depth	42	44.20	1.05	1.131	0.2621	ns	0.97
Residuals	1756	1634.13	0.93				35.89

Table 5.3. Results of a multifactor ANOVA applied to the simulation results of passive particles assigned a specific gravity of 1.048 g cm^{-3} released inshore, indicating the contributions of different explanatory variables to transport success. ns: not significant (p>0.05); **: p<0.01; ***: p < 0.001;% Var:% variability in the data explained by each variable, calculated as a percentage of SS_{TOTAL}.

Factor	df	SS	MS	*F*-value	p (> *F*)	Significance	% Var.
Release area	2	1721.72	860.86	754.464	< 2.2e−16	***	29.87
Month	11	98.30	8.94	7.832	2.15e−13	***	1.70
Year	6	22.41	3.74	3.274	0.003319	**	0.38
Recruitment depth	7	387.26	55.32	48.486	< 2.2e−16	***	6.72
Release area × Month	22	92.77	4.22	3.696	1.66e−08	***	1.61
Release area × Year	12	20.94	1.75	1.530	0.106539	ns	0.36
Release area × Recruitment depth	14	259.13	18.51	16.222	< 2.2e−16	***	4.49
Month × Year	66	335.91	5.09	4.461	< 2.2e−16	***	5.82
Month × Recruitment depth	77	794.47	10.32	9.043	< 2.2e−16	***	13.78
Year × Recruitment depth	42	25.61	0.61	0.534	0.993895	ns	0.44
Residuals	1756	2003.64	1.14				34.77

Table 5.4. Results of a multifactor ANOVA applied to the simulation results of neutrally buoyant passive particles released offshore, indicating the contributions of different explanatory variables to transport success. ns: not significant (p>0.05); **: p,0.01; **: p<0.01; ***: p < 0.001;% Var:% variability in the data explained by each variable, calculated as a percentage of SS_{TOTAL}.

Factor	df	SS	MS	*F*-value	p (> *F*)	Significance	% Var.
Release area	2	227.21	113.60	165.853	< 2.2e−16	***	6.89
Month	11	169.87	15.44	22.545	< 2.2e−16	***	5.15
Year	6	5.58	0.93	1.359	0.22778	ns	0.16
Recruitment depth	7	880.69	125.81	183.677	< 2.2e−16	***	26.72
Release area × Month	22	56.41	2.56	3.743	1.14e−08	***	1.71
Release area × Year	12	18.28	1.52	2.224	0.008934	**	0.55
Release area × Recruitment depth	14	435.30	31.09	45.393	< 2.2e−16	***	13.21
Month × Year	66	202.08	3.06	4.470	< 2.2e−16	***	6.13
Month × Recruitment depth	77	83.64	1.09	1.586	0.001117	**	2.53
Year × Recruitment depth	42	13.08	0.31	0.455	0.999022	ns	0.39
Residuals	1756	1202.81	0.68				36.50

Table 5.5. Results of a multifactor ANOVA applied to the simulation results of passive particles assigned a specific gravity of 1.048 g cm^{-3} released offshore, indicating the contributions of different explanatory variables to transport success. ns: not significant ($p>0.05$); *: $p < 0.05$; ***: $p < 0.001$;% Var:% variability in the data explained by each variable, calculated as a percentage of SS_{TOTAL}.

Factor	df	SS	MS	*F*-value	p (> *F*)	Significance	% Var.
Release area	2	517.15	258.58	261.212	< 2.2e−16	***	9.85
Month	11	540.20	49.11	49.609	< 2.2e−16	***	10.29
Year	6	39.25	6.54	6.609	6.37e−07	***	0.74
Recruitment depth	7	858.72	122.67	123.925	< 2.2e−16	***	16.35
Release area × Month	22	181.10	8.23	8.316	< 2.2e−16	***	3.45
Release area × Year	12	41.78	3.48	3.517	3.57e−05	***	0.79
Release area × Recruitment depth	14	216.03	15.43	15.588	< 2.2e−16	***	4.11
Month × Year	66	672.46	10.19	10.293	< 2.2e−16	***	12.81
Month × Recruitment depth	77	381.02	4.95	4.999	< 2.2e−16	***	7.25
Year × Recruitment depth	42	62.93	1.50	1.514	0.01896	*	1.19
Residuals	1756	1738.29	0.99				33.11

Table 5.6. Results of a multifactor ANOVA applied to the simulation results of neutrally-buoyant particles performing DVM released inshore, indicating the contributions of different explanatory variables to transport success. ns: not significant (p>0.05); **: $p < 0.01$; ***: $p < 0.001$;% Var:% variability in the data explained by each variable, calculated as a percentage of SS_{TOTAL}.

Factor	df	SS	MS	*F*-value	p (> *F*)	Significance	% Var.
Release area	2	63.70	31.85	123.211	< 2.2e−16	***	44.50
Month	11	4.23	0.39	1.489	0.142691	ns	2.90
Year	6	0.38	0.06	0.245	0.960577	ns	0.20
Release area × Month	22	7.82	0.36	1.374	0.138662	ns	5.40
Release area × Year	12	2.47	0.21	0.796	0.654350	ns	1.70
Month × Year	66	30.23	0.46	1.772	0.002808	**	21.10
Residuals	132	34.12	0.26				23.80

Table 5.7. Results of a multifactor ANOVA applied to the simulation data of neutrally-buoyant particles performing DVM released offshore, indicating the contributions of different explanatory variables to transport success. ns: not significant ($p>0.05$); *: $p < 0.05$; ***: $p < 0.001$;% Var:% variability in the data explained by each variable, calculated as a percentage of SS_{TOTAL}.

Factor	df	SS	MS	*F*-value	p (> *F*)	Significance	% Var.
Release area	2	108.84	54.42	94.191	< 2.2e−16	***	13.20
Month	11	56.98	5.18	8.966	6.93e−15	***	6.90
Year	6	9.78	1.63	2.820	0.01029	*	1.10
DVM depth range	2	23.26	11.63	20.130	3.47e−09	***	2.80
Release area × Month	22	45.44	2.07	3.575	1.02e−07	***	5.50
Release area × Year	12	13.44	1.12	1.938	0.02765	*	1.60
Release area × DVM depth range	4	3.31	0.83	1.432	0.22193	ns	0.40
Month × Year	66	198.65	3.01	5.210	< 2.2e−16	***	24.10
Month × DVM depth range	22	14.59	0.66	1.148	0.29055	ns	1.70
Year × DVM depth range	12	3.59	0.30	0.519	0.90340	ns	0.40
Residuals	596	344.34	0.58				41.80

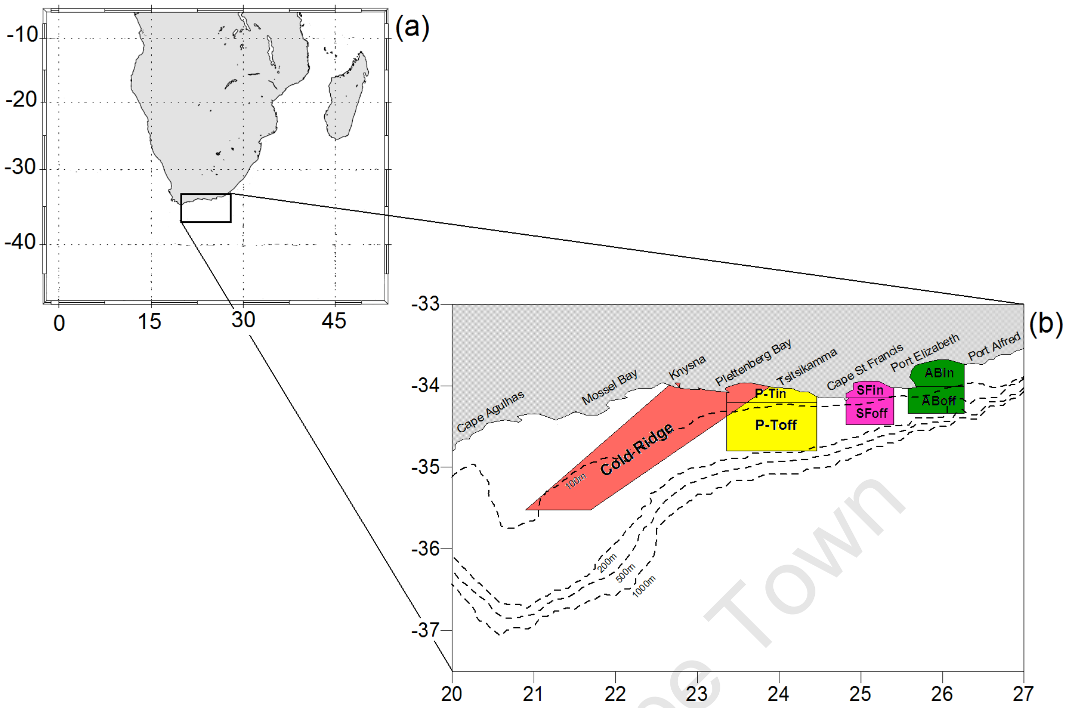


Fig. 5.1. (a) SAfE domain. (b) Study area showing the position of the cold ridge and the release areas. P-T_in: Plettenberg-Tsitsikamma inshore, P-T_off: Plettenberg-Tsitsikamma offshore, SF_in: St Francis inshore, SF_off: St Francis offshore, AB_in: Algoa Bay inshore, AB_off: Algoa Bay offshore.

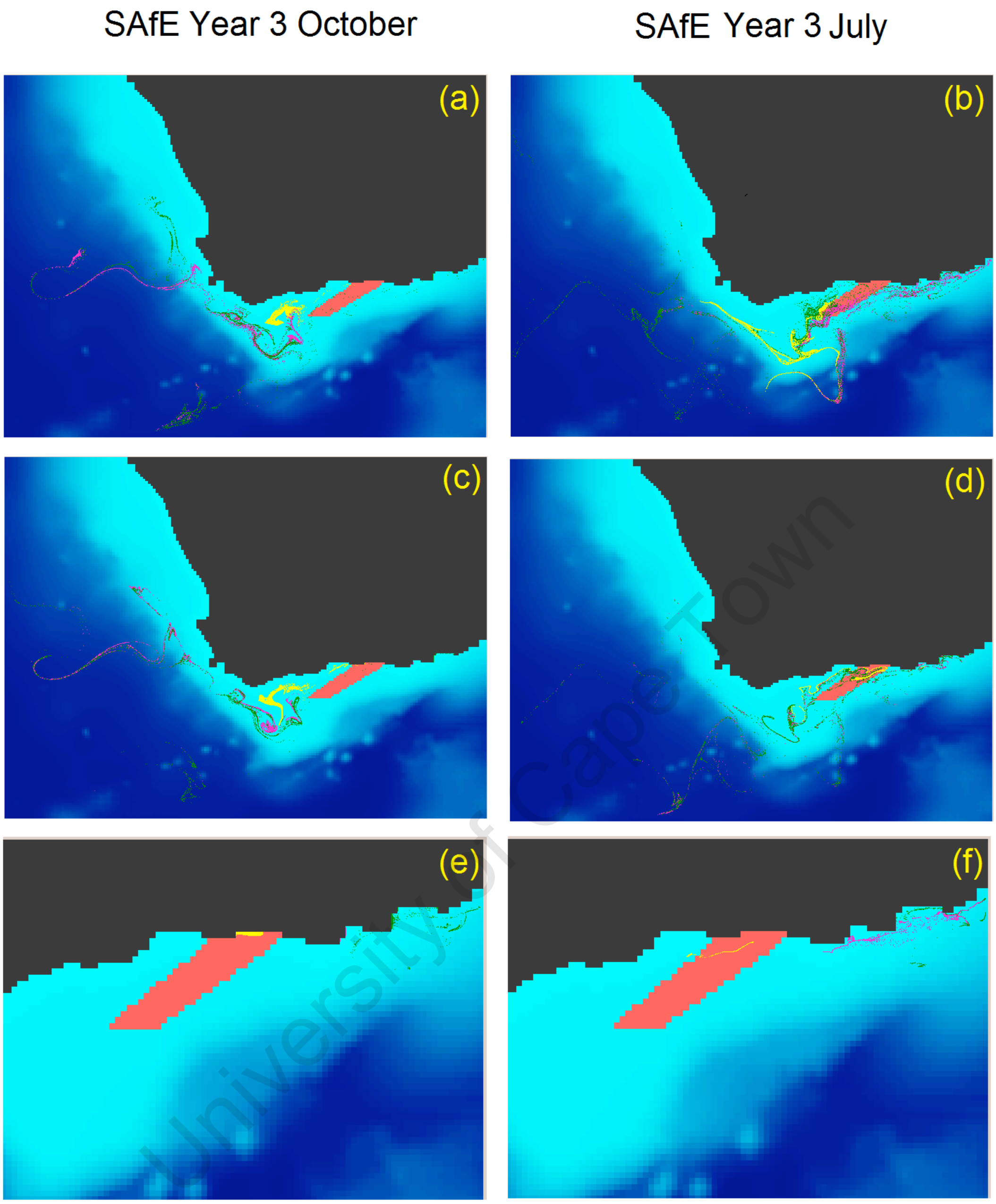


Fig. 5.2. Dispersal end points of particles after 40-d simulations from inshore release areas off P-T (yellow), SF (pink) and AB (green) in a warm (October) and a cold (July) month in model year 3. (a) – (b): Lagrangian transport, neutrally buoyant particles. (c) – (d): DVM (10–30 m), (e) – (f) Lagrangian transport, particles assigned a specific gravity of 1.048 g cm^{-3}. Note the different scale in panels (e) and (f). The orange shaded area is the assumed nursery area.

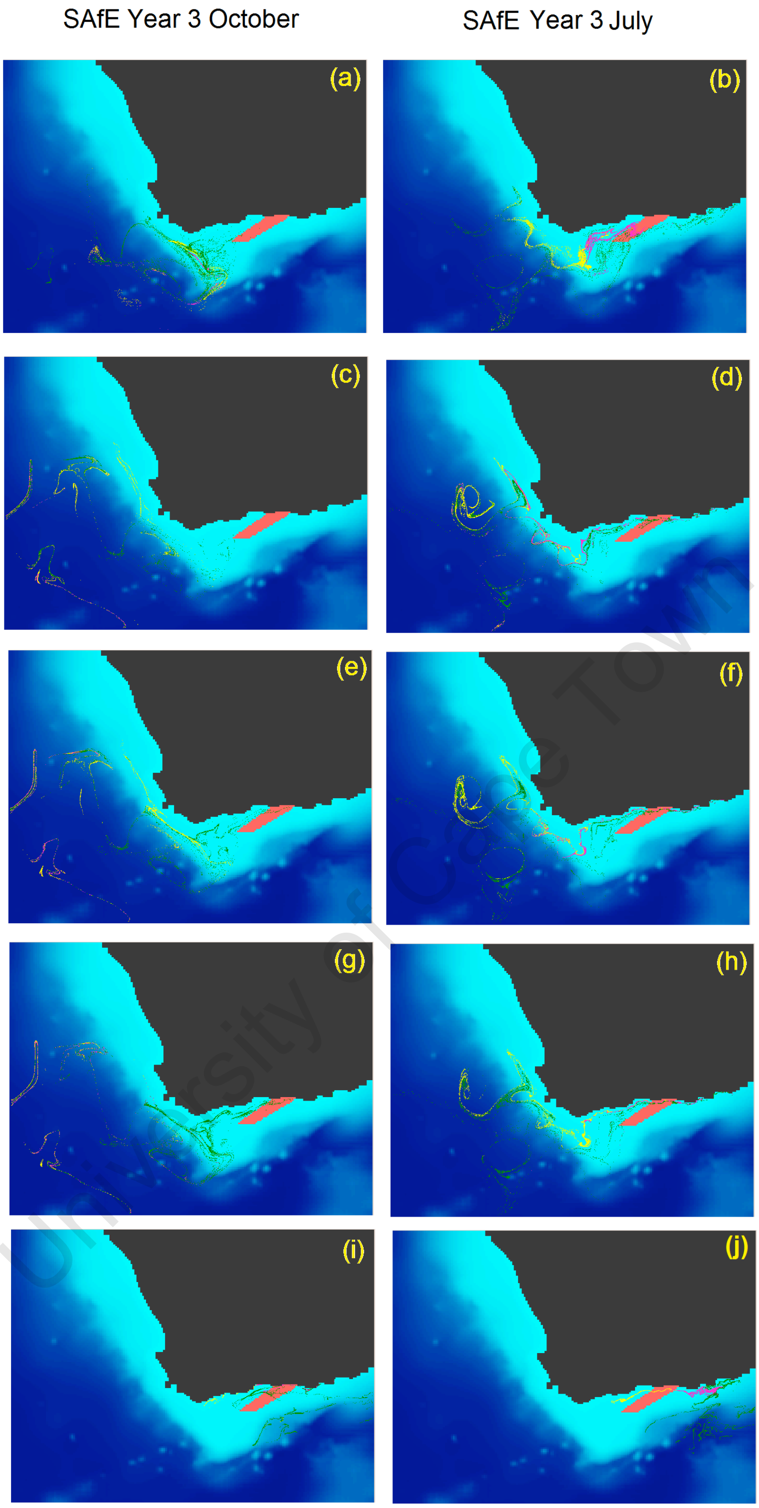


Fig. 5.3. Dispersal end points of particles after 40-d simulations from offshore release areas off P-T (yellow), SF (pink) and AB (green) in a warm (October) and a cold (July) month. (a) – (b): Lagrangian transport scenario, neutrally buoyant particles. (c) – (d): DVM (10–30 m). (e) – (f): DVM (10–60 m). (g) – (h): DVM (10–90 m). (i) – (j): Lagrangian transport scenario, particles assigned a specific gravity of 1.048 g cm^{-3}. The orange shaded area is the assumed nursery area.

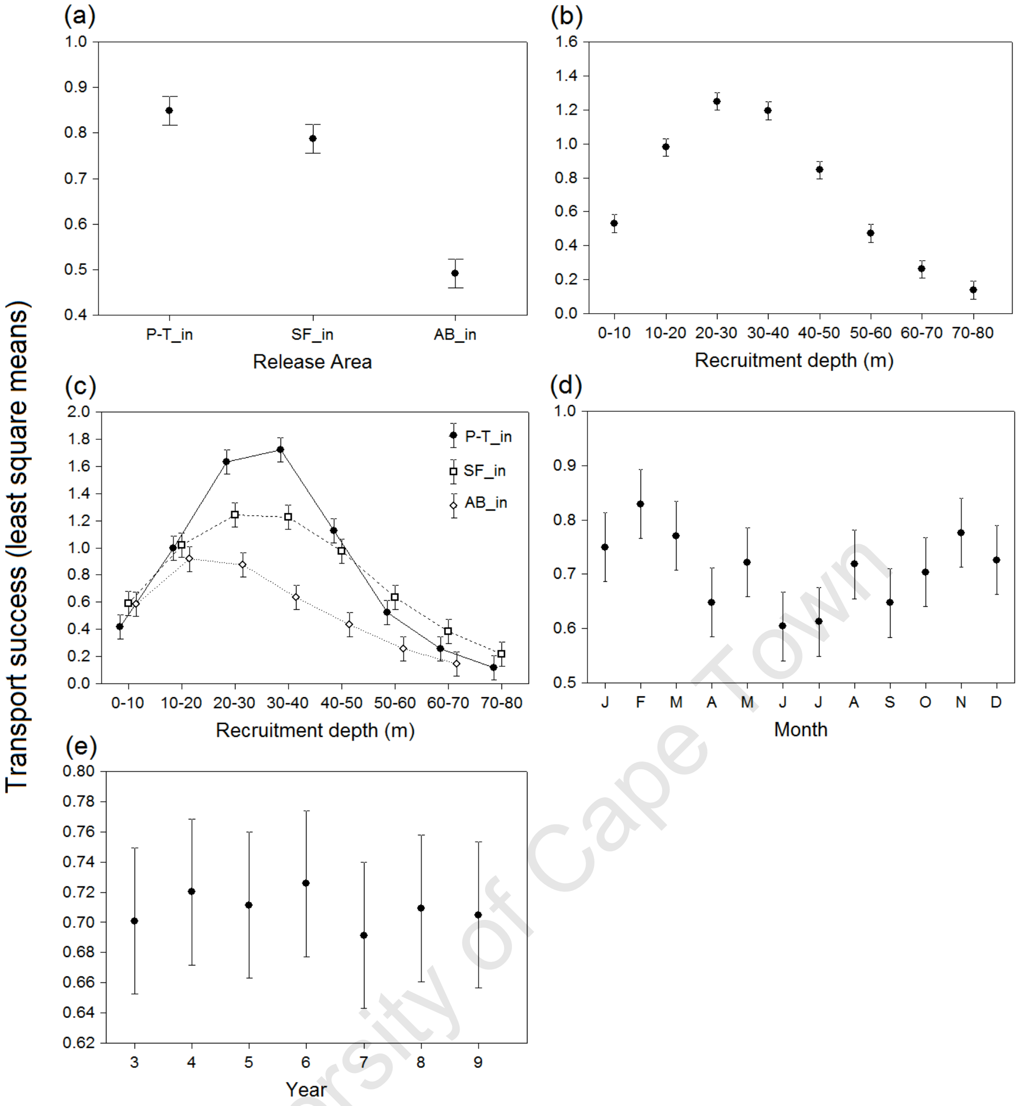


Fig. 5.4. Results of the multifactor ANOVA applied to the simulation results showing transport success for neutrally buoyant passive particles released inshore. The least squares means (± 95% C.I.) are shown for different effects and interactions: (a) release area, (b) recruitment depth, (c) release area × recruitment depth interaction (d) month and (e) year.

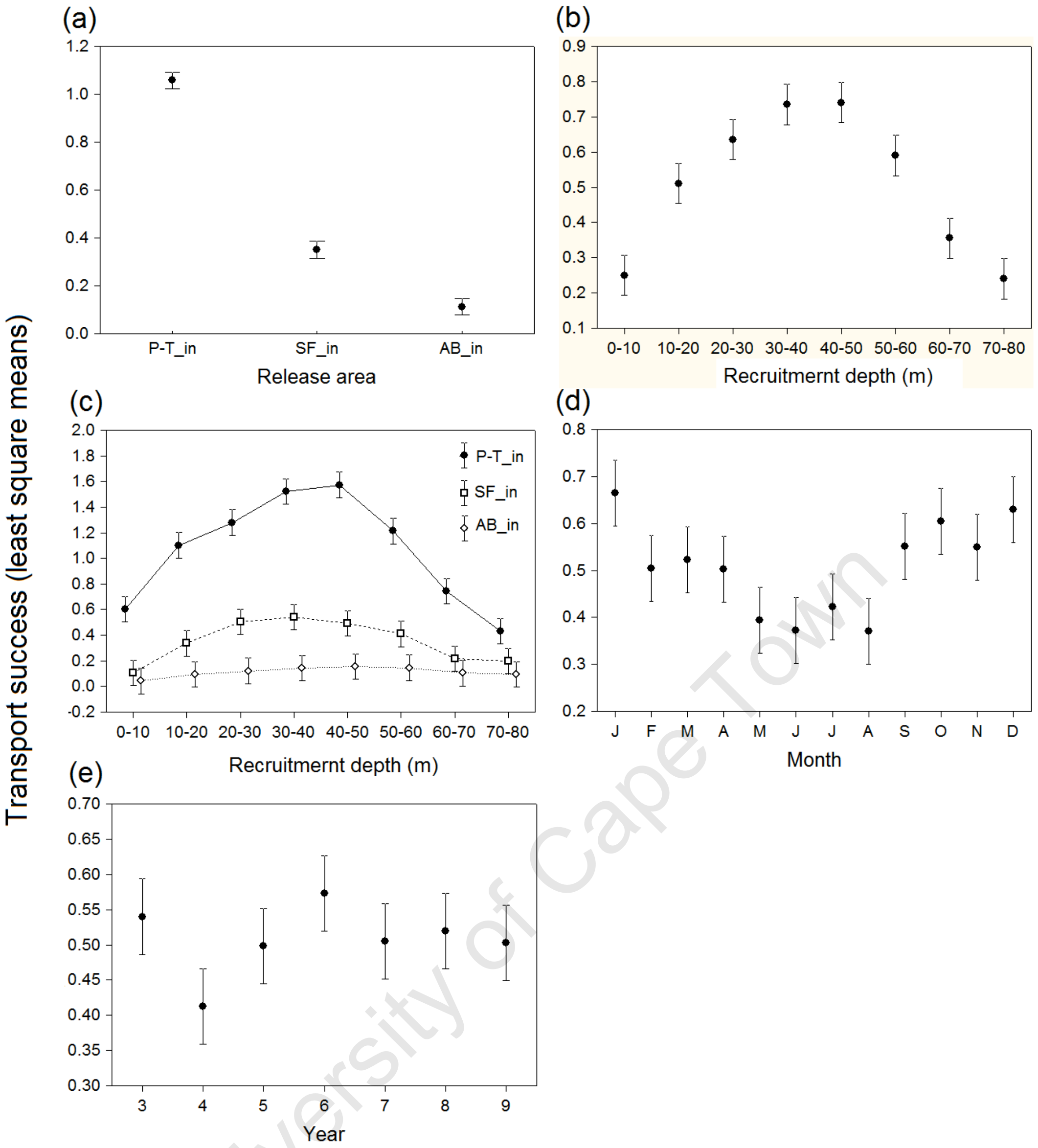


Fig. 5.5. Results of the multifactor ANOVA applied to the simulation results showing transport success for 1.048 g cm^{-3} passive particles released inshore. The least squares means (± 95% C.I.) are shown for different effects and interactions: (a) release area, (b) recruitment depth, (c) release area × recruitment depth interaction (d) month and (e) year.

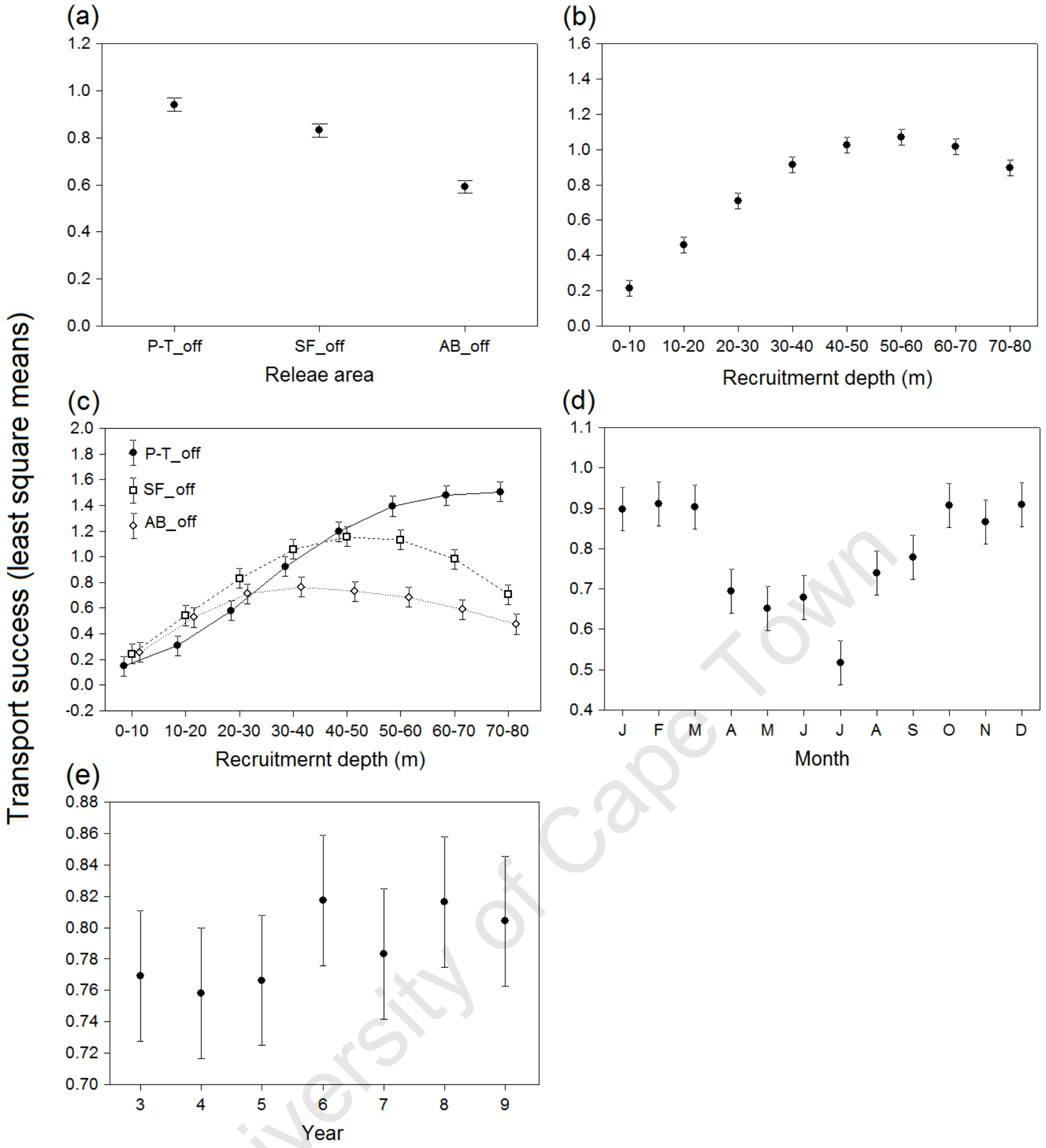


Fig. 5.6. Results of the multifactor ANOVA applied to the simulation results showing transport success for neutrally buoyant passive particles released offshore. The least squares means (± 95% C.I.) are shown for different effects and interactions: (a) release area, (b) recruitment depth, (c) release area × recruitment depth interaction (d) month and (e) year.

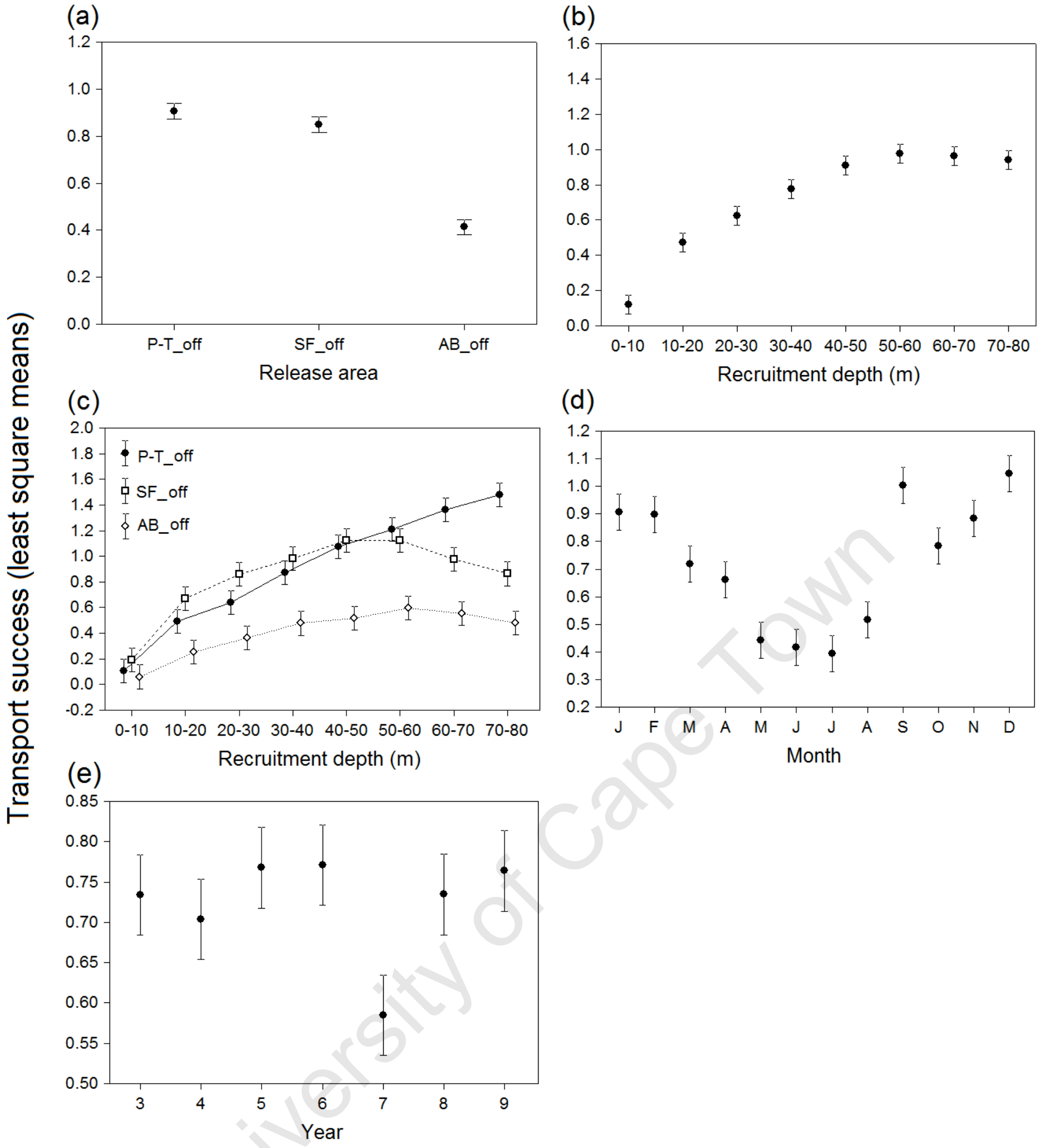


Fig. 5.7. Results of the multifactor ANOVA applied to the simulation results showing transport success for 1.048 g cm^{-3} passive particles released offshore. The least squares means (± 95% C.I.) are shown for different effects and interactions: (a) release area, (b) recruitment depth, (c) release area × recruitment depth interaction (d) month and (e) year.

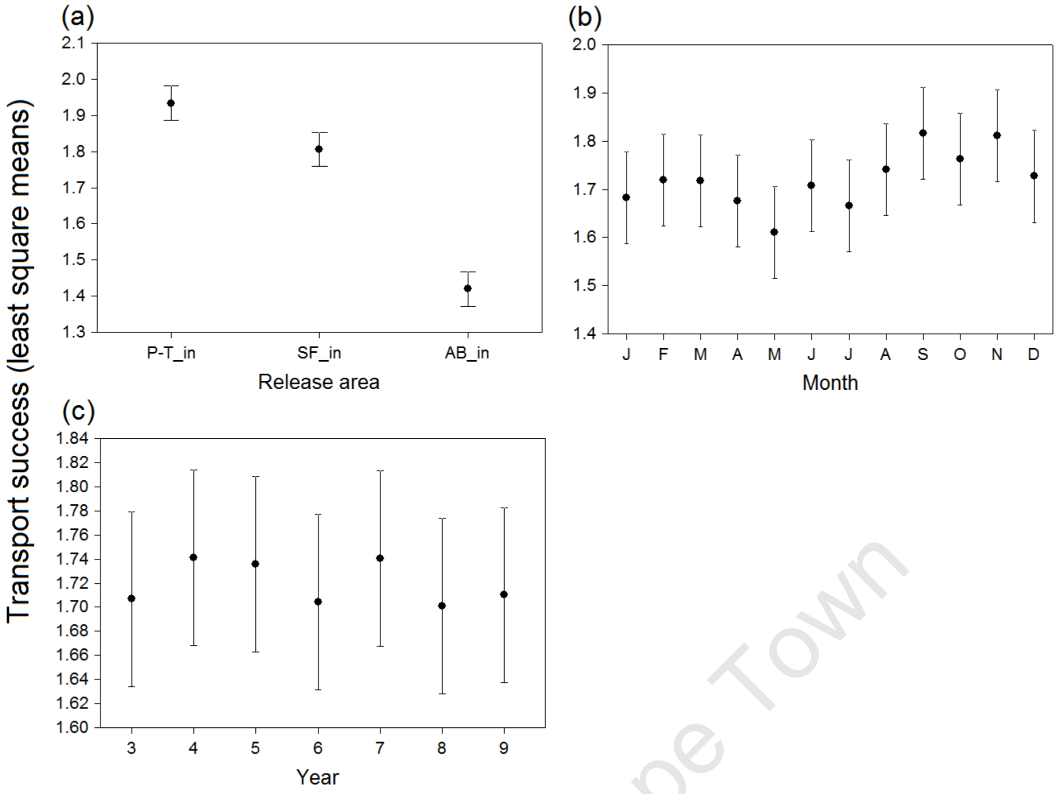


Fig. 5.8. Results of the multifactor ANOVA applied to the simulation results showing transport success for neutrally-buoyant particles released offshore and undergoing DVM. The least squares means (± 95% C.I.) are shown for different effects and interactions: (a) release area, (b) month and (c) year.

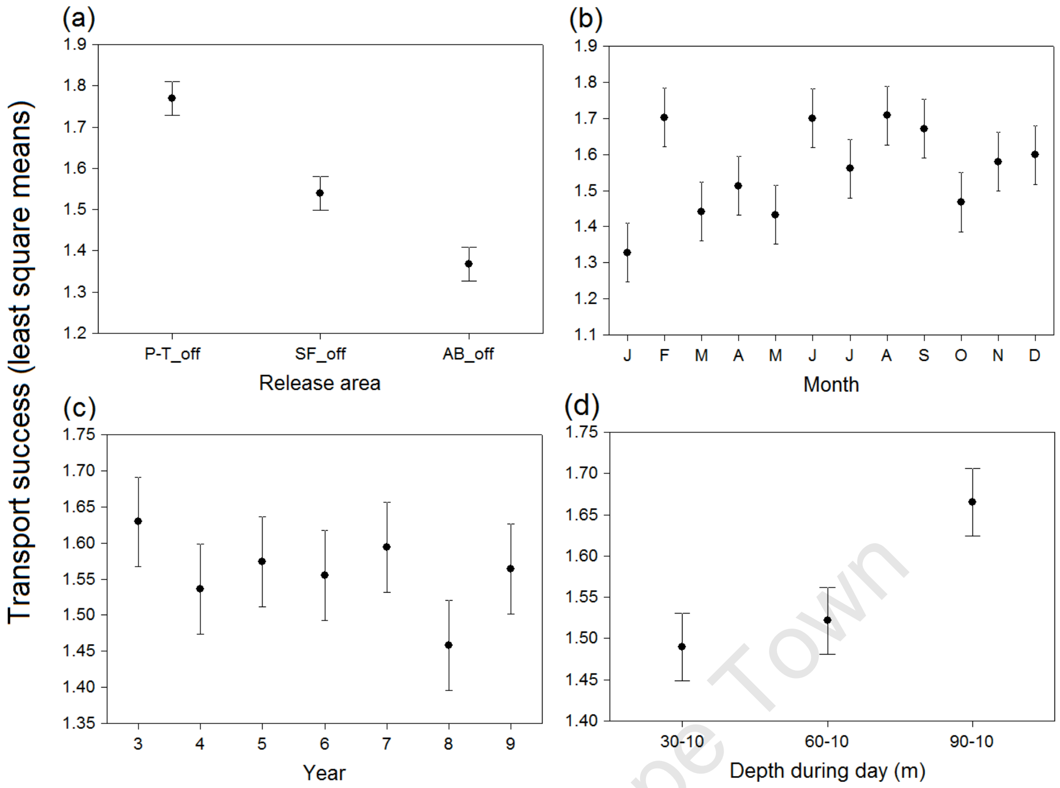


Fig. 5.9. Results of the multifactor ANOVA applied to the simulation results showing transport success for neutrally-buoyant particles released offshore and undergoing DVM. The least squares means (± 95% C.I.) are shown for different effects and interactions: (a) release area, (b) month, (c) year and (d) DVM scenario.

Chapter 6 — Synthesis

6.1. Hypotheses tested and Thesis findings

The null hypothesis regarding the temperature independence of yolk utilization (Chapter 3) in chokka squid paralarvae can confidently be rejected, since data showed clearly that yolk utilization rates change with temperature — with rates being faster in warmer than in colder water. The results support the WTH because paralarvae were shown to survive 5–6 d without food depending only on their yolk reserves. This would give them sufficient time to reach the site of favoured food (i.e. the cold ridge). While extrapolation of these findings to reality can be questionable, they do appear to be supported by field data and other studies. For example Roberts (2005) quantitatively linked years of high chokka squid yields to low summer sea temperatures in the previous year, i.e. good recruitment is the result of colder water.. This is interesting because from the yolk utilization perspective, paralarvae would survive longer on their yolk reserves when temperatures are lower. In turn this would allow the paralarvae to have more time for the development of learning-dependent predatory skills (Chen et al., 1996). However the trade-off is that growth would be slow in cold water and paralarvae would remain more susceptible to predation for a longer period of time. Notwithstanding the latter, it is plausible that a greater number of paralarvae could survive the 'critical period' (see Vidal et al., 2002) in cold water which would strengthen recruitment. This may also be one of the reasons underlying the existence of the deep water spawning grounds (Oosthuizen and Roberts, 2009).

In Chapter 4 it was shown that specific gravity (density) is closely related to the paralarval yolk content, but also that the paralarvae were always negatively buoyant even when their yolk was completely exhausted. In other words "H_A*: Specific gravity is influenced by the yolk content and affects dispersal" was proven correct. Furthermore* changes in specific gravity during the yolk utilization phase appear to depart from the exponential decay model in a systematic way, with some indication of a discontinuity after four days. Although more research is needed, this is attributed to selective utilization of the yolk components during the yolk utilization phase to fully assess this hypothesis. Also, the rate of change in specific gravity may vary with temperature, as the yolk utilization is temperature dependent (Chapter 3). Specific gravity of paralarvae was shown to influence dispersal using a simple ROMS–IBM model. For the duration of their yolk reserves (i.e. 7-d at 14°C; Chapter 4), model paralarvae (particles) assigned realistic specific gravity values demonstrated lower dispersal rates and remained closer to the shore than their neutrally-buoyant counterparts. In fact, the latter were advected into the open ocean at the southern tip of the Agulhas Bank. Therefore it would appear that the negative buoyancy of paralarvae will improve survival by maintaining them in productive shelf waters and mitigating advective losses. Hence, the null hypothesis advanced for Chapter 4 must be rejected. In the context of the WTH, this may indicate that lengthier transport times to reach the cold ridge region,, and paralarvae would need to find enough food to avoid starvation during the passive transport.

Swimming velocities of cephalopod paralarvae (several species and sizes) ranges from 0.003 to 0.25 m s^{-1} (see Chapter 5), which is lower than typical shelf current velocities of ~ 0.25 m s^{-1} (Boyd and Shillington, 1994). This suggests that at least some of their dispersal will be passive and related to water movements (Boletzky, 2003). Moreover, because current velocities tend to be greater in surface waters, biologically-mediated vertical position will further influence the transport of paralarvae. This was explored for the case of chokka squid paralarvae using the ROMS–IBM modelling approach that incorporated (a) paralarval *specific gravity* as calculated in Chapter 4 and (b) *diel vertical migration* behaviour data based on information from other loliginid species. Other parameters were also included in the simulations such as *release area* (i.e. distance from the cold ridge) and *Year* and *Month* (i.e. timing and seasonality in transport). Results showed that the most important parameter influencing paralarval transport was the release area. This suggests that birth site fidelity (i.e. adult spawning behaviour) is more important for successful transport than the remaining parameters. Also the higher specific gravity clearly favours the retention of paralarvae on the Agulhas Bank on the spawning grounds, confirming the findings of Chapter 4. Diel vertical migration behaviour however, enhanced transport to the nursery ground in the vicinity of the cold ridge — confirming that the vertical position in the water column is important for paralarval transport variability. It was also found that upwelling events appear to facilitate dispersion by moving paralarvae towards the surface of the water column and therefore exposing them to the faster surface currents. This means that the null hypothesis of no differences in transport in relation to passive Lagrangian scenarios must be rejected on the basis of the findings of this model (Chapter 5), and that "H_A: *Paralarval transport is affected by their biologically-mediated vertical position in the water column"* has been proven.

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6.2. Concluding remarks

Squid stocks around the world typically display considerable interannual fluctuations (Boyle and Rodhouse, 2005) and chokka squid is no exception. Historical landings for chokka squid from 1985 to 2006 have oscillated between 2500 and 10 000 mt (Marine and Coastal Management, Commercial Squid Fishery Catch Data, 2008). These fluctuations are likely linked to recruitment success among years which, as pointed out already, is strongly influenced by oceanographic conditions and food availability (Vidal et al., 2005; Roberts, 2005). Indeed, Roberts (2005) quantitatively linked low summer sea temperature to high annual landings and biomass in the following year.

While temperature is no doubt important, the transport of paralarvae by currents also appears to play an important role. This was suggested by the historical 'crash' in jig catches experienced in 2001. In fact it was suggested by Roberts and Mullon (in press) that the exceptionally low catches in 2001 were the result of an early retroflection of the Agulhas Current in 2000. This was thought to have caused the removal of substantial numbers of paralarvae through shelf water leakage to the open ocean, leading to poor recruitment. In other words, the transport mechanism underlying the 'Westward Transport Hypothesis' was likely disrupted by anomalous oceanographic conditions.

The data presented in this thesis are not able to accurately forecast recruitment of chokka squid in the Agulhas Bank ecosystem, but they contribute to understanding the recruitment dynamics of the species. Temperature influences on yolk utilization alone, for instance, cannot guarantee recruitment success, but it could limit recruitment despite other conditions being favourable. This may be the reason for high recruitment success following low sea temperature years, and also part of the strategy underpinning the existence of deep water spawning grounds.

The ROMS–IBM simulations presented in this thesis suggest that passive paralarval transport during early life stages is key to recruitment. In fact, the large scale losses of paralarvae to the open ocean proposed by the previous ROMS–IBM simulations carried out by Roberts and Mullon (in press) — where paralarvae (particles) were treated as passive Lagrangian, neutrally-buoyant drifters — seem unlikely on the basis of my findings.

Unlike opportunistic pelagic spawners such as sardine and anchovy, which spawn across broad regions (Parada et al., 2003; Miller et al., 2006), chokka squid restrict spawning activity to localized sites (Roberts and Sauer, 1994). Those sites seem to be not only conducive to mating and embryonic development, but also ‘strategically’ positioned to favour successful transport to the nursery area. This may be the reason for those areas being repeatedly used every year by spawning squid, and also why spawning area, rather than paralarval vertical position, was the most important variable affecting transport success in the ROMS–IBM simulations (Chapters 4 and 5). Paralarval transport, however, is likely to be affected by biologically-mediated vertical position. If swimming behaviour is ignored, then retention (specific gravity) and differential transport by depth (diel vertical migration) could be secondary (although important) factors influencing paralarval dispersal and, ultimately, recruitment.

A schematic model of the early life history of chokka squid on the Agulhas Bank, based on the results of this thesis, is depicted in Fig. 6.1. Model results suggest that retention on the inshore embayments would be greater due to the greater specific gravity of paralarvae. On the other hand, virtual paralarvae spawned offshore would be predominantly advected westwards, regardless of their specific gravity proprieties. Finally, those virtual paralarvae located close to the shelf break (~ 200 m isobaths) will tend to be lost from the ecosystem through entrainment in the fast flowing Agulhas Current.

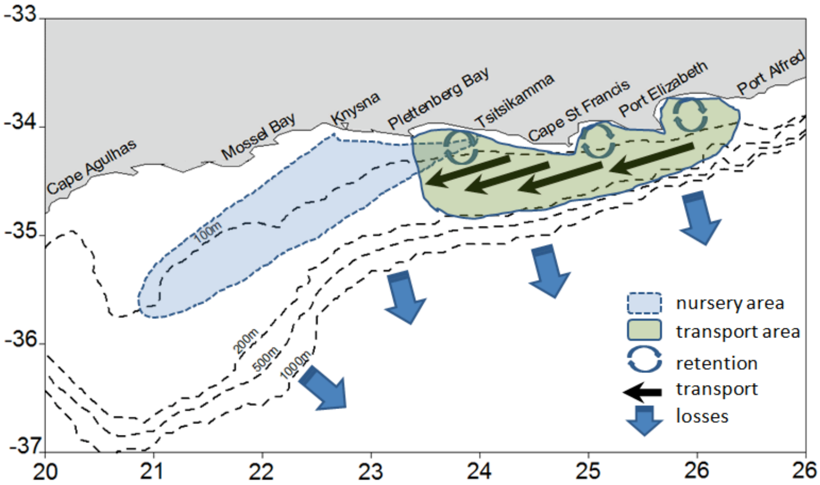

Fig. 6.1. A conceptual model of early life history of chokka squid (*Loligo reynaudii*) in the Agulhas Bank.

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