INTRODUCTION

Across its geographical range, which extends through the Mediterranean Sea and the waters of the Eastern Atlantic to the north-west coast of Africa (Roper et al. 1984, Pawson 1995), *Sepia officinalis* displays a high degree of life cycle plasticity which allows it to survive in a wide range of environmental conditions and to respond to the wide range of physico-chemical cues encountered within these environments (Pierce et al. 2010). The eggs of the common cuttlefish *S. officinalis* (Linnaeus, 1758) are typically attached to upright structures on the seabed including seaweeds, seagrasses and worm tubes (e.g. see review by Bloore et al. 2013). Following mass mortality of the adults (Boletzky 1983), these newly laid, benthic eggs are left to develop without the benefit of parental care (e.g. Guerra 2006). Undergoing direct development, with no larval or planktonic phase *S. officinalis* hatchlings emerge as miniature replicas of the adults (mantle length 6-9 mm; Boletzky 1983), identical in both structure and function, and immediately adopting a necto-benthic mode of life (Boletzky 2003, Young & Harman 1988). During these first phases of the lifecycle of *S. officinalis*, which comprise embryogenesis, hatching and the transition to first feeding, these early life stages (ELS) are relatively vulnerable (Robin et al. 2014). As the eggs are fixed to structures on the seabed, their successful development to hatching is almost entirely dictated by the ambient conditions encountered (biological, chemical and physical) at the spawning ground during embryonic development (e.g. see review by Bloore et al. 2013). However, other factors such as maternal effects (e.g. the size and quality of the egg and its yolk content and maternal nutritional status) are also able to directly or indirectly affect offspring survival and recruitment success (Bouchaud & Galois 1990, Bouchaud 1991a, Steer et al. 2004).

The spawning grounds of this species often occur in hydrodynamically unstable conditions within shallow or intertidal coastal waters (e.g. Challier et al. 2005, Bloore 2012). The optimal water conditions for successful embryonic development of *S. officinalis* are considered to be a salinity of 28 or greater, a temperature of between 9.5 and 20 °C and a dissolved oxygen concentration close to saturation (Mangold-Wirz 1963, Paulij et al. 1990, Boucaud-Camou & Boismery 1991, Nixon & Mangold 1998). However, eggs laid at different times or locations will experience a wide range of environmental conditions. Water temperature, salinity, light intensity, photoperiod, oxygen saturation and water pollution are all part of a suite of physico-chemical factors and cues that can

**THE CURRENT AND CHANGING ROLE OF PHYSICO-CHEMICAL FACTORS AND CUES IN THE EMBRYONIC AND EARLY LIFE STAGE DEVELOPMENT OF THE COMMON CUTTLEFISH (SEPIA OFFICINALIS)**

**ISOBEL S.M. BLOOR**

School of Ocean Sciences, Bangor University, Askew Street, Menai Bridge, Anglesey, LL59 5AB, UK.

ismbloor@gmail.com

**ABSTRACT.** – The spawning grounds of *Sepia officinalis* generally occur within shallow or intertidal coastal waters which experience hydrodynamically unstable conditions. Embryonic and early life stages (ELS), laid at different times or locations, can experience a wide range of physico-chemical and environmental conditions. Water temperature, salinity, light intensity, photoperiod, oxygen saturation and pollution are all part of a suite of physico-chemical factors and cues that can influence biological parameters during the ELS development of *S. officinalis*. These biological parameters include, but are not limited to, the duration of embryonic development, the rate of hatching, the rate of post-embryonic growth and the survival of early life stages. In order for embryonic and early life stage *S. officinalis* to survive in the hydrodynamically unstable conditions encountered at spawning grounds an early sensory system that is capable of regulating their development in response to osmoregulation, oxygenation or dehydration stresses, caused by physico-chemical factors, is essential. The degree to which it can respond or adapt to the physico-chemical cues encountered may account for a significant proportion of the variation in temporal (e.g. among year) and spatial (e.g. among spawning sites) survival of embryonic and early life stage *S. officinalis*. Recent research focusing on the potential impacts of climate change (e.g. ocean warming and ocean acidification) on the magnitude of these physico-chemical cues and how this may affect embryonic and ELS development of *S. officinalis* is also discussed in order to help us to understand the future impacts of climate change on these vulnerable life stages.
influence biological parameters during the development of ELSs *S. officinalis* such as the duration of embryonic development, the rate of hatching, the rate of post-embryonic growth and the survival of ELSs (Blanc 1998, Kouta & Boucaud-Camou 2003, Chaillet et al. 2005, Bloor et al. 2013). A requirement for embryonic and ELS *S. officinalis* to have an early sensory system that is capable of regulating its development in response to osmoregulation, oxygenation or dehydration stresses may therefore be essential to survival (Navet et al. 2014). The degree to which it can respond or adapt to the physico-chemical cues encountered may account for a significant proportion of the variation in temporal (e.g. among year) and spatial (e.g. among spawning sites) survival of embryonic and ELS *S. officinalis* (e.g. Bloor et al. 2013).

Newly laid *Sepia* eggs are protected by a multiple layered encapsulation (egg membrane) and a solution known as perivitelline fluid (PVF) that together provide a physical and chemical buffer between the embryo and the environment (Boletzky 1983). This provides at least some protection from the negative effects of temperature changes, osmosis or desiccation caused by variations in salinity or water coverage (e.g. uncovered in the intertidal zone) (e.g. Navet et al. 2014). However, as embryonic development progresses the eggs swell causing this protective envelope to become thinner and its permeability to seawater, and thus the various elements, pollutants and dissolved gasses it contains, to increase (Wolf et al. 1985, Boletzky 1993). Post-hatching ELS *S. officinalis* often remain inshore for several months in the warm coastal water and therefore remain vulnerable to the hydrodynamically unstable conditions encountered in these shallow waters. Following hatching they lack the protection afforded to them during embryogenesis from the outer egg membrane and the PVF and so require other methods of responding to changes in the physico-chemical conditions of the ambient environment.

The potential effects of climate change on the earth’s oceans include increased acidity as a result of increased pCO2 (ocean acidification), increased water temperatures (ocean warming) and reduced salinity (e.g. Caldeira & Wickett 2003, IPCC 2007, Feely et al. 2009). Globally average sea-surface pH has already fallen by 0.1 units since the industrial revolution and is predicted to fall a further 0.3 units by 2050 and 0.4 units by 2100 (Caldeira & Wickett 2003, 2005, Feely et al. 2009). Global sea surface temperatures are also predicted to increase by 2-3 °C by the end of the century (Meethil et al. 2007). Both warming and acidification, via changes in the magnitude of physico-chemical factors or cues, have the potential to drastically affect the physiology and development of ELS cuttlefish.

The objective of this review is to present the current state of biological knowledge on embryonic and ELS development and the processes by which the early sensory systems allow cuttlefish to adapt to variations in physico-chemical variables through life cycle plasticity and how this may help us to understand the future impacts of climate change on these vulnerable life stages. For this purpose the review is split into two main sections, Part 1 examines the current physico-chemical factors and cues, both within the egg capsule during embryogenesis, and within the surrounding oceanographic environment during the initial ELSs, which can affect development, hatching, growth and survival of ELS *S. officinalis*. Part 2 then examines and discusses the effects of potential changes in the magnitudes of physico-chemical factors and cues on embryonic and ELSs, within the context of a changing environment as predicted by the effects of ocean warming and ocean acidification.

**THE EFFECTS OF PHYSICO-CHEMICAL PARAMETERS ON ELS S. OFFICINALIS**

**Water temperature**

Water temperature is an important regulating factor in the life cycle of *S. officinalis*. Across its geographical distribution the temperature limit for this species ranges from approximately 10 °C to 30 °C (Domingues et al. 2001, Richard 1971). Spatio-temporal variations in the dynamic water temperatures experienced by embryonic and ELS cephalopods can affect not only the duration of embryogenesis, but also yolk utilisation efficiency *in ovo*, the size at hatching, the quantity and quality of the inner yolk sac available post-hatching and the eventual size that an adult can reach (e.g. Ambrose 1988, Bouchaud 1991a, Boletzky 1994). Cephalopods are poikilothermic organisms and these physiological responses are due, at least in part, to the controlling effects that temperature has on metabolism (Vidal et al. 2002).

**Embryogenesis and hatching**

An inverse relationship exists between the duration of embryonic development in *S. officinalis*, and environmental temperature (Mangold-Wirz 1963, Choe 1966, Boletzky 1975, Palmegiano & D’Apote 1983, Bouchaud & Daguzaun 1989). The duration of embryogenesis decreases as water temperature increases towards 24 °C (i.e. at 20 °C embryogenesis occurs in 40-45 days) (Boletzky 1983, Bouchaud 1991b) and increases as water temperature decreases towards 15 °C (i.e. at 15 °C embryogenesis occurs in 80-90 days). Whilst embryogenesis can continue at lower water temperatures (i.e. at 12 °C embryogenesis occurs in 5 months), it is known to cease at temperatures below 9 °C, but can restart again should the water temperature subsequently increase (Bouchaud & Daguzaun 1989, 1990).

In *S. officinalis*, embryonic development is fuelled by maternally derived energy reserves stored as yolk within
the egg. During development the yolk is divided within two reserves known as the outer and inner yolk sacs (e.g. Boletzky 1989, Sykes *et al*. 2009). The outer (or external) yolk-sac is the primary yolk reserve used to fuel embryogenesis during the early stages *in ovo*. This outer yolk sac has been referred to as an 'organ' which plays additional roles in the circulatory and respiratory systems as well as agitating or stirring the PVF around the embryo (e.g. Boletzky 1989, 2003). The later stages of embryonic development are characterised by an accumulation or transfer of yolk from the outer to the inner yolk sac (Boletzky 1983). The outer yolk sac, which is attached to the embryo in the region of the mouth is lost at hatching along with any unused yolk stored within it (e.g. Boletzky 1975, Boucher-Rodoni *et al*. 1987). The inner yolk sac equips hatchlings with an important nutritional resource which is utilised in the first 24-48 h of independent life following hatching to prevent immediate starvation (Wells 1958, Boletzky 1989, 1994). It is likely that variations in the size of the inner yolk sac between different sized hatchlings affect their capacity for growth and survival during this critical time period (Boletzky 1994).

In addition to the duration of embryonic development, size at hatching and yolk volume are also inversely correlated with incubation temperature in *S. officinalis* (Bouchaud 1991a). At higher temperatures cuttlefish develop faster and hatch at a smaller size with a larger fraction of the available yolk in the outer yolk-sac prior to hatching and less time for transfer of yolk from the outer to the inner yolk sac than is the case for embryos incubated at lower temperatures over longer time periods (Bouchaud & Daguzan 1989, 1990). The reduced duration of embryogenesis for eggs incubated at higher temperatures means that there is less time available for yolk consumption, resulting in the consumption of a smaller fraction of the available yolk in the outer yolk sac prior to hatching and less time for transfer of yolk from the outer to the inner yolk sac than is the case for embryos incubated at lower temperatures over longer time periods (Bouchaud & Daguzan 1990). This means that not only do these individuals have a reduced survival potential due to their smaller size but they also have less time following hatching, than those incubated at lower temperatures which hatch at a larger size and with a greater volume of yolk contained in the inner yolk sac, to find a suitable food source before their yolk reserves run out and they die.

Bouchaud 1991a, Boletzky 1994). A study by Bouchaud (1991a) found that cuttlefish hatch with outer yolk-sacs of various sizes in relation to the incubation temperature. For water temperatures of 15, 18, 21 and 24 °C the external yolk-sac (ES %) represents 25.5 %, 34.0 %, 43.0 % and 51.7 %, respectively, of the dry weight of eggs, at the beginning of development (Bouchaud 1991a). Bouchaud (1991a) also found that the fraction of yolk utilised for growth is highest at an incubation temperature of 15 °C (energy for growth was 367 J) whilst an incubation temperature of 18 °C the fraction of yolk utilised in catabolic expenditure is minimum (energy used for catabolism was 108.9 J). As such a temperature somewhere between the range of 15 and 18 °C, where the fraction of yolk used for growth would be highest and the fraction used for catabolism lowest, could be considered optimal for embryonic development in *S. officinalis* (Bouchaud 1991a). It has also been suggested that the quantity of yolk contained in the inner yolk sac at hatching may influence the maturation of the central nervous system (Dickel *et al*. 1997).

**Early life stage growth and survival**

*S. officinalis* is poikilothermic (*i.e.* body temperature varies with the temperature of its surroundings) which means that their metabolic rate will rise and fall directly with changes in water temperature, which in turn will create a corresponding rise in feeding and growth rates (Forsythe 1993). Thus water temperature also has a significant effect on the growth rate of ELS *S. officinalis* during their exponential growth phase and can create large variation in eventual adult size. The ‘Forsythe Effect’ states that during the exponential growth phase of ELS cephalopods, when individuals are small and most vulnerable to predation, small changes in water temperature (*e.g.* 1 °C) can create significant increases in growth rate. The Forsythe Effect was first demonstrated by Forsythe (1993) using data from laboratory studies to simulate the effect of temperature on the growth of ELS cephalopods. Simulations showed that an increase in water temperature of only 1 °C could create a twofold increase in body weight over a 90 day period and a 2 °C increase a fivefold increase in body weight (Forsythe 1993). As hatching of *S. officinalis* usually occurs in warm shallow coastal waters often over a period of gradually warming water temperatures (*e.g.* English Channel; hatching occurs in warm shallow waters during the spring and summer), each monthly cohort will encounter warmer water temperatures during their exponential growth phase and thus grow significantly faster than cohorts that hatched only weeks earlier. This optimizes the effects that temperature can exert on the exponential growth rates of these ELS, enabling younger cohorts to surpass older cohorts in size, prior to reaching 1 year of age (Forsythe 1993). Thus the growth rate of ELS *S. officinalis* can vary significantly as a function of differences in the water temperatures (both spatially and
temporally) that they encounter at the location of their spawning (Challier et al. 2005). To date there are only a few studies available on the link between temperature and survival of ELS S. officinalis. A study by Forsythe et al. 2002 investigated the effects of crowding on growth and survival of ELS S. officinalis reared at two temperatures (17 and 25 ºC). The results indicated that growth rate was significantly higher in the 25 ºC treatment compared to 17 ºC, with stocking density having no significant effect on gross growth efficiency at either temperature. In addition, the study found high survival rates for cuttlefish reared at high (equivalent to 400 Sepia m⁻²) and low (equivalent to 100 Sepia m⁻²) stocking densities at both temperature regimes (Forsythe et al. 2002). Survival of the high density cuttlefish groups was 96.7 % (± 0.03 %) and 70 % (± 10.4 %) at 17 and 25 ºC, respectively (although the lower survival rate of the latter group was explained by an accidental interruption of aeration to one of the three trial groups resulting in a high mortality event; the other two trial groups had survival rates of 100 %). Survival of the low density cuttlefish groups was 86.7 % (± 2.1 %) and 93.3 % (± 0.51 %) at 17 and 25 ºC, respectively (Forsythe et al. 2002). A study by Grigoriou & Richardson (2004) investigated the survival rates of hatchlings from eggs collected from three different locations (Plymouth, North Wales, Southampton) maintained for 35 days at 19 ºC (all groups fed to excess) and found no significant differences in growth rates among the groups and 100 % survival rates in all cases. The study also investigated the survival rates of cuttlefish which were initially maintained at ambient seawater temperature (7-16 ºC) for 6 months post-hatching with minimal food (to delay growth and development) and then subsequently transferred to one of two treatments where they were maintained at either 11 ºC or 19 ºC (with both groups fed to excess) (Grigoriou & Richardson 2004). Cuttlefish transferred to the 19 ºC treatment showed a rapid and significant increase in weight and growth rate (overall Instantaneous Growth Rate of 3.46 ± 0.08 % BWday⁻¹) to those transferred to 11 ºC (overall Instantaneous Growth Rate of 0.69 ± 0.06 % BWday⁻¹). Growth rates in the group transferred to 19 ºC were also similar to those reared at 19 ºC with a sufficient food supply shortly after post-hatching. Cuttlefish transferred to 19 ºC achieved 100 % survival rates whilst those transferred to 11 ºC achieved only 73 % survival rates (Grigoriou & Richardson 2004). The results of these studies indicate that in addition to greater growth rates of ELS cuttlefish reared at higher temperatures, survival rates may also be higher at increased water temperatures (e.g. 19 or 25 ºC) relative to lower temperatures (11 or 17 ºC).

**Light intensity and photoperiod**

The eggs of S. officinalis are often laid in relatively shallow waters (< 40 m; Boletzky 1983). At this depth daylight is only slightly attenuated and around 10 % of the original light intensity can still be detected (Poole & Atkins 1937) and light can act as an important regulating cue for ELS S. officinalis.

**Embryogenesis and hatching**

The egg capsules of S. officinalis are stained with black ink during the laying process (Boletzky 1983). As embryonic development progresses the egg capsule becomes more penetrable to light as the volume of the perivitelline fluid inside the egg increases causing the egg to swell which results in a corresponding reduction in the thickness of the black outer membrane of the egg capsules (Paulij et al. 1991). As the egg capsule becomes increasingly more translucent during late embryogenesis, the embryo becomes capable of detecting changes in the light-dark cycle enabling the hatching rhythm to be driven by external light-dark conditions (Paulij et al. 1991). Under laboratory conditions embryos maintained under a continuous light source (i.e. in the absence of a light-dark cycle) were found to have an extended time to hatching and exhibited no hatching rhythm with embryos emerging from their capsules as soon as development was complete, irrespective of the time of day (Paulij et al. 1991). In this way the transition from light to dark has been shown to be an important synchronising cue for hatching in embryos of S. officinalis enabling them to hatch shortly after the onset of darkness (Paulij et al. 1991). The ecological benefits of synchronising hatching in the darkness are thought to include a reduction in predation risk from fish species (Paulij et al. 1991).

In addition, translucent eggs, in which the outer black membrane had not been stained with ink during spawning, which were maintained in the laboratory under a standard light-dark cycle did exhibit a hatching rhythm, although the time to hatching was reduced (Paulij et al. 1991). The authors suggested that this reduced hatching time may be explained by the embryos heightened sensitivity to external light as a result of the lack of pigmentation in the outer egg membrane (Paulij et al. 1991). A recent study by Mäthger et al. (2010) detected opsin genes, which can produce a visual pigment called opsin that allows light to be absorbed for the process of vision, in the skin of adult S. officinalis. Although the exact physiological functions of these dermal opsin genes remain to be established it is possible that the surface of the skin may play a role in light-sensing (Mäthger et al. 2010). If these opsin genes are also present in embryos, then the black pigmentation found in the outer membrane of the eggs of S. officinalis may act to protect the embryos from atmospheric light at spawning sites which are often shallow or intertidal areas with low light attenuation. Thus the selection of a black outer egg membrane during evolution would make sense to ensure the proper development and maturation during...
embryogenesis in these ecological conditions (Bassaglia et al. 2013).

**Early life stage growth and survival**

Once hatched, *S. officinalis* may use visual cues for prey and predator detection and navigation. A study by Sykes et al. (2014) which investigated the effects of three light intensities (100, 350 and 1200 lux) on the rearing performance of cuttlefish during the first 50 days post-hatching found that light intensity is an important factor for growth and survival. The authors found that 100 lux was the optimal light intensity to promote highest absolute values of biomass and lowest mortality rates during the first 50 days post hatching, whilst the highest levels of mortality were observed in cuttlefish reared at 1200 lux during the first 10 days post-hatching (Sykes et al. 2014). The authors suggest that this may be a result of the visual system of cuttlefish, indicating that higher light intensities may interfere with prey contrast during the first 10 days post-hatching thus decreasing the ability of hatchlings to capture prey. A light intensity of 100 lux, obtained with daylight spectrum bulbs, is therefore suggested as the optimal setup for rearing cuttlefish hatchlings, in laboratories or aquaculture, during the first 50 days post-hatching in order to promote higher growth and survival rates and lower energetic costs (Sykes et al. 2014).

Given their nectobenthic lifestyle, cuttlefish are likely to experience low visibility conditions on a regular basis. A study by Cartron et al. (2013a, 2013b) suggest that cuttlefish use polarized vision to help improve object detection (e.g. prey and predators) in turbid waters. The authors presented computer generated stimuli (polarized patterns and unpolarized intensity contrasted images) to *S. prashanum* and *S. pharaonis* and tested their responses at different levels of water turbidity. Both species performed equally well at detecting predators using intensity vs polarization contrasts when tested in clear waters. However, when the turbidity of the water increased, although the cuttlefish were still able to detect objects with polarization contrast they were unable to detect those with intensity contrast alone, suggesting that cuttlefish can also use polarized vision to improve object detection in turbid waters (Cartron et al. 2013a, b). The ability to see further into turbid water and to better detect an approaching object would be beneficial for their survival in turbid conditions in coastal areas.

**Salinity**

The salinity of the ambient seawater during embryogenesis and hatching also imposes physiological limits on the development of embryonic and ELS *S. officinalis*. The volume of riverine input, recorded rainfall and currents can all affect the degree of salinity at a site. As the eggs are fixed to structures on the seabed for the duration of embryogenesis they are subject to any local fluctuations in salinity (e.g. river outflow, rainfall etc.) that may occur during this period. In addition, a degree of egg laying is also known to occur within the intertidal zone where eggs are exposed during low tide to osmotic stress (e.g. Bonnau et al. 2013). The degree of osmotic stress that they experience as a result of external salinity variation is dependent upon individual adaptations (Bonnau et al. 2013) and embryonic and ELS *S. officinalis* are relatively tolerant to variations in salinity.

**Embryogenesis and hatching**

Salinity can influence the duration of incubation in *S. officinalis* as well as hatching success (Paulij et al. 1990, Blanc 1998). It is considered that a salinity of 25 or above is generally required for normal embryonic development to occur in *S. officinalis* (Boletzky 1983, Paulij et al. 1990, Blanc 1998). In laboratory, studies examining the combined effects of temperature and salinity on hatching success Palmegiano & D’Apoté (1983) found that over the range 25-33, salinity had a statistically significant effect within complete inhibition of hatching below 25 and with no additional interactive effect observed between the two parameters. These results were supported by Paulij et al. (1990) who also found that hatching terminated at salinities of 23.9 and below and Blanc (1998) who found that no hatching occurred at salinities of 20 or 25. Salinity was also found to influence the duration of embryogenesis with a significantly reduced rate found in embryos developed at salinities of 28.7 and below and malformations occurring at salinities of 22.4 and below (Paulij et al. 1990). It is likely that the processes required to deal with the osmotic stress incurred at these low salinities creates a large energy demand thereby decreasing the proportion of energy available for development and growth (Paulij et al. 1990).

The outer membranes of the egg capsule provide some protection to the embryo from variations in salinity by preventing physical damage due to desiccation and osmotic stress. In the later phases of embryonic development the egg swells and the outer membrane becomes thinner and permeable to seawater, which enables various ions and respiratory gasses to be supplied to the embryo (Bonnau et al. 2013). In the hydrodynamically unstable shallow coastal waters where eggs and ELS develop an ion transport system in the membrane of the egg capsule, if present, could help limit the potential damage from variable salinities (Bonnau et al. 2013). Recent studies have begun to investigate the process for osmoregulation in *S. officinalis* embryos and ELS. The sodium-potassium pump is known to be a ubiquitous enzyme in the animal kingdom and is based on the antiport of K+ ions into the cells and transport of Na+ out of the cell and is well established as being involved in a variety of physiological processes such as osmoregulation (e.g. Dean 1941). Hu et
of 94.8 ± 18.5 and 421.8 ± 102.3 µmolATP gFM⁻¹ n⁻¹, respectively. Such high concentrations of NA⁺/K⁺-ATPase might be important in coping with the challenging abiotic conditions (low pH, high pCO₂) that these organisms encounter inside their eggs, indicating the importance of osmoregulation process during development (Hu et al. 2010). A second study by Bonnau et al. (2013) investigated the embryonic outer yolk sac as a putative site for osmoregulation in S. officinalis embryos. Their initial results indicate that ionocyte-like cells and NA⁺/K⁺-ATPases were localised in the yolk epithelium and further research is now required to understand the role of ion transport systems of this yolk epithelium in the osmoregulation processes in S. officinalis embryos (Bonnau et al. 2013).

Early life stage growth and survival

The ELS of S. officinalis are considered to be relatively tolerant to variations in salinity. A study by Blanc (1998) tested the effect of a range of salinities (30, 35 and natural variation 33-36) on the growth and survival of S. officinalis hatchlings. The author found that size and weight of hatchlings was greater for those incubated at a salinity of 30 compared to those incubated at a salinity of 35 or within a natural salinity fluctuation range (salinity 33-36). In addition differences in the growth rates of ELSs incubated at different salinities were also observed (Blanc 1998). The study also found that growth rates of ELSs were higher for the group cultured at a salinity of 30 (up to 16 weeks post-hatching). The growth curves of the groups cultured at a salinity of 35 or within natural variation (33-36) were similar and showed a relatively slow initial growth. A plateau begins at the tenth week for the 35 group. In contrast for ELSs cultured at a salinity of 30 the growth was initially very rapid, plateauing begins at week 7 with individuals attaining an average dorsal mantle length (DML) of 41.84 mm (± 4.58) approximately 6 weeks earlier than those raised in natural salinity conditions (Blanc 1998). Conversely the survival rate of ELSs was actually higher for groups cultured at a higher salinity (natural variation 33-36 and 35 salinity groups). After 5 weeks the survival of ELSs cultured in natural salinity conditions was the best (65 %), then the survival rate of those cultured at a salinity of 35 (55 %) and those cultured at a salinity of 30 had the lowest survival rate (45 %). After 7 weeks all the groups display a mortality rate of less than 50 % (Blanc 1998). In addition, observations from the Western Mediterranean and the NW Atlantic have shown that juveniles can survive for some time at salinities 18 ± 2 if slowly acclimatised (Boletzky 1983, Guerra & Castro 1988). However following hatching ELS can respond to changes in salinity through local migration although such a response would require a mechanism for juveniles to detect ambient salinity changes.

Oxygen saturation

The dissolved oxygen content of the water surrounding S. officinalis eggs is another determining factor in embryonic and ELS survival and must be close to saturation in order to produce optimal rates of development (Boletzky 1983). The capacity of hemocyanin (the oxygen transporter used in S. officinalis circulatory systems) for carrying oxygen is limited (oxygen-binding capacity of 3 nM) relying on fully oxygenating their pigment at the gills and releasing the majority of bound oxygen during each passage through the capillary beds (Melzner et al. 2006a). To maintain sufficient oxygen levels S. officinalis tends to occur in areas with oxygen saturation as close to 100 % as possible. Dissolved oxygen concentration within the egg capsule can become a limiting factor towards the end of embryonic development as the egg capsule acts as a barrier to the diffusion of dissolved gases (Cronin & Seymour 2000, Gutowska & Melzner 2009). Oxygen consumption rates increase during embryonic development and the morphology of the egg capsule in combination with ambient dissolved oxygen limit the rate of embryonic oxygen consumption in aquatic environments (Strathmann & Chaffee 1984, Strathmann & Strathmann 1995, Cohen & Strathmann 1996, Cronin & Seymour 2000). Water has a low diffusivity and capacitance of oxygen and unless developing embryos possess a system for active oxygen uptake they must obtain oxygen from the surrounding water by diffusion into the egg capsule which is a slow process and only feasible for small organisms (Cronin & Seymour 2000).

Embryogenesis and hatching

In cuttlefish perivitelline fluid (PVF) pCO₂ is known to increase with increasing embryonic mass (Gutowska & Melzner 2009). The rapid increase in oxygen consumption before and during hatching may result from the high energetic cost of both the post-organogenetic embryonic growth phase and the hatching process (e.g. Lacoue-Labarthe et al. 2010); under normal environmental conditions critical pO₂ concentrations can be reached just prior to hatching (Cronin & Seymour 2000, Gutowska & Melzner 2009). It is suspected that hypoxia-induced metabolic suppression may occur to maintain pO₂ concentrations above any critical minimum until the embryo is mature enough to hatch and that in late embryonic stages this progressive depletion of oxygen concentrations within the egg capsule may act as a cue or trigger for hatching in some species once a threshold of low pO₂ is reached (Pimentel et al. 2012, Rosa et al. 2013). For cuttlefish species, including S. officinalis, the critical minimum oxygen
threshold that may trigger hatching has been placed at around 5.8 kPa (Cronin & Seymour 2000).

**Early life stage growth and survival**

Johansen et al. (1982) studied the O$_2$ uptake in relation to body weight and concluded that the respiration rate of *S. officinalis* could be separated on the basis of size with an isometric scaling for the respiration of small ELS cuttlefish (0.12-0.15 g) and an allometric scaling for larger cuttlefish (100-1,500 g). Whilst it has been suggested that ELS cephalopods may be able to live for limited time periods at reduced oxygen concentrations via metabolic depression; the absence or low abundance of *S. officinalis* from areas with low oxygen concentrations is generally explained by low tolerance to such conditions (Guerra 2006).

**Elemental uptake**

ELS *S. officinalis* require a range of minerals in order to maintain their normal metabolic and physiological functions, which are known as essential elements (Villanueva & Bustamante 2006). These essential elements form the components of hormones, enzymes and structural proteins as well as being required for important functions such as the formation of skeletal structure and the regulation of acid-base equilibrium (e.g. Williams 1981). Both essential and non-essential (i.e. those not required by the animal for survival and growth) elements can be taken up by organisms and the range of elements that comprise *S. officinalis* hatchlings have been quantified and classified into three groups: major essential elements (Ca, K, Mg, Na, P, S); minor essential elements (As, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sr, Zn) and non-essential elements (Ag, Al, Ba, Cd, Hg, Pb) (Villanueva & Bustamante 2006).

Cephalopods are known to have a high capacity to accumulate such elements as metals, including various toxic elements such as Ag or Cd (e.g. Bustamante et al. 2002, 2004). The eggs of *S. officinalis* which are generally laid in shallow, coastal waters are vulnerable to acute or chronic exposure to contaminants which enter the marine environment from a variety of anthropogenic sources including agriculture, domestic and industrial (Boyle & Boletzky 1996, Bustamante et al. 2006, Lacoue-Labarthe et al. 2009). This risk of exposure is not just present during embryogenesis, when the eggs are fixed to structures on the seabed, but also during the ELS as individuals remain in coastal waters until their first autumn migration offshore (Lacoue-Labarthe et al. 2010). However, despite the real and continuous exposure to contamination that early life stage *S. officinalis* often experience the physiological perturbations on ELS development, survival and growth have been rarely investigated (Le Pabic et al. 2015a). Whilst a range of elements (both essential and non-essential) are naturally found in the marine environment they can be considered as contaminants if they are foreign to the natural system or if they are naturally occurring but present in unnatural concentrations. Recent studies investigating the effects of contaminant exposure on ELS cuttlefish have largely focused on the trace metal Zinc (Zn) which is an essential nutrient for living organisms, has been reported at very high concentrations in heavily polluted areas around the world (e.g. Liu & Wang 2013) and is known to be efficiently bioaccumulated in *S. officinalis* (Bustamante et al. 2002, Lacoue-Labarthe et al. 2010).

**Embryogenesis and hatching**

A number of studies have suggested that the bio-accumulation of trace elements may have important effects on embryonic development in *S. officinalis*. For example, calcium (Ca) contained in the yolk is thought to provide an essential reserve required for metabolic processes (Boletzky 1989) while a requirement for copper (Cu) in embryonic and ELS *S. officinalis* is thought to be related to the haemocyanin requirements for oxygen transport (Villanueva & Bustamante 2006) and embryos raised in artificial seawater without strontium (Sr) were found to have high levels of abnormal hatching (Hanlon et al. 1989). The egg capsule is known to act as a partially selective permeable barrier to a range of essential and non-essential trace elements that are dissolved in the surrounding seawater (Bustamante et al. 2004, Lacoue-Labarthe et al. 2012). As a result, the quality of the ambient seawater at spawning sites and the degree to which it is contaminated has the potential to impact the successful embryonic development of eggs.

The incorporation of chemicals (e.g. trace elements) and gases into the embryo during development can be regulated to some degree by the egg capsule which acts as a protective barrier (Bustamante et al. 2006, Lacoue-Labarthe et al. 2009). The degree of permeability of the egg capsule is thought to be specific for each element (Bustamante et al. 2002, 2004, 2006, Lacoue-Labarthe et al. 2008). The toxic effects of trace elements on cuttlefish embryos and ELSs has been studied on only a few metals (e.g. Ag, Cd, Co and Zn; Bustamante et al. 2002, 2004, 2006). Whilst the egg shell is known to prevent many non-essential metals (e.g. Cd, Co, Cs, Pb, Zn or V) from being absorbed by the embryo (Bustamante et al. 2002, 2006, Miramand et al. 2006, Lacoue-Labarthe et al. 2009), several toxic elements (e.g. Zn and Ag) are able to permeate the egg shell and be absorbed (Bustamante et al. 2004). The survival and growth of cuttlefish embryos and ELS is thought to be negatively affected by the uptake of such toxic metals through the outer membrane of the egg (Guerra 2006). It appears that the physico-chemical properties of each element rather than the metabolic needs of the embryo determine their capability to pass through the egg shell, with non-biologically essential elements such
as Ag, known for its enhanced embryotoxicity, permeating the eggshell (Bustamante et al. 2006). The permeability of the eggshell to different elements is also known to vary through the course of embryogenesis as the diffusion and retention properties of the egg capsule change as the volume of fluid in the egg increases (Cronin & Seymour 2000, Lacoue-Labarthe et al. 2009).

Bustamante et al. (2006) described the three main pathways that trace elements and contaminants are taken up by cuttlefish embryos, although the specific mechanisms and processes for regulation of uptake are not well known. The three pathways include:

**Pathway 1:** Elements are adsorbed onto the outer membrane of the egg capsule shielding the embryo from direct exposure (e.g. $^{241}$Am and $^{109}$Cd).

**Pathway 2:** Elements are adsorbed onto the outer membrane of the egg capsule providing temporary protection from direct exposure but once all of the specific and non-specific binding sites are full the contaminants are able to permeate across the membrane and be absorbed by the embryo (e.g. $^{110}$mAg and $^{65}$Zn).

**Pathway 3:** Elements are able to permeate the outer membrane of the egg capsule in both directions allowing the embryo direct access for absorption (e.g. $^{134}$Cs).

**Early life stage growth and survival**

Following hatching ELS cuttlefish no longer have protection provided by the egg capsule or PVF and can therefore encounter different abiotic conditions than they did as embryos, even if they remain within the same location. Recent work by Le Pabic et al. (2015a, b) have begun to develop our knowledge of the effects of contaminant exposure on the physiology of embryonic and ELS cuttlefish. Le Pabic et al. (2015a) investigated the tolerance limits of ELS *S. officinalis* for Zn, which globally is one of the trace metals most concentrated in coastal waters. ELS cuttlefish appear highly sensitive to Zn (in terms of lethal dose) compared to rates reported for other aquatic organisms with the authors estimating a Zn-mortality threshold for *S. officinalis* ELS of between 185 and 230 µg l$^{-1}$, during the first two weeks post-hatching (Le Pabic et al. 2015a). A result that may be indicative of changes in the physiology of Zn management during this initial two week period of ELS development (Le Pabic et al. 2015a). In addition the study also investigated the effects of sub-lethal exposure of Zn on growth and behaviour and found that a Zn-threshold of 108 µg l$^{-1}$ (and above) resulted in growth reductions of ELS, associated with enzymatic perturbations, after 5 weeks of exposure post-hatching. Notably, effects on size appeared only after 5-week exposure and subsequent to a decrease in cathepsin activity after 3-weeks of exposure, underlining an impact of Zn on the digestive system even when the digestive gland is mature (Le Pabic et al. 2015a). This size loss likely resulted also from Zn perturbations of other physiological traits (e.g. trace element and calcium homeostasis or acid-base balance) that were not investigated as part of this study (Le Pabic et al. 2015a). The result also indicated a negative impact of Zn exposure on behaviour with non-lethal exposure inducing an increase in the number of tentacle strikes necessary to catch a shrimp. This behaviour disruption suggests a Zn-induced disruption of the central nervous system and/or vision organs and is consistent with previous findings with adult cuttlefish (Bustamante et al. 2002). The authors suggest that this phenomenon may be linked to calcium ion dysregulation because of the ability of Zn$^{2+}$ to mimic this element and inhibit its pump (Bal latori 2002). These results indicate a relatively important sensitivity of ELS cuttlefish to Zn (Le Pabic et al. 2015a).

A second study by Le Pabic et al. (2015b) investigated the regulation of metals in ELS *S. officinalis* as well as the effects of physiological modifications to the bioaccumulation and detoxification process associated with digestive gland maturation. In the first stage of the study the presence, concentration changes and subcellular distributions of 13 elements (Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V and Zn) was quantified in ELS cuttlefish during the first 2 months post-hatching (i.e. the period corresponding to their coastal life stage) (Le Pabic et al. 2015b). The results showed that important changes of metal concentrations occurred in cuttlefish after hatching. For example, the non-essential elements Ag and Cd had the highest accumulation (with around 10- and 4-fold increase, respectively, during the first month post-hatching), correlating with the digestive gland maturation. Whilst soluble fractions of most essential trace metals (i.e. Co, Cr, Cu, Fe, Se, Zn) slowly increased consistently with the progressive needs of cuttlefish nutrition during this period (Le Pabic et al. 2015b). In the second stage of the study the effect of Zn exposure on metal regulation and distribution and the ability of ELS *S. officinalis* to regulate the effects of exposure to dissolved Zn, such as oxidative stress, was investigated. The results highlighted the potential for exposure to a single metal (e.g. Zn) to alter the subcellular distribution of elements, indicating that ELS cuttlefish have a low ability to regulate dissolved zinc accumulation. Zn exposure was for example found to cause a concentration-dependent Mn depletion in ELS, and an increase of the soluble fraction of Ag, Cd, Cu without accumulation modifications, suggesting substitution of these elements (i.e. Mn, Ag, Cd, Cu) by Zn. In addition, exposure to 52 µg l$^{-1}$ of Zn (a concentration lower or of the same order than values registered in coastal environments worldwide) caused oxidative stress in the cuttlefish digestive gland with damage to this tissue recorded after 6 weeks of exposure (Le Pabic et al. 2015b). Altogether these perturbations are consistent with a high sensitivity of ELS cuttlefish to Zn and suggest a low plasticity of ELS *S. officinalis* to regulate the effects of Zn exposure such as oxidative stress (Le Pabic et al. 2015b).
The effects of long-term exposure of contaminants on the physiology of ELS cuttlefish are not well known. The expected benefits for ELS from warmer waters and increased prey abundance within the coastal waters where they spend the first couple of months of life could be negated by the costs associated with the negative impacts of exposure to contaminants on the essential physiological processes (e.g. maturation of the respiratory system (e.g. Strobel et al. 2012), the central nervous system (e.g. Dickel et al. 1997) and the digestive gland (e.g. Yim & Boucaud-Camou 1980) that occur during the first few weeks of this critical life phase (Le Pabic et al. 2015a). Further studies on other pollutants are now a priority in order to characterise the breadth of cuttlefish sensitivity to anthropogenic contamination.

DISCUSSION AROUND THE POTENTIAL CHANGES IN MAGNITUDES OF PHYSICO-CHEMICAL FACTORS AND EFFECTS ON ELS

Global warming and ocean acidification
Impact of increasing pCO₂ (ocean acidification)

As levels of carbon dioxide (CO₂) rise, the earth’s oceans act as a buffer and absorb increasing quantities of CO₂ from the atmosphere, altering the chemistry of the seawater and causing it to become more acidic (e.g. Zeebe & Wolf-Gladrow 2001). Current predictions estimate that the expected increase in atmospheric CO₂ will cause a reduction in the pH of surface ocean waters by 0.3 units by 2050 and 0.4 units by 2100 (Calderia & Wickett 2003, 2005, Feely et al. 2009). For calcifying marine organisms like Sepia officinalis the potential negative effects of ocean acidification on their calcification and metabolic rate are of particular concern (Gutowska et al. 2008, Pörtner 2008).

Embryogenesis and hatching

Ocean acidification will generate additive effects on the development of Sepia officinalis embryos by increasing the already acidic condition of the PVF within the egg. Under normocapnia (or a state of normal atmospheric CO₂ pressure) embryos already encounter stringent abiotic conditions inside the egg capsule with changes in the pCO₂ ranging from 0.4 kPa in the early stages of embryogenesis and significantly reducing to 0.04 kPa in the later stages (Bassaglia et al. 2013). Studies by Hu et al. (2010, 2011a) have shown that in late stage embryos all ion-regulatory epithelia (e.g. epidermal, yolk-sac and gill ionocytes) are already functional. Early physiological maturation of ion-regulating epithelia combined with a protective egg capsule may have been co-selected in Sepia as a response to large variations in the external physico-chemical factors encountered during ELS development in the hydrodynamically unstable shallow coastal and intertidal waters where eggs of this species are generally laid (Bassaglia et al. 2013). However, these mechanisms will not necessarily provide Sepia embryos with the mechanisms to cope with elevated environmental seawater pCO₂ levels under situations of hypercapnia (or a state of excessive atmospheric CO₂ pressure), which are predicted to occur as a result of ocean acidification, and that will occur in addition to the constraints that the embryos are already exposed to due to the naturally high pCO₂ that occurs inside the egg (Hu et al. 2011b). Hu et al. (2011b) explored the molecular control and the physiological regulation of the Sepia embryo after long-term exposure to moderate environmental hypercapnia. They found a downregulation of ion-regulatory and metabolic genes in late stage embryos showing the sensitivity of the embryo to elevated seawater pCO₂. A second study by Hu et al. (2011b) found that in terms of gill membranes, late-stage embryos experienced a significant reduction in somatic growth and cases of incomplete development. The high sensitivity observed during embryonic development may occur as a result of additive effects of any increase in environmental pCO₂ in combination with naturally high pCO₂ (up to 0.4 kPa) within the PVF already experienced by the embryo leading to hypercapnic conditions inside the egg (Hu et al. 2011a). This finding has important consequences on the ability of embryonic cuttlefish to adapt to these changes and may affect durably the biological cycle of this species (Navet et al. 2014).

Early life stage growth and survival

Gutowska et al. (2008) showed that over a 6 week period ELS Sepia officinalis, which is an active mollusc with a high metabolic rate, can maintain its metabolism and growth capacity under elevated CO₂ conditions (~4000 and 6000 ppm), maintaining similar rates to control subjects (metabolic rate of 0.09 µmol O₂ g⁻¹ min⁻¹ and an increase of 4 % BWd⁻¹). These results correspond with more recent findings from Hu et al. (2011a) that also demonstrated that ELS growth rates were not significantly influenced by exposure to elevated seawater pCO₂ (0.37 kPa) over a 6 week period. This suggests that active cephalopods like Sepia officinalis may have a certain level of pre-adaptation to long-term increases in CO₂ levels enabling them to maintain (or increase) growth, metabolism and calcification rates (Gutowska et al. 2008) and may reflect the high ionic regulation capacities of cuttlefish (Hu et al. 2011a, b). This ability to efficiently regulate extracellular pH could be one explanation for the increased tolerance of some organisms to projected increases in future pCO₂ (Melzner et al. 2009). However, these processes, controlled by ion-transporters fuelled by ATP-dependent pumps may be energetically costly to maintain in acidified conditions (Hu et al. 2011a).
In addition to physiological impacts, internal calcified structures such as cuttlebones and statoliths may also be affected by reduced seawater pH. Studies of the effects of reduced seawater pH on the cuttlebone calcification of ELS cephalopods have so far generally reported unchanged or increased calcification rates under high seawater pCO₂ (e.g. Gutowska et al. 2010a, b). A study by Gutowska et al. (2008) on S. officinalis showed maintained calcification and growth in ELS after 40 days of exposure to high pCO₂. In a second study, Gutowska et al. (2010b) showed a 20-50% increase of the CaCO₃ fraction in ELS cuttlebones after exposure to high pCO₂ (~6000 µatm), along with a structural change in the calcified matrix. Both of these studies used much higher pCO₂ than the IPCC predictions for 2100 (IPCC 2007). A further study by Dorey et al. (2012) using realistic scenarios of predicted levels of pH change showed that a decrease of 0.25-0.5 units in the pH of seawater (hypercapnia) also resulted in the hypercalcification of cuttlebones in ELS S. officinalis by 17-80%, corroborating the result of Gutowska et al. (2010b). However, these increased calcification rates led to the formation of a more porous cuttlebone which has been suggested would negatively impact the lightweight structure and buoyancy regulatory functions of the cuttlebone (Gutowska et al. 2008, 2010b), higher rates of calcification may also cause thicker densities of the cuttlebone potentially leading to negative effects on locomotion as well (Gutowska et al. 2008, 2010b).

**Impact of increasing water temperatures (ocean warming)**

By the end of the next century, global mean sea surface temperatures (SST) are expected to rise substantially (e.g. 2-3 °C by the end of the century; Meehl et al. 2007). As temperature is an important factor influencing embryonic development and survival, ocean warming will likely have both direct impacts on the growth, development and metabolism of S. officinalis embryonic and ELSs, as well as indirect impacts occurring as a result of changes to the abundance and activity rates of their prey and predators.

**Embryogenesis and hatching**

In terms of growth rates there is already a well-established inverse relationship between temperature and the duration of embryonic development for S. officinalis as discussed in Part 1 of this review. In the context of global warming this could mean that hatchlings will emerge faster but also at a smaller size. Thus, there may be a substantial reduction in the size of cephalopod hatchlings emerging from inshore spawning grounds unless females are able to compensate for the reduction in hatching size by producing larger (and therefore) fewer eggs (Pecl & Jackson 2008).

**Early life stage growth and survival**

For growth rates of ELS cuttlefish under the regime of elevated temperature predicted by global warming there is the potential for ELS growth rates to either increase or decrease depending on whether any corresponding impacts on food availability occur. If no negative impact on food availability is observed then it is likely ELS growth rates will increase as temperature increases (e.g. Forsythe 1993).

Despite the potential gains from a scenario of increased growth rates of ELSs the opposing effects of increased temperatures on hatching size may still lead to a smaller size at age for adults. This is because the starting size of hatchlings is known to be crucial in determining final adult size despite the exponential growth rate experienced during ELSs. For example, a 0.023 g cephalopod hatching growing at 10% body weight per day would be 186 g after three months, whereas a 0.057 g hatching growing at the same rate would be 462 g after the same time period (see review by Pecl & Jackson 2008). As a function of increased growth rate, it is likely that lifecycle duration would also decrease with individuals maturing faster and at a smaller size (e.g. Sepioteuthis lessoniana; Jackson & Molchaniwskyj 2002).

The oxygen binding properties of haemocyanin are also known to be temperature dependent and the optimal affinity and oxygen transfer functions can only be maintained within a given thermal window of approximately 11-23 °C (Melzner et al. 2007b). For example, at water temperatures of 17 °C, at rest, haemocyanin releases around 80% of bound oxygen as it passes through the body (Johansen et al. 1982). Outside of the thermal window haemocyanin becomes desaturated in the cold and saturated in the warm (Melzner et al. 2007b). In addition the functioning of haemocyanin outside of the thermal window becomes severely impaired and can lead to hypoxemia (progressive internal hypoxia) and death (Melzner et al. 2007b). This phenomenon is known as the oxygen limitation of thermal tolerance and has been investigated in detail for S. officinalis (Melzner et al. 2006a, b, 2007a). Oxygen limitation of thermal tolerance for haemocyanin may pose problems for this species under the predicted scenarios of ocean warming and/or the combined effects of climate change (increased water temperature and decreased oxygen saturation). In terms of ELS the results of studies using S. officinalis have shown that smaller individuals are more hypoxia tolerant under thermal stress than larger ones, indicating a wider thermal window for smaller individuals (Johansen et al. 1982, De Wachter et al. 1988, Melzner et al. 2007a).
**Interacting effects of climate change (ocean warming and acidification)**

Global changes such as ocean acidification are not occurring in isolation and the potential interaction of reduced pH with effects of ocean warming, eutrophication and hypoxia (oxygen deficiency arising from global warming and eutrophication) are currently of international concern (e.g. Pörtner et al. 2005).

**Embryogenesis and hatching**

Although our understanding of the interacting effects of climate change on cuttlefish is not well developed at present, a study by Rosa et al. (2013) has found that future warming and acidification scenarios (e.g. 22 °C; pH 7.5) led to shorter embryonic periods, lower survival rates and the enhancement of premature hatching in *S. officinalis* when compared to current scenarios (e.g. 18 °C; pH 8.1). As expected increased temperature significantly decreased the embryonic period, from 48-49 days at 18 °C to 32-34 days at 22 °C, but reducing pH did not elicit any significant change (Rosa et al. 2013). Although embryonic survival rates were not significantly affected by pH at 18 °C (i.e. there was no difference between the normocapnic and hypercapnic treatments with survival rates of 93.7 % and 90.3 %, respectively), the future warming and acidification scenarios led to significantly lower survival rates (22 °C; pH 7.5: 31.8 %). (Rosa et al. 2013). Premature hatching was also found to increase significantly from present-day conditions (35.2 % at pH 8.0 and 41.0 % at pH 7.5) to the future warming and acidification scenario of 22 °C temperature and a pH of 7.5 reaching 100 % (i.e. all newborn juveniles still have unconsumed yolk inside the egg capsule (Rosa et al. 2013).

At the intermediate embryonic stages, the study also found that routine metabolic rates (RMR) increased during the embryonic period, but environmental hypercapnia significantly depressed energy expenditure rates of pre-hatchlings (independently of temperature) with lower RMR at the pre-hatching stage ranging between 1.3 (18 °C, pH 7.5), 2.2 µmol g⁻¹ h⁻¹ (22 °C, pH 7.5) and 5.5 µmol g⁻¹ h⁻¹ (18 °C, pH 8.0) (Rosa et al. 2013). Based on thermal sensitivity data the authors argue that the metabolic depression is a short-term method of extending the timeframe over which unfavorable conditions inside the egg can be withstood and can be achieved by reducing protein synthesis and thus growth (Rosa et al. 2013). Although metabolic depression is considered to be a sublethal reversible process, it is likely to only be an effective adaptive strategy for short-term survival of marine organisms from hypercapnia and hypoxia and not under persistent elevations of CO₂ (e.g. Pörtner et al. 2004). Thus the already stressful abiotic conditions inside *S. officinalis* eggs are likely to be aggravated by the effects of climate change and may act as a main trigger for premature hatch-

ing and thus smaller post-hatching body sizes; effects which may challenge the survival and fitness of early life stages (Rosa et al. 2013).

**Early life stage growth and survival**

To date there is very little information available on the interacting effects of climate change on ELS *S. officinalis*.

**CONCLUSION**

The common cuttlefish (*S. officinalis*) is a short-lived, fast-growing species that is vulnerable to changes in environmental conditions. This is of particular importance during its critical and vulnerable ELSs which often encounter hydrodynamically unstable conditions in the inshore or intertidal coastal waters of spawning grounds. The effects of the environment (in terms of physico-chemical cues) on embryonic and ELS cuttlefish can affect not only individual parameters such as duration of embryogenesis, rate of hatching, rate of post-embryonic growth and rate of mortality (e.g. Blanc 1998, Koueta & Boucaud-Camou 2003, Boletzky et al. 2006) but at a larger-scale it can also affect the distribution and abundance of the exploited stock (Pierce et al. 2008).

As discussed in this review, although optimal water conditions for successful embryonic development of *S. officinalis* are known, eggs laid at different times or locations can experience a wide range of environmental conditions. *S. officinalis* is known to have an early embryonic sensory system likely related to the perception of external cues and display a high degree of life cycle plasticity which allows it to survive by responding to the wide range of physico-chemical cues they encounter (Pierce et al. 2010). Research has shown that cuttlefish embryos are able to perceive their environment through at least tactile, chemical and visual sensory modalities (Dar-maillaq et al. 2008, Guibé et al. 2010) which operate *in ovo* and are functional from about 3 weeks before hatching (Dar-maillaq et al. 2008, Guibé et al. 2010, Romagny et al. 2012). Because of the importance of environmental signals for biological cycles, the molecular regulation in response to variations during development could confer adaptive advantage (Navet et al. 2014). It is known that some post-hatching behaviours are plastic and dependent on individual embryonic sensory experience (Dar-maillaq et al. 2008, Guibé et al. 2010). The early sensory system of *S. officinalis* embryos may therefore intervene when certain environmental conditions are encountered to regulate development in response to dehydration, oxygenation or osmoregulation stresses.

Increasingly studies are focusing on determining how the synergistic (or antagonistic) impacts of temperature, acidification and other environmental variables (i.e. nutri-
ents, hypoxia and salinity) may affect embryonic and ELS cephalopods. Strategies that enable species to persist in changing environments (e.g. climate change) include: the ability to shift their distribution to track optimal conditions, to adjust their phenotypes via plasticity and/or to adapt to novel stresses (Anderson et al., 2012, Bellard et al., 2012). Further research is therefore required to determine whether the early sensory system (e.g. visual, tactile and chemical senses) of *S. officinalis* together with its capacity for life cycle plasticity may be sufficient to allow the embryonic and ELS to adapt to the changing climate or whether the phenotypic plasticity that enables cuttlefish to adapt and respond in the short-term, to the wide range of local environmental conditions in hydrodynamically unstable coastal waters will be sufficient to respond to longer term, global changes in temperature and acidity and other stressors that will result from climate change.

While *S. officinalis* remains one of the better studied cephalopod species the full impact of physico-chemical cues on the embryonic and ELS development of this species in the wild, both in the context of current and changing environmental conditions, are still far from well understood and a wide range of research projects – e.g.

- Electronic tagging studies (acoustic and data storage tagging) to investigate changes in movement and migration patterns as a result of increasing temperatures;
- investigation into the potential role that surface skin opsins may play in the early embryonic sensory system;
- physiological studies to investigate the mixed effects of physico-chemical variables (e.g. lowered pH and oxygen saturation) on embryonic and ELSs;
- pollution studies to investigate the impacts of climate change on elemental uptake in embryonic and ELS;
- longer-term studies to assess the impacts of hypercalcification on survival rates of ELS;
- longer-term intergenerational studies to examine the effects of climate change on the lifecycle of cephalopods as a whole (i.e. will females begin to produce fewer but larger eggs overtime to compensate for reduction in hatching size)

– are now required to enable an accurate understanding of how the complicated interactions of physico-chemical variables may affect the ability of cuttlefish to respond and adapt to them (e.g. ion regulatory pumps, metabolic depression, elevated calcification rates, etc.) during the critical phases of embryonic and ELS development.

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Vie Milieu, 2016, 66 (1)


Received on September 16, 2015
Accepted on December 2, 2015
Associate editor: S Boletzky