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# Impact of ocean warming on the early ontogeny of cephalopods: a metabolic approach

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**Abstract** The impact of a realistic warming scenario on the metabolic physiology of early cephalopod (squid *Loligo vulgaris* and cuttlefish *Sepia officinalis*) life stages was investigated. During exposure to the warming conditions (19 °C for the western coast of Portugal in 2100), the increase in oxygen consumption rates throughout embryogenesis was much steeper in squid (28-fold increase) than in cuttlefish (11-fold increase). The elevated catabolic activity–accelerated oxygen depletion within egg capsules, which exacerbated metabolic suppression toward the end of embryogenesis. Squid late-stage embryos appear to be more impacted by warming via metabolic suppression than cuttlefish embryos. At all temperature scenarios, the transition from encapsulated embryos to planktonic paralarvae implied metabolic increments higher than 100 %. Contrary to the nektobenthic strategy of cuttlefish newborns, the planktonic squid paralarvae rely predominantly on pulsed jet locomotion that dramatically increases their energy requirements. In the future, hatchlings will require more food per unit body size and, thus, feeding intake success will be crucial, especially for squid with high metabolic rates and low levels of metabolic reserves.

## Introduction

Ocean warming has emerged as a major environmental threat to healthy marine ecosystems. Most research on biological climate-related impacts has been conducted on late ontogenetic stages (i.e., adult organisms), albeit early stages (embryos and hatchlings) are expected to be the most vulnerable to ocean changes (Kurihara 2008; Byrne 2011) and could constitute the bottleneck for species survival.

Temperature affects almost every aspect of the early life history of marine species, such as developmental time, yolk utilization efficiency, as well as body size and weight (McMahon and Summers 1971; Pechenick 1987; Pechenick et al. 1990; Kamler 1992; Boidron-Metairon 1995). It is known that above a certain temperature (also known as "pejus temperature"—Tp), oxygen delivery is maximal and cannot further rise to cover elevated metabolic demands (Frederich and Pörtner 2000; Pörtner 2001, 2002; Pörtner et al. 2004, 2005). Although survival is not immediately threatened beyond Tp, the ability to perform higher functions (e.g., feeding, growth, and reproduction) becomes limited (Pörtner and Knust 2007), which will influence the overall fitness and survival success of the species, especially on a long-term perspective.

Cephalopods are marine keystone species that play an important role as prey and predators in coastal and oceanic ecosystems (Boyle and Rodhouse 2005; Rosa et al. 2008). Active cephalopods (like muscular squids) are known to have one of the highest metabolic rates in the entire animal kingdom due to their energetically inefficient mode of locomotion—jet propulsion (O'Dor and Webber 1991; Rosa and Seibel 2008). In fact, an equally sized squid exceeds the metabolic demand of a fast-swimming fish by several times (Rosa and Seibel 2008), which may pose

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serious problems in a future warming scenario. Yet, their life strategy "live fast, die young" with short generation times might enable cephalopods to adjust quickly to changing environmental conditions and improve their chances of survival (Boyle and Rodhouse 2005; Pecl and Jackson 2008; Tian 2009; Rosa and Seibel 2010).

Coastal cephalopod embryos develop within benthic egg capsules that act as physical protection and a barrier to the diffusion of dissolved gases. The metabolic demand of embryos rises during development (i.e., cellular growth, organogenesis, and muscular activity; Sasaki et al. 1986; Wolf et al. 1985) leading to an accumulation of metabolic $CO_2$ (including pH reduction) and a simultaneous drop in $pO_2$ (down to levels of 5–6 kPa; Cronin and Seymour 2000; Gutowska and Melzner 2009). Oxygen depletion within mollusk eggs is partially compensated by egg swelling (i.e., increased surface area, reduced egg wall thickness; Cronin and Seymour 2000), but progresses during development and might even act as the main trigger for hatching. In the future, as elevated temperatures result in increased metabolic rates, oxygen levels within egg capsules are expected to be depleted faster causing major challenges for cephalopod embryos.

In the last decades, an increasing warming trend has been observed in the coasts of Western Portugal (2.7 °C century$^{-1}$; Relvas et al. 2007) and an additional warming of 2 °C is expected by 2100 (Santos et al. 2002). In the present study, we investigate, for the first time, the impact of a realistic warming scenario on the early ontogenetic metabolism of two cephalopod species with different early life cycles, namely the squid *Loligo vulgaris* and the cuttlefish *Sepia officinalis*. We determined oxygen consumption rates (OCRs) and thermal sensitivity ($Q_{10}$ values) during embryogenesis and also quantified the metabolic increment associated with planktonic (squid) and nektobenthic (cuttlefish) transitions from late embryos to hatchlings.

## Materials and methods

### Collection of eggs

Egg clutches of *L. vulgaris* (in stage I; Naef 1928) were obtained from fisherman traps placed at 30 m depth near Figueira da Foz, Portugal (Fig. 1), in March 2010 and April 2011. Egg masses of *S. officinalis* (also in stage I; Naef 1928) were harvested near Cascais, Portugal (Fig. 1), between April and May 2010. After collection, eggs of *L. vulgaris* and *S. officinalis* were immediately transferred to the aquaculture facilities in Laboratório Marítimo da Guia, Cascais and placed at the experimental conditions mentioned below.

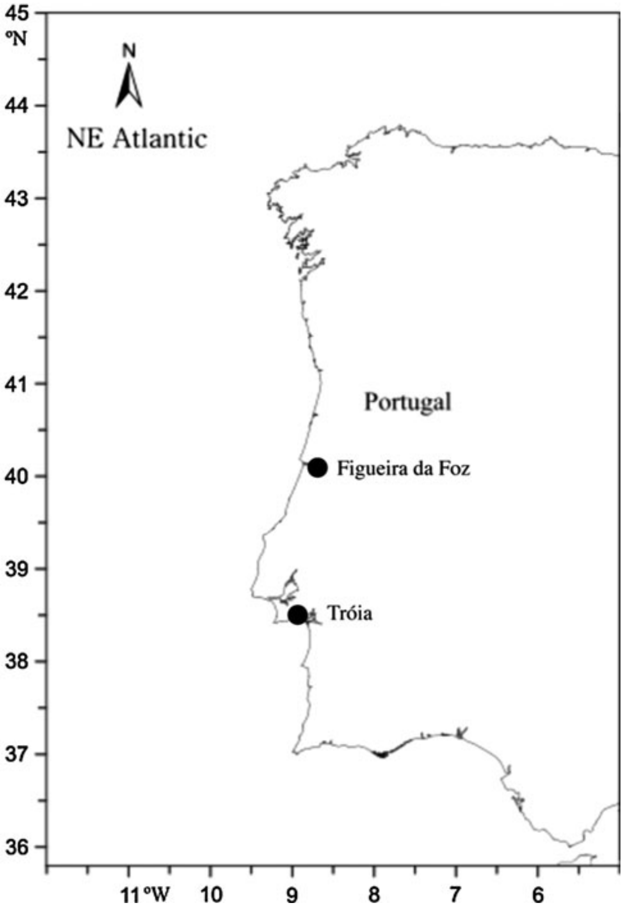

**Fig. 1** Map of the two sampling areas in the western Portuguese coast. Egg clutches of *L. vulgaris* and *S. officinalis* were sampled in Figueira da Foz and Tróia, respectively

### Egg incubation

Egg clutches and masses were placed in four recirculating systems, each containing 25 separate glass aquariums (volume of 54 L) and a collective sump of 270 L. The closed systems were filled with UV-sterilized and filtered (series 20, 10, 5, and 1 μm) seawater, and tanks were illuminated with a photoperiod of 14 h light/10 h dark. Water quality was ensured using wet–dry filters (bioballs), protein skimmers (Schuran, Jülich, Germany), and 30-W UV-sterilizers (TMC, Chorleywood, UK). Ammonia and nitrite were monitored regularly and kept below detectable levels. pH showed average values of 8.1 ± 0.1. Salinity throughout the experiment was 34.0 ± 1.0, and temperatures (13.0 ± 0.2 °C, 15.0 ± 0.2 °C, 17.0 ± 0.2 °C and 19.0 ± 0.2 °C) were controlled via Heilea chillers (Guangdong, China).

Eggs were reared until hatching at four different temperatures (27.17 ± 0.99 days at 13 °C; 23.57 ± 0.83 days at 15 °C; 21.33 ± 1.43 days at 17 °C; 14.10 ± 1.94 days at 19 °C), namely: (1) 13 °C—the mean sea bottom temperature in winter (wSBT), at 30 m depth, in western coast of Portugal (Moreno et al. 2005, 2009); (2) 15 °C—the mean winter sea surface temperature (wSST), and also the expected warming scenario for wSBT in 2100 (+2 °C; Santos et al. 2002); (3) 17 °C—the mean summer sea surface temperature (sSST); and (4) 19 °C—the future sSST warming scenario for the western coast of Portugal in 2100 (+2 °C; Santos et al. 2002).

## Developmental stages and oxygen consumption rates

Five (I–V) and four (I–IV) developmental stages were established for *L. vulgaris* (Table 1) and *S. officinalis* (Table 2), respectively, based on Naef's descriptions (1928). Oxygen consumption measurements (routine metabolic rates) were determined according to Seibel et al. (2007) and Rosa et al. (2009). Eggs and hatchlings were incubated in sealed water-jacketed respirometry chambers (RC300 Respiration cell, Strathkelvin, North Lanarkshire, Scotland) containing filtered seawater mixed with antibiotics (50 mg $L^{-1}$ streptomycin) to avoid bacterial respiration. For each species, 4 replicates were used per stage and temperature. Water volumes were adjusted in relation to animal mass (up to 4 mL) in order to minimize locomotion and stress but still allow spontaneous and routine activity rates of the hatchlings. Bacterial controls were conducted in parallel to correct for possible bacterial respiratory activity. Respiration chambers were placed in water baths (Lauda, Lauda-Königshofen, Germany) to control temperature. Oxygen concentrations were recorded with Clarke-type $O_2$ electrodes connected to a multichannel oxygen interface (Strathkelvin, North Lanarkshire, Scotland). The duration of respiratory runs varied from 12 to 24 h. In pre-hatchlings (stage IV and III of *L. vulgaris* and *S. officinalis*, respectively), the yolk sac could be dissected from its embryo to determine the embryo wet weight (without yolk sac, chorion and perivitelline fluid).

For each developmental stage and species, thermal sensitivity ($Q_{10}$) was determined using the standard equation:

$$Q_{10} = [R(T_2)/R(T_1)] * 10/(T_2 - T_1), \quad (1)$$

where $R(T_2)$ and $R(T_1)$ represent the oxygen consumption rates at temperatures $T_2$ and $T_1$, respectively.

The metabolic increment (%) of the transition from late embryos to early hatchlings (stages IV–V in *L. vulgaris* and stages III–IV in *S. officinalis*) was calculated using the following equation:

$$\text{Metabolic increment (\%)} = ((\text{OCR}_\text{H} - \text{OCR}_\text{LE})/\text{OCR}_\text{LE}) * 100, \quad (2)$$

where $\text{OCR}_\text{LE}$ represents the oxygen consumption rate of late embryos and $\text{OCR}_\text{H}$ that of early hatchlings.

## Statistical analysis

Two-way ANOVAs were conducted to detect significant differences in OCRs between developmental stages and temperatures during embryogenesis of *L. vulgaris* and

**Table 1** Description of *L. vulgaris* developmental stages based on Naef (1928)

| Stages | Naef stages | Main characteristics |
|---|---|---|
| I | V–X | Embryonic body and the yolk envelope are distinct from each other. Organ rudiments become increasingly distinct and prominent |
| II | XI–XIII | Cephalic section shows the eye stalks at the peak of their development and the fin appeared. On the arms and on the mantle, the first yellow-brownish chromatophores appear |
| III | XIV–XVIII | The eyes become directed more anteriorly and the typically larval head shape progressively appears. The funnel tube is completely closed and is inserted in the mantle. The number of chromatophores increase |
| IV | XIX | Embryo is reaching the state of a viable juvenile. Embryonic development is characterized by a strong size increase, especially in longitudinal axis. Outer yolk sac is rapidly reduced |
| V | XX | Newly hatched paralarva |

**Table 2** Description of *S. officinalis* developmental stages based on Naef (1928)

| Stages | Naef stages | Main characteristics |
|---|---|---|
| I | VII–X | The rudiments appear as folds and ridges, producing a picture very similar to *Loligo*. The arm rudiments appear bipartite as in all other sepioids |
| II | XI–XV | On the eye balls, a delicate circular ridge forms the iris fold rudiment. The gill rudiments are differentiated. Clearly differentiation of the arms. The fin appears |
| III | XVI–XIX | On the mantle the chromatophores appear. The rotation of the ink sac begins. Eyes get darker |
| IV | XX | Newly hatched juvenile |

*S. officinalis*. A three-way ANOVA was used to compare OCRs between species, temperatures, and developmental stages (embryos and hatchlings). Subsequently, Tukey's post-hoc tests were performed. Linear and polynomial regressions were conducted to assess the correlations between metabolic costs and temperatures between late embryos and hatchlings. All values are expressed as mean ± SD. All statistical analyses were performed for a significance level of 0.05, using Statistica 10.0 software (StatSoft Inc., Tulsa, USA).

## Results

### Embryogenesis

As expected, oxygen consumption rates (OCR) of *L. vulgaris* and *S. officinalis* were significantly increased over the course of development and were also significantly different between the two cephalopod species. For example, in the summer warming scenario (19 °C), the squid OCR increased significantly from 0.8 μmol $O_2$ $h^{-1}g^{-1}$ (Stage I) to 24.1 μmol $O_2$ $h^{-1}g^{-1}$ (Stage IV) throughout embryogenesis (Fig. 2; Table 3). On the other hand, cuttlefish OCR varied significantly between 0.49 μmol $O_2$ $h^{-1}g^{-1}$ (Stage I) and 5.86 μmol $O_2$ $h^{-1}g^{-1}$ (Stage III; Fig. 3; Table 4). Therefore, the OCR increase during embryonic development was much steeper in *L. vulgaris* (28-fold increase) than in *S. officinalis* (11-fold increase). Yet, unexpectedly, this metabolic boost was even more pronounced in the winter warming scenario (15 °C; *L. vulgaris*: 43-fold; *S. officinalis*: 14-fold). These findings became clearer after analyzing the thermal sensitivity data. The $Q_{10}$ values of the embryonic stages ranged mainly between 2 and 3, except for the late embryos (*L. vulgaris*: stage IV and *S. officinalis*: stage III; Table 5). In squid late

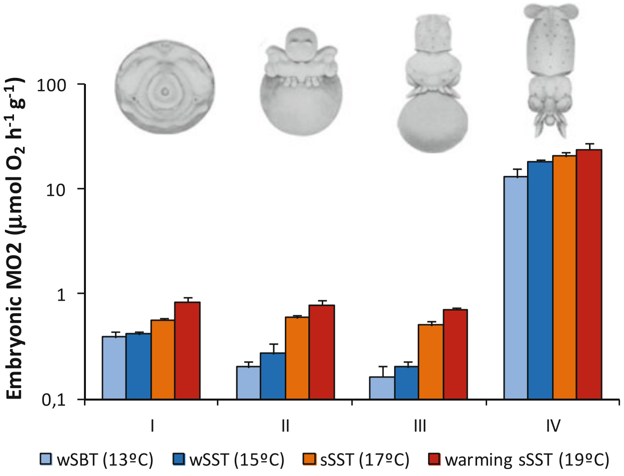

**Fig. 2** Metabolic rates ($MO_2$, μmol $O_2$ $g^{-1}$ $h^{-1}$) of *L. vulgaris*' embryos (from stage I to IV) at four different temperatures: 13 °C (winter sea bottom temperature, wSBT), 15 °C (winter sea surface temperature, wSST), 17 °C (summer sea surface temperature, sSST), and 19 °C (summer SST warming scenario in 2100). Values are means of three measurements ± SD

**Table 3** Results of two-way ANOVA evaluating the effects of embryonic stage and temperature on oxygen consumption during embryonic development of *L. vulgaris*

| | *df* | MS | *F* | *p* |
|---|---|---|---|---|
| Stage (S) | 3 | 1,040.59 | 3,688.78 | 0.000 |
| Temperature (T) | 3 | 21.22 | 75.23 | 0.000 |
| SxT | 9 | 14.69 | 52.06 | 0.000 |
| Error | 32 | 0.28 | | |

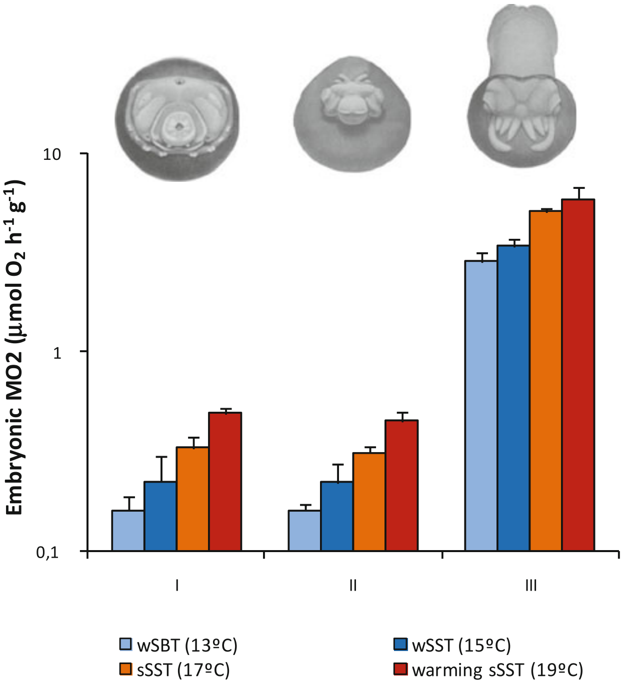

**Fig. 3** Metabolic rates ($MO_2$, μmol $O_2$ $g^{-1}$ $h^{-1}$) of *S. officinalis*' embryos (from stage I to III) at four different temperatures: 13 °C (winter sea bottom temperature, wSBT), 15 °C (winter sea surface temperature, wSST), 17 °C (summer sea surface temperature, sSST), and 19 °C (summer SST warming scenario in 2100). Values are means of four measurements ± SD

**Table 4** Results of two-way ANOVA evaluating the effects of embryonic stage and temperature on oxygen consumption during embryonic development of *S. officinalis*

| | *df* | MS | *F* | *p* |
|---|---|---|---|---|
| Stage (S) | 2 | 85.35 | 897.30 | 0.000 |
| Temperature (T) | 3 | 3.74 | 39.37 | 0.000 |
| SxT | 6 | 2.16 | 22.76 | 0.000 |
| Error | 36 | 0.10 | | |

**Table 5** Thermal sensitivity ($Q_{10}$) of *L. vulgaris* and *S. officinalis* during early development

| Temperature interval (°C) | Developmental stage | *L. vulgaris* | *S. officinalis* |
|---|---|---|---|
| 13–15 | I | 1.996 | 1.960 |
| | II | 1.896 | 1.928 |
| | III | 2.000 | 2.036 |
| | IV | 2.811 | 2.000 |
| | V | 1.987 | – |
| 15–17 | I | 2.012 | 2.170 |
| | II | 2.000 | 1.932 |
| | III | 1.996 | 1.929 |
| | IV | 1.247 | 2.002 |
| | V | 2.310 | – |
| 17–19 | I | 2.290 | 2.219 |
| | II | 2.035 | 2.107 |
| | III | 1.973 | 1.588 |
| | IV | 1.360 | 2.355 |
| | V | 2.354 | – |

embryos, a pronounced $Q_{10}$ drop above 15 °C was detected (Fig. 4). For the cuttlefish, this drop was only noticed at the last temperature interval (17–19 °C; Fig. 4).

### Transition from late embryos to hatchlings

The OCR values for squid late embryos varied between 13.0 µmol $O_2$ $h^{-1}g^{-1}$ (at 13 °C) and 24.1 µmol $O_2$ $h^{-1}g^{-1}$ (at 19 °C) (Fig. 5, left panel), while the cuttlefish OCRs ranged between 2.85 µmol $O_2$ $h^{-1}g^{-1}$ (at 13 °C) and 5.86 µmol $O_2$ $h^{-1}g^{-1}$ (at 19 °C) (Fig. 4, right panel). After hatching, the squid planktonic paralarvae displayed significantly higher OCR values, ranging between 28.0 µmol $O_2$ $h^{-1}g^{-1}$ (at 13 °C) and 59.1 µmol $O_2$ $h^{-1}g^{-1}$ (at 19 °C; Fig. 5). On the other hand, the nektobenthic cuttlefish hatchling revealed significantly lower values, namely 3.84 µmol $O_2$ $h^{-1}g^{-1}$ at 13 °C and 11.1 µmol $O_2$ $h^{-1}g^{-1}$ at 19 °C (Fig. 5; Table 6). The interspecific differences of the metabolic increment (as percentage) of this transition are shown in Fig. 5. Not surprisingly, the metabolic increments were positively correlated with temperature, but while *L. vulgaris* showed a linear relationship ($y = 10.122x + 101.59$, $R^2 = 0.9107$, $p < 0.05$; Fig. 6), *S. officinalis* revealed a polynomial one ($y = 23.084e^{0.2783x}$, $R^2 = 0.6648$, $p < 0.05$; Fig. 6). Squid metabolic increment ranged from 116 (at 13 °C) to 145 % (at 19 °C), whereas the cuttlefish increment did not vary significantly at the lower temperatures (~30 %, 13–17 °C). Nevertheless, cuttlefish metabolic increment at the predicted summer warming condition (19 °C) went up to 90 %, but still it did not achieved the values observed for the squid planktonic transition (Fig. 6).

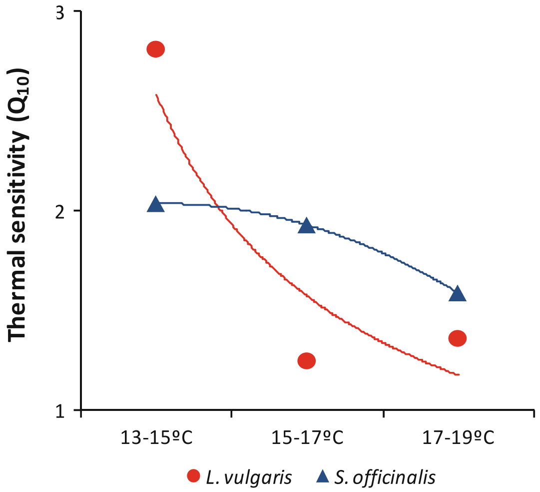

**Fig. 4** Thermal sensitivity ($Q_{10}$) of late embryonic stages of *L. vulgaris* (stage V) and *S. officinalis* (stage IV) at different temperature intervals. $Q_{10}$ values between 2 and 3 indicate active metabolic regulation; $Q_{10}$ values inferior to 1.5 suggest active metabolic suppression

## Discussion

### Warming and embryonic metabolism

Oxygen demand is known to increase over the course of embryonic development (Marvel and Fisher 1948; Wolf et al. 1985; Cronin and Seymour 2000; Brante 2006; Moran and Woods 2007; Brante et al. 2008), especially at the onset of organogenesis (Lemaire 1971; 1971). In both species, oxygen consumption rates (OCR) significantly increased in the later stage of embryonic development (stage IV in *L. vulgaris* and stage III in *S. officinalis*). Nevertheless, the OCRs of squid embryonic stages were significantly higher than those found in the cuttlefish (Figs. 2, 3). Squid's gills structures are known to be less developed than the cuttlefish and their capacity to exchange ions and extract oxygen are reduced (Fioroni 1990; Nixon and Mangold 1998; Hu et al. 2010). Yet, it is worth noting that $O_2$ uptake of early embryos (especially stages II and III for cuttlefish and squid, respectively) might be underestimated, as their yolk could not be dissected and OCR calculations of late embryos do not contain chorion and perivitelline fluid wet weights. Despite this, the rise in $O_2$ demands with progressing embryogenesis is striking and known to cause critical $pO_2$ levels within egg capsules (Cronin and Seymour 2000; Gutowska and Melzner 2009).

Not surprisingly, increased temperatures led to higher OCRs at all embryonic stages of *L. vulgaris* and *S. officinalis*. Environmental temperature dictates growth and oxygen consumption rates, as metabolically active enzymes are highly sensitive to temperature changes. Temperature can increase to a point as long as cardiac and ventilatory

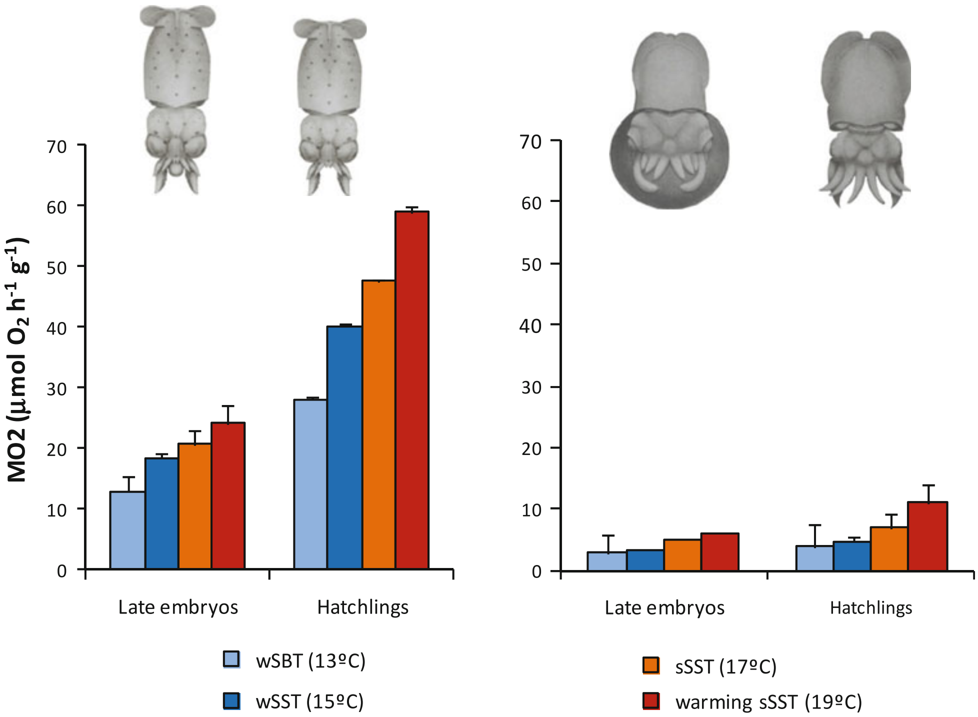

**Fig. 5** Metabolic rates ($MO_2$ µmol $O_2$ $g^{-1}$ $h^{-1}$) of pre-hatching embryos and hatchlings of *L. vulgaris* (*panel A*) and *S. officinalis* (*panel B*) at four different temperatures: 13 °C (winter sea bottom temperature, wSBT), 15 °C (winter sea surface temperature, wSST), 17 °C (summer sea surface temperature, sSST), and 19 °C (summer SST warming scenario in 2100). Values are means of four measurements ± SD

**Table 6** Results of three-way ANOVA evaluating the effects of species, developmental stage, and temperature on the oxygen consumption rates of pre-hatching embryos and hatchlings of *L. vulgaris* and *S. officinalis*

| | *df* | MS | *F* | *p* |
|---|---|---|---|---|
| Species (Sp) | 1 | 9,405.44 | 14,043.84 | 0.000 |
| Stage (St) | 1 | 2,593.99 | 3,873.25 | 0.000 |
| Temperature (T) | 3 | 387.94 | 579.25 | 0.000 |
| SpxSt | 1 | 1,778.70 | 2,655.89 | 0.000 |
| SpxT | 3 | 132.78 | 198.27 | 0.000 |
| StxT | 3 | 77.75 | 116.09 | 0.000 |
| SpxStxT | 3 | 29.97 | 44.75 | 0.000 |
| Error | 40 | 0.67 | | |

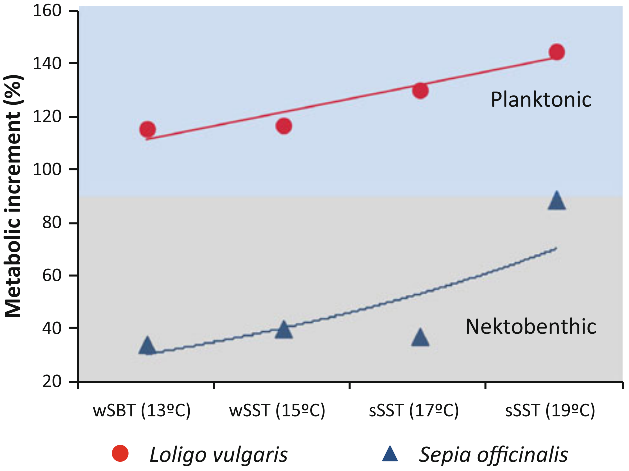

**Fig. 6** Metabolic increment (%) in the planktonic and nektobenthic transition from late embryos to hatchlings of *L. vulgaris* and *S. officinalis*, respectively, at four different temperatures: 13 °C (winter sea bottom temperature, wSBT), 15 °C (winter sea surface temperature, wSST), 17 °C (summer sea surface temperature, sSST), and 19 °C (summer SST warming scenario in 2100). Values are mean ± SD

adjustments can keep pace with increased metabolic demands (Pörtner and Knust 2007), but beyond this temperature, anaerobic respiration sets in together with protein denaturation, permanent inactivation of enzymes, growth cessation, and eventual death (Wang and Overgaard 2007; Katersky and Carter 2007). As expected, increased temperatures led to a reduction in squid developmental time (as in Bouchaud 1991; McMahon and Summers 1971; Boletzky 1994; Steer et al. 2003; Rosa et al. 2012, with concomitant negative effects on survival, growth and hatching Rosa et al. 2012; see summary in Table 7). In cuttlefish, it is known that at lower temperatures (15 °C), 41 % of the egg yolk is used for growth, and only 10 % is used for catabolic processes (such as respiration or excretion), while yolk utilization at higher temperatures (24 °C) is in deficit for growth (15 %) and in excess for catabolism (52 %), resulting in smaller hatchling size (Bouchaud 1991).

At all temperature scenarios, the enhancement in embryonic OCR was much steeper in *L. vulgaris* than in *S. officinalis*. Yet, unexpectedly, this metabolic boost was more pronounced in the winter rather than in the future

**Table 7** Impact of environmental warming in the development time, abnormalities, and survival rates of *L. vulgaris* embryos [source: Rosa et al. 2012]

| Variable | Descriptions |
|---|---|
| Development time | Embryogenesis was shortened in *circa* 13 days from present-day winter conditions (13 °C) to near-future summer warming (19 °C) |
| Abnormalities | The incidence of abnormalities increased significantly with temperature; the higher percentage (44 %) was found in late embryos exposed to the summer warming scenario |
| Survival | Survival rates decreased up to 71 % at the projected near-future ocean warming |
| Hatching | The number of paralarvae that hatched prematurely was significantly higher in the warming scenario (8.3 %) than at present-day temperatures (0 %) |

summer scenario. Concomitantly, squid late embryos showed a pronounced $Q_{10}$ drop above 15 °C; for the cuttlefish, this drop was only noticed at the last temperature interval (Fig. 4). Elevated catabolic activity, and consequently OCR, seemed to accelerate oxygen depletion within egg capsules causing an oxygen deficiency in late embryos. In the future, one may argue that, in order to avoid such warming related-physiological impairments, both species may lay their eggs in deeper and cooler environments. Yet, it should be kept in mind that cuttlefish habitat depth is limited by resistance of chambered shells (phragmocones) of calcium carbonate to implosion (Denton and Gilpin-Brown 1961a, 1961b). Their cuttlebones are known to implode between 150 and 200 m (Ward and Boletzky 1984). Also, thought squids do not have such constraints, their planktonic hatchlings will eventually have to face some ecophysiological challenges at shallower depths (more details in section "Planktonic versus nektobenthic transition in a warming scenario").

## Metabolic suppression and oxygen deficiency in late embryos

In many ectotherms at normal operating temperature, metabolic demand for oxygen increases substantially with temperature ($Q_{10} = 2$–3), whereas oxygen uptake by diffusion, a physical process, increases more slowly ($Q_{10} < 2$; Moran and Woods 2007), and therefore can lead to a relative oxygen shortage (Woods 1999; Pörtner 2001; Woods and Hill 2004). In adult cephalopods, $Q_{10}$ values usually range between 2 and 3 (O'Dor and Wells 1987; Rosa and Seibel 2008, 2010), which is in agreement with our data with early stages, except for pre-hatchlings. In fact, the late embryos of squid (at temperature intervals of 15–17 °C and 17–19 °C) and cuttlefish (at 17–19 °C) showed thermal sensitivity values below 1.5, which might be a consequence of active metabolic suppression (Thorp and Covich 2001), or instead, indicative that such temperatures are already outside squid's temperature tolerance window (i.e., beyond $T_c$), and therefore, a consequence of oxygen deficiency (Pörtner 2001, 2002; Pörtner et al. 2004, 2005). On the other hand, hatchlings did not show such thermal sensitivity trends. This might be due to the more developed gill structures (Hu et al. 2010) at that stage of development, or a response to the improved abiotic conditions outside the egg.

It is known that to enable rising $O_2$ fluxes by means of diffusion throughout embryonic development, many molluskan eggs swell, leading to enhanced surface areas and reduced egg wall thicknesses (Wolf et al. 1985; Cronin and Seymour 2000). Nonetheless, egg swelling does not prevent $pO_2$ from consistently falling (e.g., *Sepia apama*: 14 kPa down to 5–6 kPa close to hatching; Cronin and Seymour 2000) and $pCO_2$ from rising (>0.3 kPa, pH < 7.5; Hu et al. 2011). Here, we show that squid late embryos seem to be more impacted by environmental warming than the cuttlefish ones. Cuttlefish hatchlings are miniature models of adults (Fioroni 1990; Nixon and Mangold 1998) and possess the general structures of adult gills (i.e., first- and second-order vessels and lamellae; Hu et al. 2010). Their higher hypoxia tolerance might also be linked to a high $O_2$-affinity pre-hemocyanin (Wolf et al. 1985; De Wachter et al. 1988) and simultaneously lower metabolic demand to enable embryos to remain much longer in the protective egg casing (De Wachter et al. 1988) and use their energy reserves more efficiently for growth and differentiation. Nonetheless, the stressful abiotic conditions inside eggs are expected to be aggravated by increasing temperature in future climate change scenarios and accelerate oxygen deficiencies that might induce metabolic suppression. This will induce deleterious effects on the growth and survival of cephalopod early stages.

## Planktonic versus nektobenthic transition in a warming scenario

The egg–paralarvae transition in squid implied metabolic increments higher than 100 % at all temperatures, whereas the cuttlefish costs did not vary significantly at the lower and intermediate temperatures (~30 %, between 13 and 17 °C). These interspecific differences at hatching seem to reflect different strategies in their early life style.

Large-egged species, such as *S. officinalis*, take longer to develop between spawning and hatching but emerge as larger, more competent hatchlings (Mangold-Wirz 1963). The nektobenthic newborns showed lower metabolic needs because of the following: (1) they rest large fractions of the day and hardly move (Aitken et al. 2005), (2) their locomotion is less dependent on jet propulsion (Aitken and O'Dor 2004), (3) they possess a buoyancy facilitating cuttlebone (Denton and Gilpin-Brown 1961a, b; Webber et al. 2000), and iv) their oxygen uptake system is highly improved (Wells et al. 1988). On the other hand, the planktonic squid paralarvae rely predominantly on a pulsed jet for locomotion (Bartol et al. 2009). This propelling mode distinguishes them from the majority of aquatic locomotors, which employ mostly oscillatory/undulatory movements. Jet propulsion is an inefficient locomotory system that obligates squids to consume much more oxygen than other nektonic predators in order to maintain the same high-activity levels (O'Dor and Webber 1986; Rosa and Seibel 2008, 2010). Furthermore, the undeveloped gill structures of squid's embryos and paralarvae limit $O_2$ uptake and ion-regulatory capacity (Hu et al. 2010).

Although the metabolic cost of squid egg–paralarvae transition was always higher, it seemed less influenced by ocean warming, varying from 116 (at 13 °C) to 145 % (at 19 °C). In opposition, cuttlefish transition did not vary significantly at the lower temperatures (~30 %, 13–17 °C), but increased up to 90 % at the predicted summer warming condition (19 °C), which suggest higher sensitivity at high temperatures. An eventual shortened development time and less developed gill structures might cause elevated stress in cuttlefish hatchlings at such temperatures. Nonetheless, hatchlings of both species will require more food per unit body size at higher temperatures (see also Pecl and Jackson 2008) and feeding failures will be disastrous, especially for squid with high metabolic rates and low levels of metabolic reserves. Furthermore, ocean warming may reduce the aerobic scope of cephalopods (Rosa and Seibel 2008) and diminish their ability to respond to external stimuli, leaving them potentially more vulnerable to predation.

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## References

Aitken JP, O'Dor RK (2004) Respirometry and swimming dynamics of the Australian cuttlefish *Sepia apama*. Mar Freshw Behav Physiol 37:217–234

Aitken JP, O'Dor RK, Jackson GD (2005) The secret life of the giant cuttlefish *Sepia apama* (Cephalopoda): behaviour and energetics in nature revealed through radio acoustic positioning and telemetry (RAPT). J Exp Mar Biol Ecol 320:77–91

Bartol IK, Krueger PS, Stewart WJ, Thompsonn JT (2009) Pulsed jet dynamics of squid hatchlings at intermediate Reynolds numbers. J Exp Biol 212:1506–1518

Boidron-Metairon IF (1995) Larval nutrition. In: Edward LMc (ed) Ecology of marine invertebrate larvae. CRC Press, Boca Raton, pp 223–248

Boletzky SV (1994) Embryonic development of cephalopods at low temperatures. Antarct Sci 6(2):139–142

Bouchaud O (1991) Energy consumption of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) during embryonic development, preliminary results. Bull Mar Sci 49(1–2):333–340

Boyle P, Rodhouse P (2005) Cephalopods: ecology and fisheries. Blackwell Science, Oxford 452 pp

Brante A (2006) An alternative mechanism to reduce intracapsular hypoxia in ovicapsules of *Fusitriton oregonensis* (Gastropoda). Mar Biol (Berl) 149:269–274

Brante A, Fernández M, Viard F (2008) Effect of oxygen conditions on intracapsular development in two calyptraeidae species with different modes of larval development. Mar Ecol Prog Ser 368:197–207

Byrne M (2011) Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. Ocean Mar Biol Annu Rev 49:1–42

Cronin ER, Seymour RS (2000) Respiration of the eggs of the giant cuttlefish *Sepia apama*. Mar Biol 136:863–870

De Wachter B, Wolf G, Richard A, Decleir W (1988) Regulation of respiration during juvenile development of *Sepia officinalis* (Mollusca: Cephalopoda). Mar Biol 97:365–371

Denton EJ, Gilpin-Brown JB (1961a) The buoyancy of the cuttlefish, *Sepia officinalis* (L.). J Mar Biol Assess UK 41:319–342

Denton EJ, Gilpin-Brown JB (1961b) The effect of light on the buoyancy of the cuttlefish. J Mar Biol Assess UK 41:343–350

Fioroni P (1990) Our recent knowledge of the development of the cuttlefish (*Sepia officinalis*). Zool Anz 224:1–25

Frederich M, Pörtner HO (2000) Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. Am J Physiol 279:1531–1538

Gutowska MA, Melzner F (2009) Abiotic conditions in cephalopod (*Sepia officinalis*) eggs: embryonic development at low pH and high pCO2. Mar Biol 156:515–519

Hu MY, Sucré E, Charmantier-Daures M, Charmantier G, Lucassen M, Himmerkus N, Melzner F (2010) Localization of ion-regulatory epithelia in embryos and hatchlings of two cephalopods. Cell Tissue Res 339(3):571–583

Hu MY, Yung-Che T, Stumpp M, Gutowska MA, Kiko R, Lucassen M, Melzner F (2011) Elevated seawater PCO2 differentially affects branchial acid-base transporters over the course of development in the cephalopod *Sepia officinalis*. Am J Physiol Regul Integr Comp Physiol 301:R1700–R1709

Kamler E (1992) Early life history of fish: an energetic approach. Chapman and Hall, London

Katersky RS, Carter CG (2007) High growth efficiency occurs over a wide temperature range for juvenile barramundi *Lates calcarifer* fed a balanced diet. Aquaculture 272:444–450

Kurihara H (2008) Effects of $CO_2$-driven ocean acidification on the early developmental stages of invertebrates. Mar Ecol Prog Ser 373:275–284

Lemaire J (1971) Etude du développenaent embryonnaire de *Sepia officinalis* L., Thèse Doctorat 3 ème cycle. Université de Lille, France, p 70

Mangold-Wirz K (1963) Biologie des céphalopods benthiguis et nectonigues de la mer catalane. Vie Millieu 13(Suppl):285

Marvel MR, Fisher KC (1948) Developmental changes in the viability of squid embryos after subjection to cyanide. Biol Bull 94:45–54

McMahon JJ, Summers WC (1971) Temperature effects on the development rate of squid (*Loligo pealei*) embryos. Biol Bull 141:561–567

Moran AL, Woods HA (2007) Oxygen in egg masses: interactive effects of temperature, age, and egg-mass morphology on oxygen supply to embryos. J Exp Biol 210:722–731

Moreno A, Pereira J, Cunha M (2005) Environmental influences on age and size at maturity of Loligo vulgaris. Aquat Living Resour 18:377–384

Moreno A, dos Santos A, Piatkowski U, Santos AMP, Cabral H (2009) Distribution of cephalopod paralarvae in relation to the regional oceanography of the western Iberia. J Plankton Res 31:73–91

Naef A (1928) Die cephalopoden. Fauna et Flora di Golfo del Napoli Monographia 35(2):186–194

Nixon M, Mangold K (1998) The early life of *Sepia officinalis*, and the contract with that of *Octopus vulgaris* (Cephalopoda). J Zool Lond 245:407–421

O'Dor RK, Webber DM (1986) The constraints on cephalopods: why squid aren't fish. Can J Zool 64:1591–1605

O'Dor RK, Webber DM (1991) Invertebrate athletes: trade—offs between transport efficiency and power density in cephalopod evolution. J Exp Biol 160:93–112

O'Dor RK, Wells MJ (1987) Energy and nutrient flow. In: Boyle PR (ed) Cephalopod life cycles, comparative reviews, vol 2. Academic Press, London, pp 109–133

Pechenick JA (1987) Environmental influences on larval survival and development. In: Giese AC et al (eds) Reproduction of marine invertebrates, vol IX. Blackwell Scientific Publications, New York, pp 551–608

Pechenick JA, Eyster LS, Widdows J, Bayne BL (1990) The influence of food concentration and temperature on growth and morphological differentiation of blue mussel *Mytilus edulis* L. larvae. J Exp Mar Biol Ecol 136:47–64

Pecl GT, Jackson GD (2008) The potential impacts of climate change on inshore squid: biology, ecology and fisheries. Rev Fish Biol Fish 18:373–385

Pörtner HO (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88:137–146

Pörtner HO (2002) Environmental and functional limits to muscular exercise and body size in marine invertebrates athletes. Comp Biochem Physiol A 133:303–321

Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315:95–97

Pörtner HO, Mark FC, Bock C (2004) Oxygen limited thermal tolerance in fish? Answers obtained by nuclear magnetic resonance techniques. Resp Physiol Neurobiol 141:243–260

Pörtner HO, Langenbuch M, Michaelidis B (2005) Synergistic effects of increased $CO_2$, temperature and hypoxia on marine animals. J Geophys Res 110:C09S10

Relvas P, Barton ED, Dubert J, Oliveira PB, Peliz A, da Silva JCB, Santos AMP (2007) Physical oceanography of the western Iberia ecosystem: latest views and challenges. Prog Oceanogr 74:149–173

Rosa R, Seibel BA (2008) Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. Proc Natl Acad Sci USA 105:20776–20780

Rosa R, Seibel BA (2010) Metabolic physiology of the Humboldt squid, *Dosidicus gigas*: implications for vertical migration in a pronounced oxygen minimum zone. Progr Oceanogr 86:72–80

Rosa R, Dierssen HM, Gonzalez L, Seibel BA (2008) Large-scale diversity patterns of cephalopods in the Atlantic open ocean and deep-sea. Ecology 89:3449–3461

Rosa R, Trueblood L, Seibel BA (2009) Ecophysiological influence on scaling of aerobic and anaerobic metabolism of pelagic Gonatid squids. Physiol Biochem Zool 82:19–429

Rosa R, Pimentel MS, Boavida-Portugal J, Teixeira T, Trübenbach K, et al. (2012) Ocean warming enhances malformations, premature hatching, metabolic suppression and oxidative stress in the early life stages of a keystone squid. PLoS ONE 7(6): e38282. doi: 10.1371/journal.pone.0038282

Santos FD, Forbes K, Moita R (2002) Climate change in Portugal scenarios. Impacts and adaptation measures-siam project. Gradiva, Lisbon

Sasaki GC, Capuzzo JM, Biesiot P (1986) Nutritional and bioenergetic consideration in development of the American lobster *Homarus americanus*. Can J Fish Aquat Sci 43:2311–2319

Seibel BA, Dymowska A, Rosenthal J (2007) Metabolic temperature compensation and coevolution of locomotory performance in pteropod molluscs. Integr Comp Biol 47:880–891

Steer MA, Moltschaniwskyj NA, Jordan AR (2003) Embryonic development of southern calamari (*Sepioteuthis australis*) within the constraints of an aggregated egg mass. Mar Freshw Res 54:217–226

Thorp JH, Covich AP (2001) Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, CA, pp 315–399

Tian Y (2009) Interannual–interdecadal variations of spear squid Loligo bleekeri abundance in the southwestern Japan Sea during 1975–2006: impact of the trawl fishing and recommendations for management under the different climate regimes. Fish Res 100:78–85

Wang T, Overgaard J (2007) The heartbreak of adapting to global warming. Science 315:49–50

Ward P, Boletzky SV (1984) Shell implosion depth and implosion morphologies in three species of Sepia (Cephalopoda) from the Mediterranean Sea. J Mar Biol Assoc UK 64:955–966

Webber DM, Aitken JP, O'Dor RK (2000) Costs of locomotion and vertic dynamics of cephalopods and fish. Physiol Biochem Zool 73:651–662

Wells J, Hanlon RT, Lee PG, Dimarco FP (1988) Respiratory and cardiac performance in *Lolliguncula brevis* (Cephalopoda, Myopsida): the effects of activity, temperature and hypoxia. J Exp Biol 138:17–36

Wolf G, Verheyen E, Vlaeminck A, Lemaire J, Decleir W (1985) Respiration of *Sepia officinalis* during embryonic and early juvenile life. Mar Biol 90:35–39

Woods HA (1999) Egg-mass size and cell size: effects of temperature on oxygen distribution. Am Zool 39:244–252

Woods HA, Hill RI (2004) Temperature-dependent oxygen limitation in insect eggs. J Exp Biol 207:2267–2276