RESEARCH NOTE

Transport of cuttlefish, Sepia officinalis, eggs under dry and damp conditions

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The cuttlefish Sepia officinalis Linnaeus, 1758 is used extensively in biomedical and environmental research (von Boletzky & Hanlon, 1983) and is the ‘working model’ in cephalopod research (Koueta et al., 2006). Cuttlefish caught by fishing gears or damaged during trawling suffer trauma, making them difficult to maintain in aquaria. To avoid reliance on wild-caught animals, some laboratories now routinely rear cuttlefish from field-collected eggs, ensuring a regular supply for experimental purposes throughout the year. Cephalopods and their eggs require careful transport to the laboratory to ensure optimum survival (Hanlon, 1990). Transport of cuttlefish can be difficult as they can release ink resulting in asphyxiation and usually only 20 cuttlefish (30–40 mm dorsal mantle length, DML) can be transported in 6 l of seawater (Hanlon, 1990), although movement of egg masses is simpler. This study investigates how different transportation conditions affect the survival of S. officinalis embryos and post-hatching juvenile growth.

Four-day-old (Stage I; see von Boletzky et al., 2006) egg masses (singly encapsulated eggs laid in dense clusters) of S. officinalis were collected using recently cleaned cuttlefish traps deployed at a depth of 8 m in Bracklesham Bay (UK). Egg masses were immersed in seawater (40 l), covered with Fucus serratus and transported 35 km by boat over 3.5 h to Portsmouth Harbour and held overnight for 15 h in a polypropylene mesh sac (50 × 80 × 50 cm, 5 mm diameter mesh) suspended at a depth of c. 60 cm from a pontoon. Egg masses were removed after 12 h, separated by hand and mixed to randomize the effects of position in the egg mass, spawning sequence, local environmental conditions and the effect of different parentage (see von Boletzky, 1983; Steer et al., 2002; Steer & Moltschaniwskyj, 2007). Egg capsules were allocated to four different treatments with five replicates per treatment. The treatments were: (1) wet: eggs immersed in 1.5 l of harbour sea water; (2) wet and aerated: eggs immersed in 1.5 l of harbour sea water and aerated; (3) damp: eggs wrapped in paper towel dampened with 90 ml of harbour sea water; (4) dry: eggs blotted dry with paper towel and emersed in air. Each replicate consisted of a polythene bag (30 × 40 × 30 cm) containing 50 eggs (250 eggs per treatment) in each polyurethane-insulated box (Campingaz, dimension 20 × 27 × 18 cm). The packing density ensured the eggs would not clump together, reducing any effect of egg position.

Four Tinytag TGI 3080 temperature loggers (Gemini Data Logger, UK) placed in one replicate from each treatment, monitored seawater or air temperature. Data on the pH, salinity and dissolved oxygen content of the seawater and relative humidity of the air inside the boxes were obtained every 2 h from a subsample of replicates and compared with the data from Portsmouth Harbour. No ammonia, nitrite or nitrate was detected using standard laboratory kits.

Boxes with the eggs and identical control boxes without eggs were arranged in a single layer in the back of a car in a 5 × 5 arrangement (Latin square design), controlling for any effect of box position within the car. Boxes were covered with a Gelert reflective blanket to reduce the effect of warming by sunlight. Sorting and packaging of the eggs into their transport containers took 2 h, during which time the eggs were stored in seawater. The eggs were transported 15 h after collection from Bracklesham Bay and were kept under experimental conditions for about 12.5 h following sorting, i.e. sorting and packing into the cool boxes (2 h), 8.5 h in transit by car from Portsmouth to Menai Bridge (502 km) and a final 2 h while the eggs were unpacked, sorted and placed into aquaria in Menai Bridge. Egg masses were therefore exposed to experimental conditions for between 8.5 and 12.5 h.

Following transport, eggs from each replicate were transferred to separate plastic mesh baskets (22 × 15 × 18 cm, 30 eggs per basket). One replicate basket with eggs from each treatment was placed in one of the five 150-l rearing tanks (A–E), containing seawater maintained at 17°C (±1°C) under subdued constant illumination. The position of the baskets within each tank was changed daily to randomize local effects. The hatchlings in each basket were recorded daily, photographed in seawater and the DML was measured (to the nearest 0.01 mm using Vernier callipers) before transfer to small (1.8 l) tanks.

Cuttlefish hatched over 40 days and the hatchlings were reared in small aquaria submerged in the main rearing tanks and fed ad libitum on mysid shrimp, Neomysis integer, for 14 days. Fourteen days post-hatching was arbitrarily considered a minimum time to investigate whether development and hatching growth had been affected by transport. At the end of the rearing trial, the DML and wet body weight (Ww) (recorded to the nearest 0.01 g) of each cuttlefish from the four different replicates in a representative tank (tank C) were measured. Significant differences in DML and Ww of hatchlings from the different treatments in tank C at birth, and 14 days after last hatching were assessed using one-way ANOVA to investigate the potential differences in hatching success, hatching frequency and hatching survival among treatments. Student t-test, Mann–Whitney U-test and Mood’s Median tests were used to check if environmental conditions differed among the treatments.

Seawater temperature differed significantly in the wet and wet-and-aerated boxes (W101, 101 = 11231.5, P = 0.033), but air temperature between the damp and dry treatments did not (χ² = 0.49, P = 0.486) (Tables 1 and 2). The median air temperatures were significantly higher in the damp and dry treatments than in seawater (χ² = 122.38, P < 0.05). No physically damaged egg capsules were recorded, although those transported either damp or dry looked ‘pinched’ (Fig. 1A) compared with those transported in seawater (Fig. 1B). Egg capsules returned to their characteristic ‘grape-like’ shape after 24 h in seawater.

Hatchlings appeared 28 days following transport and continued to appear until Day 68; most eggs hatched between 40 and 60 days following transport. Thus some early hatchlings...
were 40 days old before the last hatchling appeared. Fifteen per cent of eggs did not hatch and eventually ruptured. There was no significant difference in the frequency of hatching (\(F = 0.01, P = 0.999\)) among treatments (Fig. 2) and no significant difference in percentage of hatchling survival among replicates within treatments (\(F = 1.09, P = 0.382\)).

Developing cuttlefish embryos can be transported for 8.5 h, damp, dry, in seawater with or without aeration, without significant deleterious effects on hatching success, growth or post-hatching survival. Tolerance of transport stress may be conferred by the layer of ink surrounding the egg combined with stable environmental conditions in which the eggs were transported. Parra, Villanueva, & Yúfera (2000) found that specific oxygen consumption rates in late stage embryos of Octopus vulgaris were significantly lower than in hatchlings. A similar reduction in oxygen consumption in S. officinalis would protect them in damp and dry conditions. The effects of transportation on cephalopod eggs or hatching success following transportation are largely unstudied, with only basic descriptions of the methods used to transport eggs of the oval squid, Sepioteuthis lessoniana, being available (e.g. Lee et al., 1994; Forsythe et al., 2001; Walsh et al., 2002).

Egg viability within each treatment ranged between 82.8 and 86.4% for eggs transported wet and dry; hatching success did not differ among the treatments. The wet and wet with aeration treatments had the lowest hatching success, whereas eggs transported damp and dry had the highest hatch rate, despite distortion of the eggs, apparently due to desiccation and their greater susceptibility to temperature fluctuations. In previous studies, hatching success in S. lessoniana ranged from 37 to 49% (Lee et al., 1994), 33 to 93% (Forsythe et al., 2001) and 34.9% (Walsh et al., 2002). Only 1.2% of the eggs of the cuttlefish Sepia pharaonis transported from Thailand to the USA at a density of 9.5–10.5 eggs \(l^{-1}\) hatched (Minton et al., 2002). Egg capsules of this species lack the encapsulating ink layer present in egg capsules of S. officinalis (Norman, 2000). In the current study, despite the eggs being the same age, hatching took place over 40 days at about 17°C, considerably longer than the 14 and 20 days reported by Richard (1975) for S. officinalis eggs reared at 20 and 15°C, respectively. The subdued continuous illumination probably extended the hatching period since the time to hatching increases and is asynchronous in S. officinalis eggs reared under constant illumination (Paulij et al., 1991).

Egg viability, hatching success and subsequent hatchling growth following transport affect the selection of an appropriate

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**Table 1.** Summary of environmental factors recorded during the transport of Sepia officinalis eggs from Portsmouth to Menai Bridge.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean seawater/air temperature (°C) ± SD</th>
<th>Air temperature range (°C)</th>
<th>Mean pH ± SD</th>
<th>Mean dissolved oxygen (% saturation) ± SD</th>
<th>Mean salinity ± SD/relative humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harbour water</td>
<td>16.8/n/a</td>
<td>n/a</td>
<td>8.54</td>
<td>69.8</td>
<td>35/n/a</td>
</tr>
<tr>
<td>Wet</td>
<td>18.81 ± 0.05/n/a</td>
<td>17.45–19.65</td>
<td>8.39 ± 0.05</td>
<td>73.06 ± 11.41*</td>
<td>33.40 ± 0.54/n/a</td>
</tr>
<tr>
<td>Wet and aerated</td>
<td>18.87 ± 0.61/n/a</td>
<td>17.31–17.91</td>
<td>8.38 ± 0.06</td>
<td>89.57 ± 4.68*</td>
<td>33.80 ± 0.44/n/a</td>
</tr>
<tr>
<td>Damp</td>
<td>n/a/19.80 ± 1.02</td>
<td>17.25–21.13</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a/63.2</td>
</tr>
<tr>
<td>Dry</td>
<td>n/a/19.80 ± 1.34</td>
<td>16.98–22.05</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a/61.0</td>
</tr>
</tbody>
</table>

\*Significant difference between variables (\(P < 0.05\)).

n/a, not applicable.

**Table 2.** Mean number of Sepia officinalis hatchlings arising from five replicate batches of eggs transported using four different treatments from Portsmouth to Menai Bridge and reared at 17°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean number of hatchlings ± SD/minimum and (maximum) (%)</th>
<th>Mean hatching DML ± SD</th>
<th>Mean hatching survival ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet and aerated</td>
<td>42.2 ± 4.2/74 (94)</td>
<td>9.2 ± 1.0</td>
<td>94.6 ± 3.59</td>
</tr>
<tr>
<td>Wet</td>
<td>42.0 ± 5.7/68 (94)</td>
<td>9.3 ± 0.9</td>
<td>88.7 ± 15.69</td>
</tr>
<tr>
<td>Damp</td>
<td>42.8 ± 5.4/68 (96)</td>
<td>9.2 ± 0.9</td>
<td>95.9 ± 4.15</td>
</tr>
<tr>
<td>Dry</td>
<td>43.2 ± 1.9/82 (92)</td>
<td>8.6 ± 1.2</td>
<td>97.8 ± 2.86</td>
</tr>
</tbody>
</table>

**Figure 1.** Eggs of Sepia officinalis following 8.5 h transport. A. Dry: eggs blotted dry with paper towel and emersed in air. B. Wet and aerated: eggs immersed in 2 l of harbour seawater. The fixating rings (arrow) were removed from some of the eggs during handling. Abbreviation: D, egg diameter.
transport method. Financial, logistic and bio-security (the reduction in threats from potential invasive species) considerations may also determine the eventual transport method selected. Reducing the volume of transport water reduces shipping weight and therefore transport costs (Bower et al., 1999) and damp and dry treatments will lower freight costs and reduce the risk of introducing invasive species. Methods described here to transport the eggs of *S. officinalis* will enable cuttlefish to be moved with minimal fatalities to research laboratories and aquaria worldwide.

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**REFERENCES**


**Figure 2.** Cumulative percentage of hatched *Sepia officinalis* eggs over 40 days at 17°C.