DOCTOR OF PHILOSOPHY

Aspects of the biology of the little cuttlefish, Sepiola atlantica and the common European cuttlefish, Sepia officinalis (Mollusca: Cephalopoda)

Jones, Nicholas James Edward

Award date:
2009

Awarding institution:
Bangor University

Aspects of the biology of the little cuttlefish, *Sepiola atlantica* and the common European cuttlefish, *Sepia officinalis* (Mollusca: Cephalopoda)

by

Nicholas James Edward Jones

A thesis presented in partial fulfilment of the requirements of Bangor University for the degree of Doctor in Philosophy

Bangor University

School of Ocean Sciences

Menai Bridge

December 2009

CONTENTS

		Page
	Summary	
	Acknowledgements	
	Declaration	
	List of Figures	
	List of Tables	
CHAPTER 1	**General Introduction**	1
	References	14
CHAPTER 2	**Distribution, ecology and reproductive biology of the little cuttlefish *Sepiola atlantica* from around Anglesey**	18
	Introduction	20
	Methods	24
	Sampling	24
	Description of sampling sites	26
	Morphometric measurements and the assessment of reproductive condition	32
	Statistical analyses	35
	Results	37
	Distribution of *Sepiola atlantica* around the coast of Anglesey	37
	Seasonal distribution of *S. atlantica* at Traeth Penrhos and Y Foryd	40
	Associated fauna	42
	Population structure	45
	Maturity	48
	Morphometry of *Sepiola atlantica*	61
	Discussion	65
	References	82
CHAPTER 3	**Laboratory culture of the little cuttlefish, *Sepiola atlantica* (Cephalopoda; Sepiolidae)**	88
	Introduction	89
	Methods	93
	Brood stock collection	93
	Aquarium husbandry	94
	Mating behaviour	95
	Egg, paralarvae and juvenile husbandry	96

	Diet	99
	Survival and growth	100
	Results	102
	Water quality during larval rearing	102
	Mating behaviour	102
	Spawning and hatching success	104
	Embryonic development	108
	Hatching and feeding behaviour	111
	Survival	113
	Growth	115
	Discussion	118
	References	132
CHAPTER 4	**Hatching success, survival and juvenile growth of cuttlefish *Sepia officinalis* following egg transport**	137
	Introduction	138
	Methods	143
	Egg collection	143
	Egg transport	143
	Egg development & juvenile rearing	146
	Results	149
	Egg Collection and Transport	149
	Egg Development	152
	Discussion	156
	References	161
CHAPTER 5	**General Discussion**	164
	References	170

SUMMARY

Aspects of the biology of the little cuttlefish *Sepiola atlantica* and the common European cuttlefish, *Sepia officinalis* were examined in order to further the understanding of the culture, ecology, husbandry and reproductive biology of both species. The distribution of *Sepiola atlantica* around Anglesey, North Wales during the summer months (April to October) was assessed. These data together with a detailed investigation of the reproductive condition and the population biology of *Sepiola atlantica* in the shallow subtidal from two sites with contrasting wave exposure were undertaken over consecutive years (2006, 2007 and 2008). *Sepiola atlantica* of a wide size range and of all maturity stages were found to make an annual seasonal inshore migration in July into shallow coastal waters. The inshore migration began when seawater temperatures started increasing in the spring with numbers of migrants peaking in July and August and then falling between September and October as seawater temperatures declined in the autumn. These data indicate that *S. atlantica* of all sizes and maturity stages congregate in the shallow waters around Anglesey to mate when environmental conditions are favourable for enhanced growth and maturation and where the concentrations of large numbers of individuals increases the opportunity and the chance of a successful mating.

Experiments were undertaken to culture *Sepiola atlantica* from eggs held in the laboratory. Wild-caught *S. atlantica* transferred to laboratory aquaria and held under controlled conditions of seawater flow, food supply and lighting laid egg masses in June, August and September. These eggs were observed throughout development and the resultant hatchling paralarvae reared on a diet of zooplankton collected from the Menai Strait. The small numbers of juveniles that developed into

adulthood were fed on shrimp, *Crangon crangon*, however none of these individuals went on to spawn in the laboratory. The life cycle of *S. atlantica* is similar to those of other frequently cultured sepioids, although there is a need for further experimentation to establish that *S. atlantica* can be reared through consecutive generations in the laboratory.

The survival of eggs and the hatching frequency, hatching success and subsequent growth of hatchlings from samples of *Sepia officinalis* eggs transported under a range of environmental conditions have been investigated. Eggs were transported for 8.5h under the following conditions: 1) Wet: in sea water, 2) Wet & aerated: eggs in sea water with continuous aeration, 3) Damp: eggs wrapped loosely in damp paper towel and 4) Dry: eggs gently blotted dry in air. Hatchlings from eggs transported "Dry" were significantly smaller in length than those transported using other treatments. The finding that there were no significant differences in hatching success, hatching frequency, growth and survival between treatments, indicates that it is feasible to transport cuttlefish eggs for periods of ~8hours using simple and cost-effective methodologies.

ACKNOWLEDGEMENTS

First and Foremost, I would like to express my sincere thanks to my supervisor, Prof. Chris Richardson for his support, advice and encouragement during my time at Bangor University. I would also like to thank the European Social Fund and the Anglesey Sea Zoo for funding this study.

A very special thank you to Berwyn Roberts for his technical support in the field, advice on how and where to catch *Sepiola atlantica* and for all the enthusiasm he has shown for this project. Also, a big thank you to Gwyn Hughes and Gwynne Parry-Jones for their technical support. Thanks also to Dr Panos Grigoriou, Dr Shelagh Malham and Dr Roland Anderson for their advice on maintaining cephalopods in captivity.

I would like to thank Dr Cara Hughes, Dr James Bussell and Eilir Morgan for their advice on the statistical procedures and analysis.

Many thanks to Dave Baldachino for supplying me with thousands of *Sepia officinalis* eggs as well as Bethany Lloyd and Dr Iain Ridgway for assisting me with the egg transport study.

Thanks to all those people who accompanied me on the numerous seine netting excursions around Anglesey; Berwyn Roberts, Dan Ward, Andy Matkin, Stefan Kolle, Bethany Lloyd, Sara Richardson, Jess Orr, Gareth Johnson, Gwynne Parry-Jones, Vickie Hickin, Joe Kenworthy, Gareth Buckley, Rachel Kingham, Joe Chapman, Patrick Thomas and especially Andy Marriott, who was always willing to give a helping hand, even on rainy Friday nights.

A special thank you to my wife, Gwen, for her love and support, for accompanying me to the labs every weekend and for missing out on a summer holiday for three years. Last and certainly not least, a very special thank you to my parents for all the support they have given me throughout my life. I could not have done this without you.

LIST OF FIGURES

Chapter 1		**Page**
Fig. 1	The little cuttlefish, *Sepiola atlantica* Orbigny, (1839-1842)	10
Fig. 2	The hectocotylus of a mature, male *Sepiola atlantica*	10
Fig. 3	Map showing the known distribution of *Sepiola atlantica*	11
Chapter 2		
Fig. 1	Map showing sites visited around the coast of the Isle of Anglesey, along with a map showing the location of the Isle of Anglesey within the UK	26
Fig. 2	Aerial photograph showing the study sites at a) Y Foryd and b) Traeth Penrhos	31
Fig. 3	Morphometric measurements undertaken on *Sepiola atlantica* including: DML, dorsal mantle length and MW, maximum mantle width	32
Fig. 4	The internal anatomy of; a) a mature (Stage IV), mated female and b) a mature (Stage IV), male *Sepiola atlantica*	33
Fig. 5	The seasonal distribution of *S. atlantica* collected from Traeth Penrhos and Y Foryd bay, Anglesey between June 2006 and September 2008	41
Fig. 6	Length-frequency distributions of juvenile, male and female *Sepiola atlantica* collected between 2006 & 2008 from a) Y Foryd and b) Traeth Penrhos	46
Fig. 7	Combined data from seine surveys conducted between July and October 2006 & 2007 to compare the monthly length-frequency distributions of *Sepiola atlantica* from Y Foryd and Traeth Penrhos	47
Fig. 8	The relationship between dorsal mantle length (DML) and maturity stage for *Sepiola atlantica* collected from a) Y Foryd and b) Traeth Penrhos	50
Fig. 9a	Cumulative percentage of male and female *Sepiola atlantica* at full maturity (Stage IV) from Y Foryd	51
Fig. 9b	Cumulative percentage of male and female *Sepiola atlantica* at full maturity (Stage IV) from Traeth Penrhos	52

Fig. 10	The relationship between wet weight (Ww), spermatophoric complex index (SCI) and gonadosomatic index (GSI) for male and female *Sepiola atlantica* collected from a) Y Foryd and b) Traeth Penrhos between 2006 & 2008	56
Fig. 11a	The relationship between wet weight and number of spermatophores for male *Sepiola atlantica* collected from Y Foryd and Traeth Penrhos between 2006 and 2008	58
Fig. 11b	The relationship between wet weight and number of advanced ova for female *Sepiola atlantica* collected from Y Foryd and Traeth Penrhos between 2006 and 2008	59
Fig. 12	The relationship between Wet weight (Ww) and dorsal mantle length (DML) of juvenile, male and female *Sepiola atlantica* collected from a) Y Foryd and b) Traeth Penrhos between 2006 and 2008	64

Chapter 3

Fig. 1	PVC crates used to house adult *S. atlantica*	95
Fig. 2	Mesh-based PVC cylinder used to maintain the eggs of *S. atlantica* following removal of the chorion	98
Fig. 3	PVC bowls used to rear *S. atlantica* through the paralarval phase to sexual maturity	98
Fig. 4	*Sepiola atlantica* in a mating embrace known as the male parallel position. The male is positioned below the female	103
Fig. 5	a) *Sepiola atlantica* eggs laid in the corner of a tank at the air-water interface b) the aerated eggs of *S. atlantica*	106
Fig. 6	The number of *S. atlantica* hatchlings recorded over a 23 day period at 14°C	108
Fig. 7	Developmental stages of laboratory reared *S. atlantica* at 14°C. A) day 9 B) day 14 C) day 17 D) day 20 E) day 29 F) day 32 G) day 34; H) hatchling	109
Fig. 8	Premature *S. atlantica* hatchling with external yolk sac still attached	110
Fig. 9	A) *S. atlantica* hatchling B) *S. atlantica* emerging from an egg distal end first C) *S. atlantica* within the egg prior to hatching	112
Fig. 10	Juvenile *S. atlantica* consuming its *Artemia* prey	112
Fig. 11	Juvenile *S. atlantica* consuming *Crangon crangon* prey	114

Fig. 12 Comparison of the survival of *Sepiola atlantica* paralarvae and juveniles reared on a diet of *Artemia* nauplii, adult *Artemia* or a zooplankton mix over a 50 day period at ambient seawater temperatures in 2006 and 2007 115

Fig. 13 A comparison of the cumulative increase in Dorsal Mantle Length (DML) of a mixed sex population (n = 5) of laboratory reared *Sepiola atlantica* and ambient seawater temperature 116

Fig. 14 Increase in dorsal mantle length (DML) of male and female *Sepiola atlantica* reared individually in the laboratory a) at 12°C to the onset of sexual maturity and b) at 15°C from the onset of sexual maturity to death 117

Chapter 4

Fig. 1 The experimental setup used to transport cuttlefish eggs from Portsmouth to Menai Bridge 145

Fig. 2 Mesh baskets used to house cuttlefish eggs following transport 147

Fig. 3 Aquarium system used to rear cuttlefish hatchlings 147

Fig. 4 Eggs of *Sepia officinalis* following 8.5h transport. A) Dry: eggs blotted dry with paper towel and emersed in air. B) Wet & aerated: eggs immersed in 2L of harbour sea water. D indicates the axis of measurement of egg diameter 151

Fig. 5 The number of *Sepia officinalis* hatchlings recorded over a 68 day period following transport of a consignment of eggs from Portsmouth to Menai Bridge 153

Fig. 6 The cumulative percentage of hatched *Sepia officinalis* eggs recorded over a 40 day period at 17°C 154

LIST OF TABLES

Chapter 2		Page
Table 1.	Summary of site locations where *Sepiola atlantica* were collected and the level of shore exposure (adapted from Ballantine, 1961)	30
Table 2.	Maturity stage scale for *Sepiola atlantica* from Yau & Boyle (1996)	34
Table 3.	Summary of the numbers of *Sepiola atlantica* collected from various locations around the coast of Anglesey between 2006 & 2008	39
Table 4a.	By-catch species observed during seine net surveys at Y Foryd between the months of June to September during 2006, 2007 and 2008 beach seine surveys	43
Table 4b.	By-catch species observed during seine net surveys at Traeth Penrhos between the months of June to September during 2006, 2007 and 2008 beach seine surveys	44
Table 5.	Mean dorsal mantle length (DML) and Wet weight (Ww) at each maturity stage for juvenile, male and female *Sepiola atlantica* collected from Y Foryd and Traeth Penrhos	49
Table 6.	Temporal variation in the number of *Sepiola atlantica* from the different locations and different stages of gonad development	54
Table 7.	Monthly percentage occurrence of mated sexually mature female *Sepiola atlantica* collected during beach seine surveys from July to October, 2006, 2007 and 2008 along with the mean number of spermatangia observed on the *bursa copulatrix* of each animal and sample number	55
Table 8.	A comparison of the spermatophoric complex indexes (SCI) and gonado somato indexes (GSI) of mature male and female *Sepiola atlantica* collected from Y Foryd and Traeth Penrhos between 2006 and 2008	57
Table 9.	The relationship between Ww and Number of spermatophores or advanced ova found in *Sepiola atlantica* collected from Y Foryd and Traeth Penrhos between 2006 & 2008, with results of correlation analysis between Ww (g) and the number of either spermatophores or advanced ova	60

Table 10.	The analysis of the increase in Wet weight with Dorsal Mantle length in juvenile, male and female *Sepiola atlantica* collected from Y Foryd between 2006-2008	61
Table 11.	Coefficient of allometry for wet weight (Ww) versus dorsal mantle length (DML) in male and female *Sepiola atlantica* from Y Foryd and Traeth Penrhos	63
Table 12.	The reported fecundity of selected female and male Sepiolid species	76

Chapter 3

Table 1.	Brood female origin, size of egg mass, temperature during embryonic development, length of development, time to first hatching and death post spawning of five wild caught female *Sepiola atlantica*	107

Chapter 4

Table 1.	Summary of environmental factors recorded during the transport of *Sepia officinalis* eggs from Portsmouth to Menai Bridge	150
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	155
Table 3.	Dorsal mantle length and mean wet body weight of *S. officinalis* hatchlings at the time of hatching and after rearing at 17°C for 14 days	155

CHAPTER 1

General Introduction

The Cephalopoda is one of the largest classes of Mollusca, consisting of approximately 700 species (Hanlon & Messenger, 1996), some of which are the most attractive of all marine invertebrates (Nixon & Young, 2003), with representatives in all marine habitats worldwide except the Black Sea (Jereb & Roper, 2005). All living cephalopods belong to one of two subclasses, the Nautiloidea or the Coleoidea (Nixon & Young, 2003). The Nautiloidea contains two genera, *Nautilus* and *Allonautilus*, which are the only living cephalopods with an external shell, while the Coleoidea contains the major groups of squids, cuttlefishes, octopods and vampire squid (Jereb & Roper, 2005) whose shell is internal, may be reduced to a rudiment or may even have disappeared (Nixon & Young, 2003). The living coleoid cephalopods are generally semelparous, with a single, terminal breeding season and short life span (1-2 years), while the Nautiloidea are multi-annual breeders with longer life spans (up to 15 years) (Hanlon & Messenger, 1996; Boyle & Rodhouse, 2005). Cephalopods are highly developed organisms, with the ability to change colour almost instantaneously using hundreds or thousands of chromatophores, pigment-filled sacs in the skin controlled by the central nervous system (Nixon & Young, 2003) that are able to rapidly expand or contract (Jereb & Roper, 2005). They have a central brain and nervous system far larger than that of any other mollusc and close in size to that of some vertebrates (fish & reptiles). Cephalopods have the ability to learn rapidly and discriminate well, with sensory organs that are both elaborate and complex (Nixon & Young, 2003), while some representatives of the class have the largest single nerve axons in the animal kingdom (Jereb & Roper, 2005). These traits led to cephalopods

being important experimental animals in biomedical research, with direct applications to humans, being valuable to behavioural, comparative neuroanatomical and neurophysiological studies (Jereb & Roper, 2005).

While efforts have been made to improve the maintenance, rearing and culture of cephalopods for both scientific research and human consumption, cephalopod fisheries have so far met the demand for the consumption of cuttlefish, octopus and squid while most wild stocks are far from depleted. Therefore there has been insufficient market pressure towards the production of cephalopods for human consumption (Boletzky & Hanlon, 1983; Hanlon, 1987). According to the FAO (2009), the global capture production of squids, cuttlefishes and octopuses has increased from a modest 580,435 tonnes per annum in 1950 to 4,375,448 tonnes in 2007, of which 351,781 tonnes consisted of cuttlefish and bobtail squid. Cephalopods are primarily maintained in captivity for scientific research into various aspects of their biology, physiology and biochemistry and a supply of healthy, laboratory maintained animals is essential to meet these demands (Boyle, 1991). However, according to the FAO (2004), the rapid decline in global fish stocks together with the technological advances in recent years and decreased prices of commonly cultured species means that the development of technology for the rearing and culture of new species is not only profitable, but necessary. Sykes et al. (2006) have predicted an increase in the global consumption of cephalopods since aspects of cephalopod biology and physiology make them good candidates for aquaculture while they also have a high commercial value. According to Sykes et al. (2006) cuttlefish are a good source of protein, essential lipids, mineral salts and vitamins and can be prepared in a number of different ways such as raw sashimi or sushi, deep-fried tempura, boiled as nimono or processed and dried as surume or saki-ika (Kunisaki, 2000). However, the

combined global aquaculture production of squids, cuttlefishes and octopuses between 1967 and 2007 was a meagre 887 tonnes (FAO, 2009) therefore it appears that there is much work to be done.

Historically, cephalopods have been thought of as being difficult to maintain in captivity (Boletzky & Hanlon, 1983) and in general, would not appear to be good aquarium subjects as they tend to be shy, cryptic, and nocturnal animals (Anderson, 2001) that are also often large, mobile, predatory animals that require considerable aquarium space and resources. Interest in the correct maintenance of cephalopods is now increasing due to their biological importance as advanced invertebrates, having comparisons with vertebrates such as; a well developed nervous system (Young, 1971; Wells, 1962; Budelmann, 1995) a bilateral brain organisation allowing specialised regions of the brain to store either visually based or tactile learning (Wells, 1978), while also exhibiting advanced behaviours (Hanlon & Messenger, 1996). The presence of free nerve endings in the skin suggests that the perception of pain is possible in cephalopods (Moltschaniwskyj, 2007). According to Mather & Anderson (2007) the ability to learn about the consequences of actions found in *Octopus vulgaris* by Wells (1978) demonstrates that octopuses need consideration under both the utilitarian and the rights-based ethical approaches. At present, there is no universal legislation governing the welfare of cephalopods in the laboratory, with *Octopus vulgaris* being the only species included under Home Office regulations in the UK. However, the status of cephalopods in the European Union legislation for animal ethics is under review, the recommendation being that all cephalopods are included in the legislation (Moltschaniwskyj, 2007).

Cephalopods are also of ecological importance as food for organisms from higher trophic levels, as well as being predators themselves. According to Smale

(1996), few fish species feed almost exclusively on cephalopods with the exception of the tawny nurse shark *Nebrius ferrugineus* and the sicklefin weasel shark *Hemigaleus microstoma*, while some deep-sea spiny dogfish may occasionally feed extensively on cephalopods. In general, cephalopods are important components in the diet of large, predatory fishes such as sharks that inhabit the continental slope and rise, while also being important prey items for pelagic swordfish and tunas (Smale, 1996). Clarke (1996) states that in excess of 80% of odontocete cetacean species and two species of baleen whales regularly feed on cephalopods, with members of the Physeteridae, Ziphiidae, Phocaenidae and Delphinidae feeding predominantly on cephalopods, largely the oceanic Ommastrephidae, Histioteuthidae, Cranchiidae and the neritic Loliginidae. Of the approximately 700 recorded species of cephalopods, less than 60 species occur regularly in the diet of cetaceans. Of the 33 species of pinnipeds, 31 are known to, or thought to include cephalopods in their diet, although none feed exclusively on cephalopods. However, some pinnipeds consume significant numbers of cephalopods and are known to be important seasonal prey items (Klages, 1996). Cephalopods that are frequently consumed by seals are benthic octopods, members of the neritic Loliginidae, the oceanic Ommastrephidae, Onychoteuthidae and Gonatidae (Klages, 1996). According to Croxall and Prince (1996), cephalopods constitute approximately 5% of the diet of seabirds, although they are as important prey items as fish or crustaceans to some albatross and petrel species. Of those squid species consumed by seabirds, members of the Ommastrephidae, Onychoteuthidae, Histioteuthidae and Gonatidae are predominantly consumed. Nixon (1987) notes that cephalopods primarily feed on a variety of crustaceans, molluscs and fishes and to a lesser extent, echinoderms, polychaetes, chaetognaths and siphonophores, being able to incorporate between 25 and 70% of their food into body tissue. Much of the

information on cephalopod diets is derived from information from; 1) stomach contents, 2) direct observation in the sea, 3) laboratory observations and 4) middens left by some neritic octopods, although all of these methods have some inherent problems (Nixon, 1987).

The maintenance, well being and captive environment of all animals held for scientific purposes is under increased scrutiny (Boyle, 1991). This will ultimately lead to a better understanding of cephalopod husbandry, yet compared to the wealth of information on the husbandry of vertebrates, very little is known of the aquarium and laboratory care of cephalopods (Boyle, 1991). This is largely because cephalopods are often treated as wild animals, they are temporary residents in laboratory aquaria and as invertebrates, little attention has been given to their correct captive care as long as their survival has been adequate for the purpose for which they were captured (Boyle, 1991). Public interest in cephalopods is also high, hence many public aquaria find it profitable to display cephalopods, the giant Pacific octopus *Enteroctopus dofleini* being the most commonly displayed cephalopod world-wide, while in the UK, the native curled or lesser octopus *Eledone cirrhosa* is the most commonly displayed species (Dunlop & King, 2009). Other native species such as *Octopus vulgaris* (Dunlop & King, 2009), *Sepia officinalis* and *Sepiola atlantica* (pers. obs) are occasionally displayed in the UK as well as the tropical *Octopus cyanea* and *Nautilus pompilius*. There is also a market for smaller cephalopods for the home aquarium such as the Sepioids *S. officinalis*, *S. bandensis*, *Metasepia pfefferi* and *Euprymna scoplopes* (Dunlop & King, 2009) although at present there is no data for the number of individuals entering the trade (pers. obs). Previously, their short lifespan, difficulties in obtaining live food, as well as preventing their escape has restricted their care to only the most dedicated aquarists (Boyle, 1991).

Members of the Cephalopoda are gonochoristic and unlike some molluscs there is no evidence of hermaphrodites or sex reversal in the class (Mangold, 1987; Hanlon & Messenger, 1996). External sexual dimorphism generally consists of the presence of a hectocotylus in males, a specialised arm used to transfer spermatophores stored in the Needham's sac, from the penis to the females (Hanlon & Messenger, 1996). Other variations may exist in size, body patterns, sucker size, shape of the gonad, colour and in some genera, the photophores (Mangold, 1987; Hanlon & Messenger, 1996). Among the sepioids, males have enlarged suckers on some of the arms while in some species the gonads are easily distinguished through the integument (Boyle, 1983). Sepioids lay single eggs embedded in oviducal jelly, fertilised at or before the point of spawning (Boyle & Rodhouse, 2005) that are spirally wrapped into a gelatinous band produced by the nidamental glands and in the sepiolids spawning is often intermittent (Mangold, 1987; Rocha et al. 2001). Transferred spermatophores are stored until spawning, which usually takes place shortly after mating. Although all male cephalopods transfer spermatophores to the females in this manner, significant differences exist in the courtship behaviour, site of sperm storage and spawning (Mangold, 1987). In shallow water species visual stimuli play an important role in courtship which generally involves specific body patterns and postures. However, no courtship behaviour has been observed in either captive or wild sepiolids, where mating is brief and violent, the male grasping the female by the head, holding her in position with his lateral arms while he places spermatophores on the *bursa copulatrix*, a large pouch lying on the visceral mass, where the spermatophores are stored as spermatangia (Mangold, 1987).

The Sepiolidae comprises three subfamilies of 'bobtail squids'; the nectobenthic Rossiinae and Sepiolinae and the pelagic Heteroteuthinae (Jereb &

Roper, 2005). While not true squids of the order Teuthoidea, the term 'squid' is commonly applied to some sepioids (Hanlon et al. 1997). Members of the Sepiolidae have a global distribution in most tropical, temperate and some Arctic waters (Norman, 2000). Of the seventy-six species of Sepiolidae, seven (*Sepiola atlantica*, *S. rondeleti*, *Sepietta neglecta*, *S. oweniana*, *Rossia macrosoma*, *R. palpebrosa* & *Neorossia caroli*) have been recorded in British waters (Jereb & Roper, 2005). Sepiolids are distributed over the continental shelf, from the intertidal zone down to depths of 1600m (Nixon & Young, 2003) where they spend much of their time buried in mud or sand, but they have also been recorded some distance off the sea floor (Shears, 1988; Nixon & Young, 2003). The wide bathymetric distribution of the Sepiolids is thought to have arisen because many populations are unable to cope with inshore catastrophic events experienced during storms when conditions in soft substrata close to shore are disturbed (Boletzky, 1983).

The Sepiolidae are characteristically small animals, ranging from 10 to 100mm in dorsal mantle length (DML), with short, rounded bodies, kidney shaped fins located laterally approximately mid way along the mantle, eight short arms, two retractable, clubbed feeding tentacles and large eyes covered by a transparent membrane (Boletzky, 1983; Boyle & Rodhouse, 2005; Jereb & Roper, 2005). The pen may be absent or reduced to a rudiment, while the funnel-mantle locking cartilage is simple and straight (Yau & Boyle, 1996). Six genera of the Sepiolidae (*Sepiola*, *Euprymna*, *Inioteuthis*, *Rondeletiola*, *Semirossia* and *Heteroteuthis*) have members which possess a photophore, or light organ, containing bioluminescent bacterial symbionts (*Vibrio fischeri*), while these photophores are lacking in *Sepietta*, *Rossia* and *Neorossia* (Herring et al. 1981; Montgomery & McFall-Ngai, 1998; Nyholm & McFall-Ngai, 1998; Foster et al. 2002; Jones & Nishiguchi, 2004). The bi-lobed light

organ is found in the centre of the mantle cavity where it is continuously bathed with seawater as a result of ventilatory activity (Ruby, 1996). The photophore is large and may extend up to 30% of the dorsal mantle length of the host and is partially embedded in the ventral surface of the ink sac (Herring et al. 1981), consisting of a complex set of tissues that support the culture of bioluminescent bacteria (Ruby & McFall-Ngai, 1992).

Several marine organisms are known to bioluminesce for intra-specific communication, prey attraction, predator evasion, anti-predation and counter illumination (Jones & Nishiguchi, 2004). Bioluminescence is used by sepiolids for counter illumination in moonlight in order to match the intensity and wavelength of down-welling light (Herring et al. 1981; Jones & Nishiguchi, 2004), using camouflage to avoid predation (Jones & Nishiguchi, 2004). During periods of bioluminescence a blue-green light is emitted ventrally, the intensity of which may vary among individuals (Herring et al. 1981), with the concentration of bacteria within the light organ depending on changes in abiotic factors such as seawater temperature and salinity (Nishiguchi, 2000; Soto et al. 2009). Interest in the mutualistic, symbiotic relationship between bobtail squid and luminescent bacteria, (largely the Hawaiian bobtail squid, *Euprymna scolopes* and *Vibrio fischeri*) has increased over recent years with much of the current research on the Sepiolidae undertaken in this field (Boettcher & Ruby, 1990; Ruby, 1996; McFall-Ngai & Ruby, 1998; Ruby & Lee, 1998; Claes & Dunlap, 2000; Nishiguchi, 2000; Nyholm et al. 2000; Foster et al. 2002; Nishiguchi et al. 2004; Mandel et al. 2009; Adin et al. 2009; Wollenberg & Ruby, 2009), with the relationship used as a model for the study of the physiological and molecular signalling between the host organism and its bacterial symbiont (Soto et al. 2009). A laboratory culture technique for *E. scolopes* was developed by Hanlon

et al. (1997) who required aposymbiotic hatchlings (i.e. hatchlings deprived of the bacterium) through much of their lives in order to study cellular and molecular aspects of the development of the symbiosis.

According to Boletzky et al. (1971), the laboratory culture of any species has two main purposes, firstly to gather data on the life history of that species, and secondly to breed individuals under controlled conditions in order to produce animals for physiological, genetic, and ethological studies. For practical reasons, the latter is of particular relevance for species that are small in size, reach sexual maturity at an early age, are readily abundant, and require little care. Among the Cephalopoda, members of the Sepiolinae match these demands perfectly. To date, several species of bobtail squid have been successfully maintained, cultured and reared in captivity, including *Sepiola robusta*, *S. rondeleti*, *S. ligulata*, *Sepietta neglecta*, *S. obscura* (Boletzky et al. 1971), *Euprymna scolopes* (Hanlon et al. 1997; Arnold et al. 1972) *E. berryi* (Choe, 1966), *E. tasmanica* (Steer et al. 2004), *E. hylleberghi* (Nabhitabhata et al. 2005) and *Rossia macrosoma* (Boletzky & Boletzky, 1973).

The little cuttlefish (or Atlantic bobtail squid), *Sepiola atlantica* Orbigny, (1839-1842) (see Figure 1) is a member of the subfamily Sepiolinae and may reach 21mm dorsal mantle length (DML) when mature. The species is distinguished from other members of the Sepiolinae by having 4-8 rows of suckers at the distal tips of the ventral arms, and a ventral, bi-lobed photophore (Yau & Boyle, 1996). Mature males have a hectocotylus (see Figure 2), along with 3-4 large suckers on the middle of the dorsal arm, followed sequentially towards the tip of the arm by 3-4 small suckers and 4 large suckers of decreasing size (Yau & Boyle, 1996).

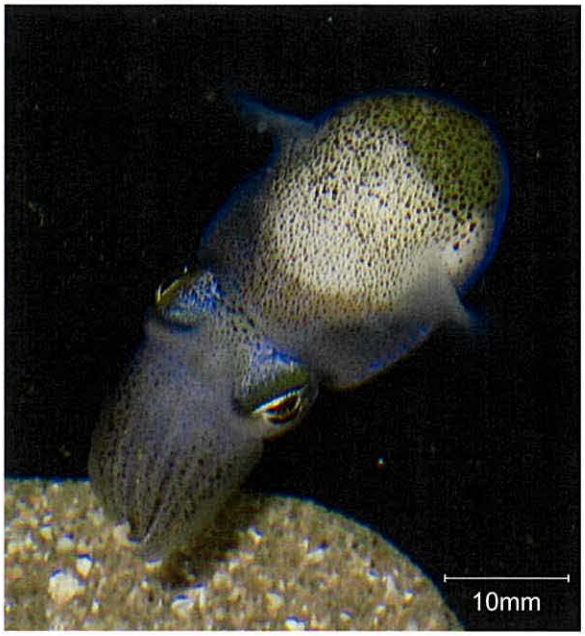


Fig. 1 The little cuttlefish, *Sepiola atlantica* Orbigny, (1839-1842).

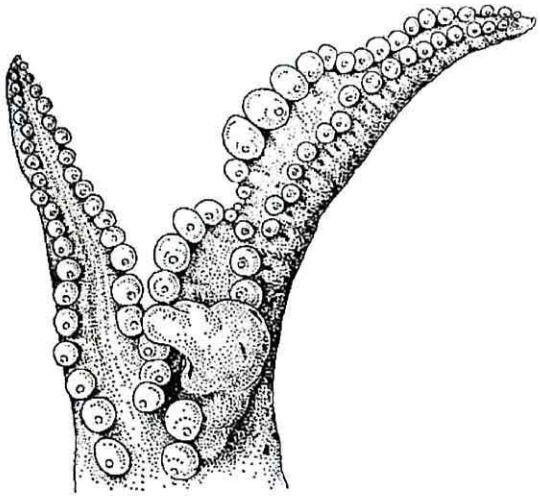


Fig. 2 The hectocotylus of a mature, male *Sepiola atlantica*. Adapted from Jereb & Roper (2005).

Sepiola atlantica is widely distributed in the northeastern Atlantic from 65°N to 35°N i.e. from Iceland, the Faroe Islands and western Norway in the north to Morocco in the south (see Figure 3), with a single specimen recorded in the Mediterranean Sea (Yau & Boyle, 1996; Collins et al. 2002, Jereb & Roper, 2005). It

is found in rock pools and the shallow intertidal zone down to 150m (Norman, 2000; Collins et al. 2001, Hayward & Ryland, 1996). Due to its wide distribution on the north European continental shelf and relatively common occurrence, *S. atlantica* is likely to occur in local fisheries yet no data are currently available (Jereb & Roper, 2005).

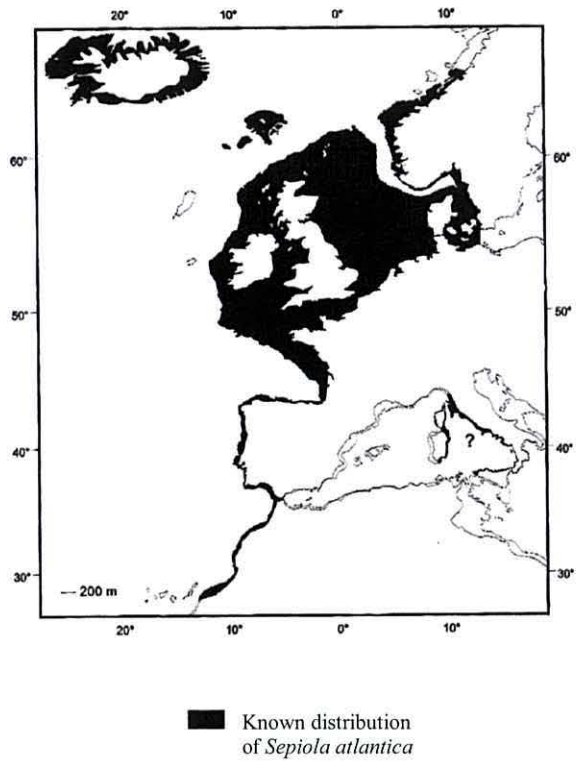


Fig. 3 Map showing the known distribution of *Sepiola atlantica*. Adapted from Jereb & Roper (2005).

Despite the wide distribution and common occurrence of the species there have been relatively few published works on the biology and ecology of *S. atlantica*. The most extensive study of *S. atlantica* was made by Yau & Boyle (1996), who examined the ecology of a resident population of *S. atlantica* in the shallow sublittoral zone of Loch Ewe on the west coast of Scotland, as well as the captive feeding behaviour of *S. atlantica*. Racovitza (1894) first described the mating behaviour of *S. atlantica* whilst Richard et al. (1979) used *S. atlantica* to examine the cycle of activity

in the accessory nidamental glands of cephalopods. Weill (1927) studied the structure and function of the spermatophore of *S. atlantica*, Adam (1934), described the radula of *S. atlantica*, while a number of studies have examined photoreceptor potentials and the structure of the eye lens of *S. atlantica* (Delamere & Duncan, 1977; Duncan & Pynsent, 1977; 1979; Jacob & Duncan, 1984; Willekens et al. 1984; Boucher-Rodoni et al. 1995). Bioluminescent activity and the structure of the photophore of *S. atlantica* have been described by Herring et al. (1981), and Nishiguchi et al. (1998; 2004), used *S. atlantica* along with other species of sepiolids as model organisms to further the understanding of the evolution of bacteriogenic light organs in squid. Hanlon (1987) has stated that in order to fully understand cephalopod life cycles, observations and experimentation in both the field and laboratory are necessary.

This PhD research project was specifically aimed at increasing the understanding of the laboratory culture, maintenance and life history of *Sepiola atlantica* and *Sepia officinalis*. The research was funded by the European Social Fund (ESF) in collaboration with Anglesey Sea Zoo with a specific remit for developing an understanding of the ecology, breeding and behaviour of selected species of cephalopods and to develop methods for improving the living conditions and display of cephalopods in aquaria. This chapter (chapter 1) is a general introduction that introduces the reader to the general aspects of cephalopods and particularly to members of the Sepiolidae. Since many cephalopod species are used in the laboratory and are components of public aquaria there is a need to develop methodologies for transporting the eggs and juveniles, often over considerable distances. In chapter 2, the distribution of *S. atlantica* around Anglesey and North Wales and their seasonal abundance, their population structure and reproductive biology in the shallow subtidal waters around the coast of Anglesey are reported.

Observations of the mating behaviour, spawning and the effects of various diets on paralarval and juvenile growth and survival together with observations of the embryonic development of the species and aspects of their aquarium behaviour are reported in chapter 3. In Chapter 4, I report on experiments that were undertaken to study the survival of the eggs, embryos and post-juvenile growth of the cuttlefish *Sepia officinalis* with a view to developing a methodology for transporting the eggs and juveniles of *S. atlantica*. This information is required in order to understand their requirements in captivity, in the hope of furthering the future possibility of culturing several consecutive generations of *S. atlantica* under laboratory conditions. Chapter 5 is a general discussion, drawing together the findings of the study and summarising the research undertaken in the thesis.

REFERENCES

Adam, W. 1934. Notes on the cephalopods: 4. The variation of the radula of *Sepiola atlantica* d'Orbigny 1839. Bulletin of the Royal Museum of Natural History of Belgium. 10 (24), 1-4

Adin, D.M; Engle, J.T.; Goldman, W.E; McFall-Ngai, M.J. & Stabb, E.V. 2009. Mutations in *amp*G and lytic transglycosylase genes affect the net release of peptidoglycan monomers from *Vibrio fischeri*. Journal of Bacteriology. 191 (7), 2012-2022

Anderson, R.C. 2001. Aquarium husbandry of pacific northwest marine invertebrates. The Seattle Aquarium, Washington. pp113-121

Arnold, J.M; Singley, C.T. & Williams-Arnol, L.D. 1972. Embryonic development and post-hatching survival of the sepiolid squid *Euprymna scolopes* under laboratory conditions. The Veliger. 14(4), 361-364

Boettcher, K.J. & Ruby, E.G. 1990. Depressed light emission by symbiotic *Vibrio fischeri* of the sepiolid squid *Euprymna scolopes*. Journal of Bacteriology. 172 (7), 3701-3706

Boletzky, S.v. 1983. *Sepia officinalis*. In: cephalopod Life Cycles Vol I. Academic Press, London. pp 31-53

Boletzky, S.v; Boletzky, M.v; Frosch, D. & Gatzi, V. 1971. Laboratory rearing of Sepiolinae (Mollusca: Cephalopoda). Marine Biology. 8, 82-87

Boletzky, S.v. & Boletzky, M.v. 1973. Observations on the embryonic development of *Rossia macrosoma* (Mollusca, Cephalopoda). Helgolander wiss. Meersunters. 25, 135-161

Boletzky, S.v. & Hanlon, R.T. 1983. A review of the laboratory maintenance, rearing and culture of cephalopod molluscs. Memoirs of the National Museum of Victoria. 44, 147-187

Boucher-Rodoni, R., Martine, M. Peduzzi, R. 1995. Preliminary results of a comparative study on antigenicity of Cephalopod eye-lens proteins: the sepiolids. Bulletin de l'Institut océanographique. 17, 93-98

Boyle, P.R. 1983. Cephalopod Life Cycles, Vol. I: Species Accounts. Academic Press, London

Boyle, P.R. 1991. The UFAW handbook on the care and management of cephalopods in the laboratory. Universities Federation for Animal Welfare, Aberdeen. pp1-63

Boyle, P.R. & Roydhouse, P. 2005. Cephalopods- Ecology & Fisheries. Blackwell publishing, Oxford. pp 369-370

Budelmann, B.U. 1995. The cephalopod nervous system: what evolution has made of the molluscan design. In: Breidback, O, Kutsch, W (Eds) The nervous system of invertebrates. An evolutionary and comparative approach. Birkhauser Verlag. pp 115-138

Choe, S. 1966. On the eggs, rearing, habits of the fry and growth of some Cephalopoda. Bulletin of Marine Science. 16(2), 330-348

Claes, M.F. & Dunlap, P.V. 2000. Aposymbiotic culture of the sepiolid squid *Euprymna scolopes*: Role of the symbiotic bacterium *Vibrio fischeri* in host animal growth, development, and light organ morphogenesis. Journal of Experimental Zoology. 286, 280-296

Clarke, M.R. 1996. Cephalopods as prey. III. Cetaceans. Philosophical Transcriptions of the Royal Society, London, B. 351, 1053-1065.

Collins, M.A; Yau, C; Allcock, L. & Thurston, M.H. 2001. Distribution of deep water benthic and bentho-pelagic cephalopods from the north-east Atlantic. Journal of the Marine Biological Association of the United Kingdom. 81, 105-117

Collins, M.A; Yau, C; Boyle, P.R; Friese, D. & Piatkowski, U. 2002. Distribution of cephalopods from plankton surveys around the British Isles. Bulletin of Marine Science. 71 (1), 239-254

Croxall, J.P. & Prince, P.A. 1996. Cephalopods as prey. I. Seabirds. Philosophical Transcriptions of the Royal Society, London, B. 351, 1023-1043.

Delamere, N.A. & Duncan, G. 1977. A comparison of ion concentrations, potentials and conductances of amphibian, bovine and cephalopod lenses. Journal of Physiology. 272, 167-186

Duncan, G. & Pynsent, P.B. 1977. Intracellular and extracellular photoreceptor potentials in the retina of the cephalopod *Sepiola atlantica*. Journal of Physiology. 267, 37-38

Duncan, G. & Pynsent, P.B. 1979. An analysis of the wave forms of photoreceptor potentials in the retina of the cephalopod *Sepiola atlantica*. Journal of Physiology. 288, 171-188

Dunlop, C. & King, N. 2009. Cephalopods: Octopuses and Cuttlefishes for the Home Aquarium. T.F.H. Publications, Inc, Neptune City. 226pp

FAO. 2004. The state of world fisheries and aquaculture. FAO Fisheries Department. Food and Agriculture Organisation of the United Nations. Rome, Italy. 153 pp

Food and Agriculture Organization of the United Nations (FAO) [Internet]. Rome, Italy. [Cited December 8, 2009]. Available from: http://www.fao.org/fishery/statistics

Foster, J.S; Boletzky, S.v. & McFall-Ngai, J. 2002. A comparison of the light organ development of *Sepiola robusta* Naef and *Euprymna scolopes* Berry (Cephalopoda: Sepiolidae). Bulletin of Marine Science. 70(1), 141-153

Hanlon, R.T. 1987. Mariculture, In: Cephalopod Life Cycles, Vol II. Academic Press, London. pp 291-299

Hanlon, R.T. & Messenger, J.B. 1996. Cephalopod Behaviour. Cambridge University Press. pp 232

Hanlon, R.T; Claes, M.F; Ashcraft, S.E. & Dunlap, P.V. 1997. Laboratory culture of the sepiolid squid *Euprymna scolopes*: A model system for bacteria-animal symbiosis. Biological Bulletin. 192, 364-374

Hayward, P.J. & Ryland, J.S. 1996. Handbook of the marine fauna of north-west Europe, Oxford University Press. pp 800

Herring, P.J; Clarke, M.R; Boletzky, S.v. & Ryan, K.P. 1981. The light organs of *Sepiola atlantica* and *Spirula spirula* (Mollusca: Cephalopoda): bacterial and intrinsic systems in the order Sepioidea. Journal of the Marine Biological Association of the United Kingdom. 61, 901-916

Jacob, T.J.C. & Duncan, G. 1984. A comparative study of the membrane permeability properties of amphibian and cephalopod mollusc lenses. Journal of Comparative Physiology B. 154, 333-341

Jereb, P. & Roper, C.F.E (eds). 2005. Cephalopods of the world: An annotated and illustrated catalogue of cephalopod species known to date. Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae). FAO Species Catalogue for Fishery Purposes. No. 4, Vol. 1. Rome, 262 pp

Jones, B.W. & Nishiguchi, M.K. 2004. Counterillumination in the Hawaiian bobtail squid, *Euprymna scolopes* Berry (Mollusca: Cephalopoda). Marine Biology. 144, 1151-1155

Kunisaki, N. 2000. Nutritional properties of squid and cuttlefish. In: (Okuzumi, M. & Jujii, T. eds) Nutritional and Functional Properties of Squid and Cuttlefish. National Cooperative Association of Squid Processors, Japan. 218pp

Klages, N.T.W. 1996. Cephalopods as prey. II. Seals. Philosophical Transcriptions of the Royal Society, London, B. 351, 1045-1052

Mandel, M.J; Wollenberg, M.S; Stabb, E.V; Visick, K.L. & Ruby, E.G. 2009. A single regulatory gene is sufficient to alter bacterial host range. Nature. 458 (7235), 215-218.

Mangold, K. 1987. Reproduction. In P.R. Boyle (ed.) Cephalopod Life Cycles, Volume II. Academic Press, London. pp 157-200

Mather, J.A. & Anderson, R.C. 2007. Ethics and invertebrates: a cephalopod perspective. Disease of Aquatic Organisms. 75, 119-129

McFall-Ngai, M.J. & Ruby, E.G. 1998. Sepiolids and Vibrios: When they first meet. BioScience. 48(4), 257-265

Moltschaniwskyj, N.A; Hall, K; Lipinski, M.R; Marian, J.E.A.R; Nishiguchi, M; Sakai, M; Shulma, D.J; Sinclair, B; Sinn, D.L; Staudinger, M; Van Gelderen, R; Villanueva, R. & Warnke, K. 2007. Ethical and welfare considerations when using cephalopods as experimental animals. Reviews in Fish Biology and Fisheries. 17, 455-476.

Montgomery, M.K. & McFall-Ngai, M.J. 1998. Late postembryonic development of the symbiotic light organ of *Euprymna scolopes* (Cephalopoda: Sepiolidae). Biological Bulletin. 195, 326-336

Nabhitabhata, J., Nilaphat, P., Promboon, P. & Jaroongpattananon, C. 2005. Life cycle of cultured bobtail squid, *Euprymna hyllebergi* Nateewathana, 1997. Phuket Marine Biological Centre Research Bulletin. 66, 351-365

Nishiguchi, M.K.2000. Temperature affects species distribution in symbiotic populations of *Vibrio* spp. Applied and Environmental Microbiology. 66 (8), 3550-3555

Nishiguchi, M.K., Lopez, J.E. & Boletzky, S. v. 2004. Enlightenment of old ideas from new investigations: more questions regarding the evolution of bacteriogenic light organs in squids. Evolution & Development. 6 (1), 41-49

Nishiguchi, M.K., Ruby, E.G. & McFall-Ngai, M.J. 1998. Competitive dominance among strains of luminous bacteria provides an unusual form of evidence for parallel evolution in sepiolid squid-*Vibrio* symbioses. Applied and Environmental Microbiology. 64 (9), 3209-3213

Nixon, M. 1987. Cephalopd diets, In: Cephalopod Life Cycles, Vol II. Academic Press, London. pp 201-219

Nixon, M. & Young, J.Z. 2003. The brains and lives of Cephalopods. Clarendon Press, Oxford. 381pp

Norman, M. 2000. Cephalopods: A world guide. Conch Books, Hachenheim. 320pp

Nyholm, S.V. & McFall-Ngai, M.J. 1998. Sampling the light organ microenvironment of *Euprymna scolopes*: Description of a population of host cells in association with the bacterial symbiont *Vibrio fischeri*. Biological Bulletin. 195, 89-97

Nyholm, S.V; Stabb, E.V; Ruby, E.G. & McFall-Ngai, M.J. 2000. Establishment of an animal-bacterial association: Recruiting symbiotic vibrios from the environment. Proceedings of the National Academy of Sciences of the United States of America. 97 (18), 10231-10235

Racovitza, É.G. 1894. Sur l'accouplement de quelques Céphalopodes *Sepiola rondeleti* (Leach), *Rossia macrosoma* (delle Chiaje) et *Octopus vulgaris* (Lam.). Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences Series D. 118, 722-724

Richard, A., Van den Branden, C. & Decleir, W. 1979. The cycle of activity in the accessory nidamental glands from cephalopods, in: Naylor, E.; Hartnoll, R.G. (Ed). Cyclic phenomena in marine plants and animals: Proceedings of the 13th European Marine Biology Symposium, Isle of Man, 27 September – 4 October 1978. European Marine Biology Symposia, 13. pp 173-180

Rocha, F., Guerrra, Á. & González, Á.F. 2001. A review of reproductive strategies in cephalopods. Biological Reviews. 76, 291-304

Ruby, E.G. 1996. Lessons from a cooperative, bacterial-animal association: the *Vibrio fischeri-Euprymna scolopes* light organ symbiosis. Annual Review in Microbiology. 50, 591-624

Ruby, E.G. & McFall-Ngai, M.J. 1992. A squid that glows in the night: development of an animal-bacterial mutualism. Journal of Bacteriology. 174, 4865-4870.

Ruby, E.G. & Lee, K.H. 1998. The *Vibrio fischeri-Euprymna scolopes* light organ association: Current ecological paradigms. Applied and Environmental Microbiology. 64 (3), 805-812

Shears. J. 1988. The use of a sand-coat in relation to feeding and diel activity in the sepiolid squid *Euprymna scolopes*. Malacologia. 29 (1), 121-133

Smale, M.J. 1996. Cephalopods as prey. IV. Fishes. Philosophical Transcriptions of the Royal Society, London. 351, 1067-1081.

Soto, W., Gutierrez, J., Remmenga, M.D. & Nishiguchi, M.K. 2009. Salinity and temperature effects on physiological responses of *Vibrio fischeri* from diverse ecological niches. Microbial Ecology. 57, 140-150

Sykes, A.V., Domingues, P.M., Correia, M. & Andrade, J.P. 2006. Cittlefish culture – State of the art and future trends. Vie et Milieu. 56 (2), 129-137

Weill, R. 1927. Recherche sur la structure, valeur systématique et le fonctionnement du spermatophore de '*Sepiola atlantica*' d'Orbigny. Bulletin Biologique de France et de Belgique. 62, 59-92

Wells, M.J. 1962. Brain and behaviour in cephalopods. Heinemann. 171pp

Wells, M.J. 1978. Octopus-physiology and behaviour of an advanced invertebrate. Chapman & Hall, London

Willekens, B., Vrensen, G., Jacob, T. & Duncan, G. 1984. The ultrastructure of the lens of the cephalopod *Sepiola*: A scanning electron microscopic studty. Tissue & Cell. 16 (6), 941-950

Wollenberg, M.S. & Ruby, E.G. 2009. Population structure of *Vibrio fischeri* within the light organs of *Euprymna scolopes* squid from two Oahu (Hawaii) populations. Applied and Environmental Microbiology. 75 (1), 193-202.

Yau, C. & Boyle, P.R. 1996. Ecology of *Sepiola atlantica* (Mollusca: Cephalopoda) in the shallow sublittoral zone. Journal of the Marine Biological Association of the United Kingdom. 76, 733-748

Young, J.Z. 1971. The anatomy of the nervous system of *Octopus vulgaris*. Clarendon Press. 690pp

CHAPTER 2

Distribution, ecology and reproductive biology of the little cuttlefish *Sepiola atlantica* from around Anglesey

ABSTRACT

The distribution of the little cuttlefish *Sepiola atlantica* in the shallow subtidal zone around Anglesey was investigated using seine netting. *Sepiola atlantica* was found in varying numbers at twelve locations around the west and east coasts of Anglesey and within the Menai Strait. Highest numbers were collected from the shores of the sheltered Y Foryd Bay and the wave-exposed Traeth Penrhos and these locations were sampled further to examine the seasonal occurrence, population and reproductive biology of *S. atlantica*. *Sepiola atlantica* migrated inshore seasonally, first appearing around Anglesey in July reaching peak abundances between July and August as seawater temperatures approached 17°C, declining in numbers between September and October as temperatures dropped below 15-16°C and finally disappearing from inshore locations in late October.

Sepiola atlantica ranged in size between 5 and 28 mm dorsal mantle length; females were significantly larger than males, with males maturing at a smaller size than females. The number of spermatangia on the *bursa copulatrix* of female *S. atlantica* varied seasonally attaining maximum numbers in October with a mean number of 22 spermatangia on the *bursa copulatrix* of Y Foryd females. The number of spermatophores and advanced ova in male and female *S. atlantica* from Y Foryd and Traeth Penrhos ranged between 1-147 and 20-137, respectively. The spermatophoric complex and gonado-somatic indices showed a high degree of variability in little cuttlefish of similar wet weight with female gonads constituting a far greater percentage of the total wet weight than the male gonads. Data presented in

this chapter suggest that *S. atlantica* of all sizes and maturity stages congregate in the shallow waters around Anglesey between July and October when environmental conditions are favourable for enhanced growth and maturation and where the high numbers of animals enhances opportunities for mating and genetic exchange.

INTRODUCTION

Reproduction is the most important phase in the life cycle of all living organisms (Cuccu et al. 2007) and an understanding of the reproductive strategy of a cephalopod species is critical in determining its entire life cycle, which in turn is key to understanding its ecology (Boyle & Rodhouse, 2005). The generally short life cycle of cephalopods means that regular annual recruitments are necessary in order to sustain a population (Boyle, 1990). Thus a complete understanding of the reproductive strategy of commercially important species is a key fisheries management tool (Pecl, 2001). It has long been recognised that cephalopods form a valuable fisheries resource worldwide and as such their populations require as much attention to that previously given to fin fish species (Amaratunga, 1987). Also an understanding of cephalopod fisheries will allow the stocks to be properly managed, conserved and exploited (Amaratunga, 1987). In order to achieve these objectives a knowledge of the population structure of a cephalopod species, along with the manner in which it interacts within an ecosystem needs to be fully understood.

Compared with other groups of cephalopods, very little is known about the distribution and life history of members of the cephalopod family Sepiolidae (see Orsi Relini & Bertuletti, 1989) which are generally smaller in size than the pelagic squid, benthic cuttlefish and octopus species. There are no target fisheries or markets for sepiolids, yet the potential exists for members of the family to be exploited since many similar sized cephalopods are caught as by-catch and sold on for human consumption (Volpi et al. 1995). Sepiolids are important links in marine food webs as they are prey for a variety of marine organisms including cetaceans, seabirds, demersal and pelagic fishes and crustaceans, some of which are commercially important species (Smale, 1996; Lefkaditou & Kaspiris, 2005; Rosa et al. 2006).

The little cuttlefish, *Sepiola atlantica*, has been recorded amongst the stomach contents of the common dolphin, *Delphinus delphis* (Silva, 1999; De Pierrepont et al. 2005), the small spotted catshark, *Scyliorhinus canicula* (Lyle, 1983), Arctic and common terns, *Sterna paradisaea* and *S. hirundo*, respectively (Pearson, 1968) and it is one of the most important sources of food for whiting, *Merlangius merlangus* in the Irish Sea (Patterson, 1985). Several members of the Sepiolidae occur in relatively shallow water and can be caught easily and readily maintained in aquaria. These characteristics have allowed the entire life cycle of several species in the family to be documented (Boletzky et al. 1971; Hanlon et al. 1997; Nabhitabhata et al. 2005). Such traits combined with their small adult size and nocturnal life style makes them excellent candidates for laboratory study (Boletzky, 1983).

Several studies have examined the reproductive biology of members of the Sepiolidae (e.g. Bergström & Summers, 1983; Summers, 1985; Yau & Boyle, 1996; Salman, 1998; Salman & Önsoy, 2004; Rosa et al. 2006; Cuccu et al. 2007; Laptikhovsky, 2008 & Önsoy et al. 2008). As early as 1922, Russell (1922) suggested that reproduction in *S. atlantica* took place over a prolonged period, with the main mating season in the North Sea between January and March and off Plymouth between July and August. However, a later paper by Stephen (1944) noted the prolonged presence of sexually mature *S. atlantica* between October and March in Scottish coastal waters. Yau and Boyle (1996) recorded the year round presence of all maturity stages of *S. atlantica* in Firemore Bay, Scotland although the majority of sexually mature animals were present between March and August with a peak of mature animals appearing in June.

The distribution of animals in a population is determined by a series of complex responses to both the physical and biological state of the environment, with

these responses allowing individuals to select those habitats that provide the optimum conditions for growth and reproduction whilst also offering the lowest risk of mortality. However, these habitats may be in a state of flux whether it is on a daily or seasonal basis. Aquatic organisms in coastal areas are affected by changes in the tides, particularly those individuals inhabiting intertidal and shallow subtidal zones. Species inhabiting these zones employ a number of strategies in order to cope with tidally phased fluctuations in habitat suitability (Gibson et al. 1996). Cephalopod populations (other than nautiloids) comprise short-lived semalparous animals at different stages in their life cycle and are strongly influenced by, and responsive to inter-annual changes in environmental conditions e.g. seawater temperature and the availability and movement of prey species. In turn, these variables influence population size, distribution and species abundance (e.g. Boyle & Boletzky, 1996; Semmens et al. 2007; Pierce et al. 2008). While several studies examining the distribution of cephalopods have documented the occurrence of members of the Sepiolidae (e.g. Villanueva, 1992; Ünsal et al. 1999; Quetglas et al. 2000; Lordan et al. 2001; Collins et al. 2001, 2002; Zumholz & Frandsen, 2006), few of them have specifically focused on the genus *Sepiola* (Orsi Relini & Bertuletti, 1989; Yau & Boyle, 1996; Jereb et al. 1997; Lefkaditou & Kaspiris, 2005; Rosa et al. 2006).

Sepiola atlantica occurs in coastal waters on the continental shelf of the north-eastern Atlantic (65°N to 35°N) from Iceland, the Faeroe Islands and western Norway south to Morocco (Jereb & Roper, 2005) yet it is absent from the Mediterranean Sea (Naef, 1916; Grimpe, 1925; Bello, 1986, 1992; Mangold & Boletzky 1988). Unusually a single mature male was recorded from a depth of 90 m in the Tyrrhenian Sea, in the Mediterranean Sea (Würtz et al. 1995). In 1996, Yau & Boyle reported on a study of a population of *S. atlantica* in the shallow sublittoral waters of Firemore

Bay, Loch Ewe, Scotland where *S. atlantica* were present annually between December and August. They speculated that the continued presence of *S. atlanitca* in the Loch was probably dependent on the annual recruitment of individuals from outside the bay as the entire population could be eradicated following severe weather and strong wave action. Collins et al. (2002) recorded *S. atlantica* in plankton tows from around the British Isles and suggested that *S. atlantica* is the most abundant species of sepiolid in British waters.

Despite its wide distribution, common occurrence and prominent role in the marine food chain, there is a distinct lack of published accounts of the biology, life cycle and ecology of *S. atlantica*, perhaps as it has little or no commercial value (Yau & Boyle, 1996). A complete understanding of the seasonal distribution, population biology and ecology of *S. atlantica* would be of benefit to those requiring specimens of a known size, sex or reproductive condition for culture, behavioural or physiological studies. The aims of the present study were: 1) to describe the distribution of *S. atlantica* around the coast of Anglesey, North Wales, 2) to compare the seasonal distribution of *S. atlantica* between a wave-exposed and sheltered shore on Anglesey and 3) to gain a better understanding of the population ecology and reproductive biology of *S. atlantica* from the coastal waters around Anglesey in order to gather information that would be of benefit to those wanting to culture the species in captivity.

METHODS

Sampling

In order to establish the distribution of *S. atlantica* around the coast of Anglesey, a sampling programme was undertaken in the summer and early autumn (between June and October 2006) during clement weather at ten locations (See Figure 1 & Table 1). *Sepiola atlantica* had previously been observed and occasionally collected from several coastal locations around Anglesey; at Traeth Llanddwyn, (Richardson, pers. comm.), at Rhosneigr and Llanddona (Roberts, pers. comm.), Moel y don and Y Foryd (Linley 2005). At the latter site, a pair of male and female *S. atlantica* was collected in a mating embrace. Offshore sampling was not undertaken due to the high cost of undertaking ship or small boat work.

From June 2006 to September 2008, two of the ten locations (Foryd Bay and Traeth Penrhos) were further sampled regularly to examine the seasonal occurrence of *S. atlantica* (See Figures 2a & 2b). The criteria used in the selection of these two sites were that *S. atlantica* was abundant at both sites, that each location was accessible all year round, and that the wave exposure at each location was different, i.e. Y Foryd bay was a sheltered site and Traeth Penrhos was wave-exposed.

Sampling was undertaken an hour before low water in order to survey as much of the subtidal zone as possible, on both neap and spring tides using a beach seine net (20 x 2.2 m, cod end mesh diameter = 5 mm) deployed by small boat (Y Foryd) and by wading (Traeth Penrhos). Sampling was not undertaken at night due to logistic and the added dangers of undertaking field work at night. *Sepiola atlantica* is also thought to be nocturnal therefore it was thought that animals would be harder to catch at night when they would be more active. Both sites were sampled three times per month, with a standard number of four tows per session thus fishing effort was

roughly equal at both sites. Each sampling visit took approximately two hours from start to finish. Sampling was restricted to water no more than 1.5 m deep ensuring that the net would fish without losing animals over the top of the net and a distance of ~40 m was left between tows in order to reduce the chances of disturbance to *S. atlantica* further along the shore. The number of *S. atlantica* in each tow was recorded together with any by-catch species collected.

Some of the *S. atlantica* collected were placed on ice for freezing and later study whilst others were transported alive back to the School of Ocean Sciences, Menai Bridge for culture experiments (see chapter 4). *Sepiola atlantica* were individually bagged, labelled and placed in a freezer at –70°C until they were required for an examination of their morphometry and reproductive condition. Global Positioning System (GPS) co-ordinates of the start and finish of each tow were recorded using a Garmin E-Trex Legend (GPS), and the depth of the tow was estimated from the boat using an Eagle CU DA 128 echo sounder. Seawater temperature during sampling was recorded continuously using a Tinytag temperature data logger and the data downloaded onto a PC upon return to the laboratory. Wind speed and direction were obtained from a web site (xcweather.co.uk) that gives live data from a weather station at Royal Air Force Valley (west Anglesey). The numbers of *S. atlantica* were recorded but not their density as it was difficult to determine the exact area fished and the fishing efficiency of the seine net.

Further additional data on subtidal populations of *S. atlantica* from around the coast of Anglesey and Gwynedd, North Wales were obtained opportunistically from collections of specimens taken from a PhD study by Al-Rashada (2009) in which a 2m beam trawl deployed from the RV 'Mya' (fore net = 2m with 15 mm mesh, cod end = 2 m with a mesh diameter of 10 mm) was towed at a speed of ~1.6 knots at

depths of between 2 and 4 metres in several coastal locations. Specimens were frozen for later examination.

Description of sampling sites

The location of each study site is shown in Figure 1. The degree of wave exposure at each site was assessed using the Ballantine 'Biologically-defined Exposure Scale' (Ballantine, 1961), while information on the littoral and sublittoral biotopes at each site was taken from Brazier et al. (1999). The exact location of each site and sample was recorded using GPS (latitude and longitude) (see Table 1). Some sampling locations required special permission to undertake scientific surveys and in these cases permission was either granted by the North Wales and Western Sea Fisheries Committee, the Forestry Commission Wales (FCW) and/or the Countryside Council for Wales (CCW).

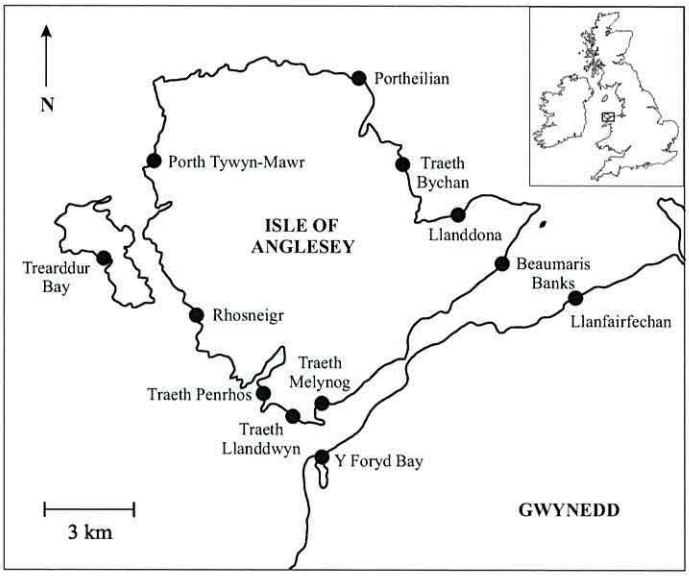


Fig. 1 Map showing sites visited around the coast of the Isle of Anglesey, along with a map showing the location (within the boxed area) of the Isle of Anglesey within the UK.

West Anglesey & Gwynedd

The distribution of *S. atlantica* was investigated at five locations on the west coast of Anglesey. This stretch of coastline is generally west and south-west facing and is exposed to prevailing winds, although the southern end of the coastline receives some shelter from the Lleyn Peninsula. While Rhoscolyn was not visited, *S. atlantica* had previously been observed there (pers. obs).

1. **Traeth Llanddwyn**: Traeth Llanddwyn lies within the Newborough Warren nature reserve and is a dynamic sandy shore that is backed by sand dunes and pine forest. The shore comprises of sublittoral fine sands with polychaetes and amphipods.

2. **Traeth Penrhos**: This south-west facing shore lies within Malltraeth Bay in the Newborough Warren nature reserve and is separated from Traeth Llanddwyn by Ynys Llanddwyn (Llanddwyn Island). The shallow sloping shore comprises mobile sand containing amphipods and polychaetes and is backed by extensive sand dunes and a pine forest and is situated close to the Cefni estuary. Permission was granted to survey this site by the Countryside Council for Wales (CCW) and the Forestry Commission Wales (FCW).

3. **Rhosneigr**: The beach at Rhosneigr is southwest facing and consists of mobile sand with amphipods and polychaetes, interspersed with patches of exposed littoral rock (with numerous rock pools) covered in fucoid algae and kelp. This beach is popular with water sports enthusiasts.

4. **Trearddur Bay**: Trearddur Bay is a west facing site with a small bay enclosed by littoral rocks covered with dense mats of fucoid algae, mussels and barnacles, while the beach is composed of sublittoral muddy sands with bivalves. This popular

location is used as a swimming beach from May to October and therefore sampling between 10.00 am and 19.00 p.m. was not permitted during the summer months for health and safety reasons although pleasure craft are regularly launched here.

5. **Porth Tywyn-mawr**: A shallow sloping shore, backed by sand dunes, fringed with littoral rock at each end of the beach, with kelp beds, mobile sand with amphipods and polychaetes and outcrops of littoral rock towards the southern end of the shore. This site is also a designated a swimming beach during the summer months, while small pleasure craft are also launched here. Wind-driven algal debris litters the shore and this made sampling with a seine net difficult.

East Anglesey

The distribution of *S. atlantica* was examined at four sites on the east coast of Anglesey. While this stretch of coastline is sheltered from prevailing winds, the coastline is influenced by moderately strong currents.

6. **Portheilian**: This small north facing bay is enclosed from the open sea by littoral rock covered by dense mats of fucoid algae and limpets. The shore is comprised of mobile sand with amphipods and polychaetes while a small stream runs into the bay causing some turbidity caused by the freshwater runoff.

7. **Traeth Bychan**: Traeth Bychan is a small bay fringed with littoral rock with dense fucoid algae with mobile sand containing amphipods and polychaetes. This bay is a popular swimming beach during the summer months while it is home to a small sailing club.

8. **Llanddona**: Llanddona lies at the eastern end of the estuarine Red Wharf Bay (Traeth Coch). The area is an extensive muddy sand shore with amphipods, polychaetes and bivalves, with some boulders sparsely covered with algae and

littorinids at the eastern end of the shore. Subtidally, the sediment is predominantly composed of fine sands.

9. **Llanfairfechan**: The shore at Llanfairfechan lies adjacent to the extensive Lafan sands close to the north east entrance to the Menai Strait. The sediment here is composed of mobile sand with amphipods and polychaetes, while subtidally the sediment is comprised of fine sands with polychaetes and amphipods.

Menai Strait

The distribution of *S. atlantica* was examined at three sites within the Menai strait. The Menai Strait is a narrow channel (250-500 m wide in some areas) that runs approximately 20 km from Penmon Point in the north-east to Aber Menai point in the south-west and separates the county of Gwynedd from the Isle of Anglesey (Ynys Mon). While the Menai Strait is sheltered from wave action, some areas experience strong tidal currents in excess of 8 knots during spring tides as water from Liverpool Bay passes through the Strait into Caernarfon Bay. With strong currents, the Menai Strait is also characterised by high turbidity and high nutrient loading from terrestrial runoff and sewage discharge. Although not visited during the course of this study, a single *S. atlantica* was collected at Moel y don by Linley (2005).

10. **Beaumaris banks**: The Beaumaris banks lie opposite Gallows point, Beaumaris, and are part of the extensive Lafan Sands. The sand bank is comprised of mobile sands with amphipods and the area experiences strong currents and some wave action while the beach shelves and water depth increases dramatically close to the shore. In the summer months (June through August) this site is regularly visited by bait collectors who fish for sand eels (*Ammodytes tobianus* and *Hyperoplus lanceolatus*).

11. **Y Foryd bay**: This north facing sandy bay lies at the eastern mouth of the Menai Strait, close to the mouth of the Afon Gwyrfai and is backed by muddy sand shores

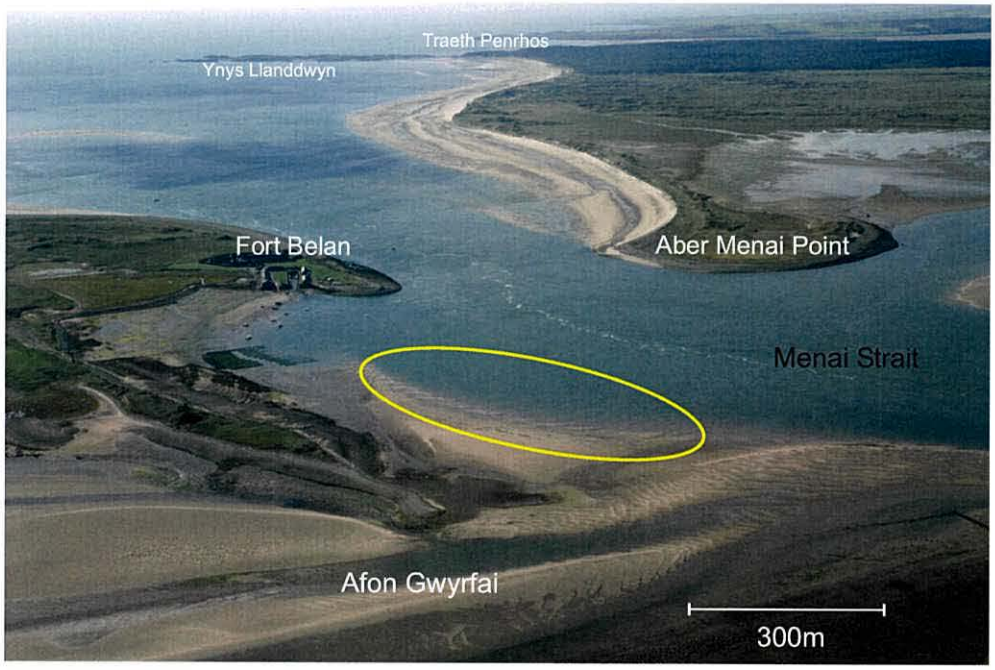
with *Zostera noltii* as well as estuarine sandy mud with polychaetes and oligochaetes. The site is sheltered from wave action, although currents in this area may be strong and the shore is comprised of mobile sand with amphipods and polychaetes. This site is visited regularly by bait collectors during the summer months. A permit to survey this site was granted by the Countryside Council for Wales (CCW).

12. **Traeth Melynog**: Traeth Melynog is an extensive, gently sloping shore comprised of areas of both muddy and mobile sands with bivalves, polychaetes and amphipods. The shore is backed by salt marsh and is subject to some freshwater input from the Afon Braint.

Table 1. Summary of site locations where *Sepiola atlantica* were collected and the level of shore exposure (adapted from Ballantine, 1961).

Site No.	Site	Latitude / Longditude	Level of exposure
1	Traeth Llanddwyn	53°08.394'N / 004°23.266'W	Fairly-sheltered
2	Traeth Penrhos	53°08.736'N / 004°24.469'W	Exposed
3	Rhosneigr	53°14.026'N / 004°31.526'W	Sheltered
4	Trearddur Bay	53°16.710'N / 004°37.110'W	Sheltered
5	Porth Tywyn-mawr	53°20.176'N / 004°34.359'W	Sheltered
6	Portheilian	53°24.692'N / 004°17.599'W	Sheltered
7	Traeth Bychan	53°20.402'N / 004°13.855'W	Sheltered
8	Llanddona	53°30.891'N / 004°14.785'W	Semi-exposed
9	Llanfairfechan	53°18.813'N / 003°58.448'W	Semi-exposed
10	Beaumaris banks	53°15.206'N / 004°05.786'W	Semi-exposed
11	Y Foryd	53°07.328'N / 004°19.337'W	Very sheltered
12	Traeth Melynog	53°08.298'N / 004°19.454'W	Very sheltered

a)



b)

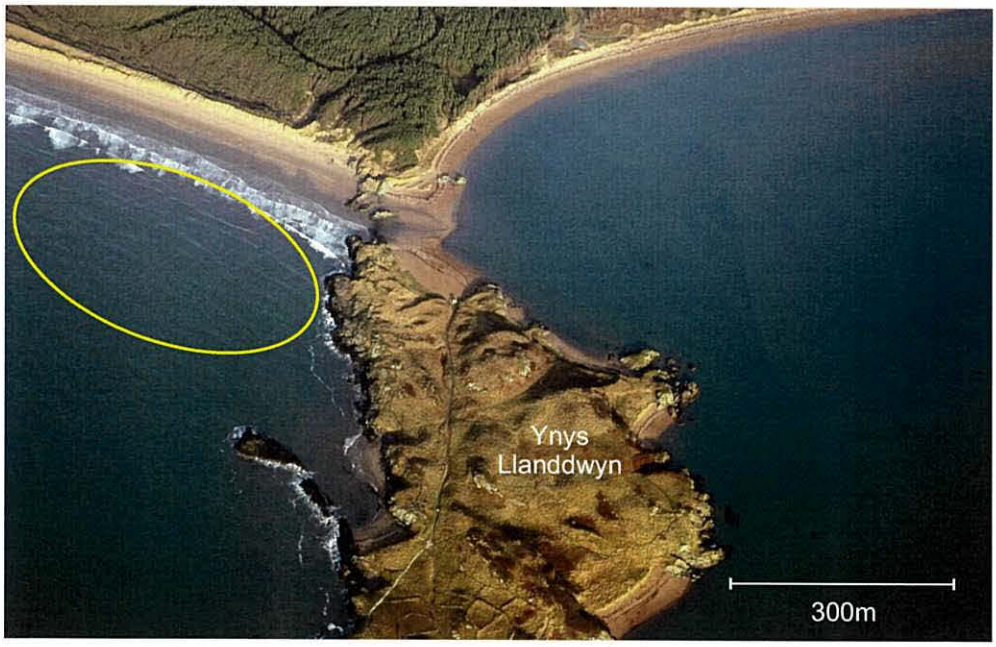


Fig. 2 Aerial photograph showing the study sites at a) Y Foryd and b) Traeth Penrhos as indicated by the area within the yellow lines. Images courtesy of Dave Roberts at pixaerial.com.

Morphometric measurements and the assessment of reproductive condition

Frozen *S. atlantica* were defrosted at room temperature for approximately one hour prior to examination. Measurements of the dorsal mantle length (DML), anterior mantle width (MW) (see Figure 3) (taken to the nearest 0.1 mm using vernier callipers) were taken from a sub-sample of 30 animals of a range of sizes from the smallest to the largest (5 to 28mm). Gender was determined and the maturity stage of each *S. atlantica* assessed using a maturity stage scale devised by Yau & Boyle (1996) that had been modified from a scale for *Loligo forbesi* (Boyle & Ngoile, 1993) (see Table 2). Total wet Weight (Ww) was determined by blotting dry each animal with tissue paper and then weighing on a top loading balance (Ohaus Analytical Plus) to the nearest 0.01 g. The internal anatomy of mature and female *S. atlantica* can be seen in Figure 4. Animals were dissected and the wet (blotted dry) gonad weight (♀ = ovary; ♂ = spermatophoric complex = spermatophoric sac, vas deferens and spermatophoric glands) of sexually mature (stage IV) animals were recorded to the nearest 0.001g using an Ohaus Adventure fine top loading balance.

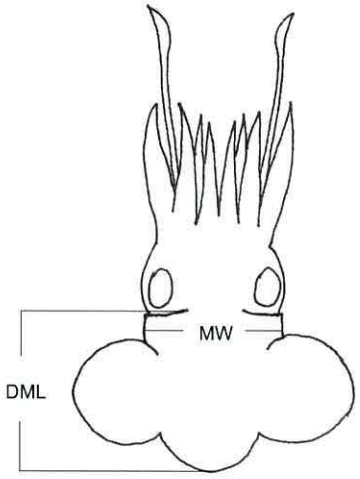
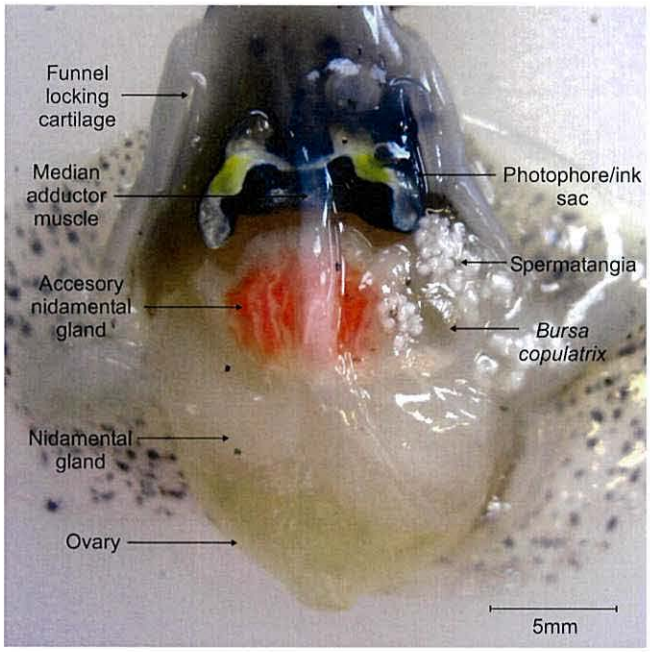


Fig. 3 Morphometric measurements undertaken on *Sepiola atlantica* including: DML, dorsal mantle length and MW, maximum mantle width. Adapted from Yau & Boyle (1996).

a)



b)

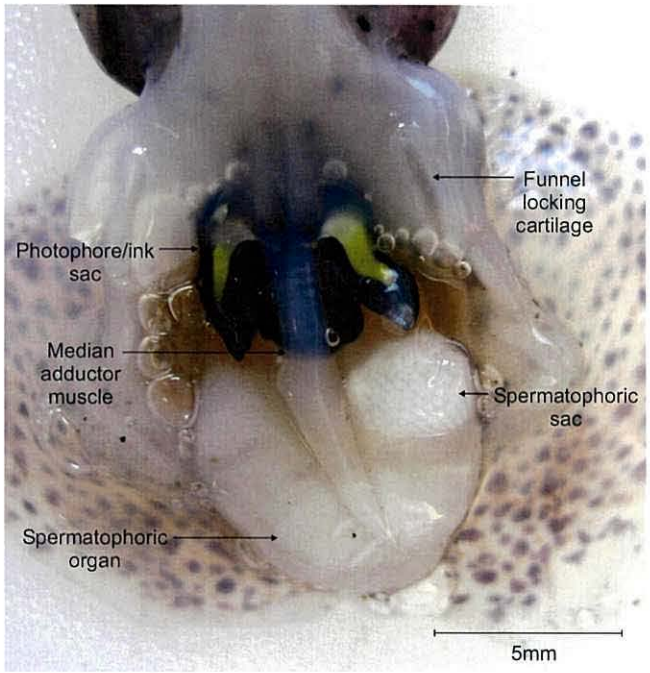


Fig. 4 The internal anatomy of; a) a mature (Stage IV), mated female and b) a mature (Stage IV), male *Sepiola atlantica*.

Table 2. Maturity stage scale for *Sepiola atlantica* from Yau & Boyle (1996).

Stage	Juveniles	
I	Reproductive parts are transparent and microscopic. The sex of the individual cannot be distinguished by eye.	
	Females	**Males**
II	The *bursa copulatrix* is just visible as a small pit on the left side of the visceral mass. The nidamental glands are small and translucent. The ovary cannot be distinguished.	The hectocotylus is just differentiating on the left dorsal arm with the formation of the basal process (or copulatory apparatus). The testis is small and translucent.
III	The nidamental glands are opaque and white, eventually becoming greatly enlarged to fill the posterior mantle. The *bursa copulatrix* occupies most of the left side of the visceral mass, appearing as a wrinkled and folded pouch. The accessory nidamental glands are anterior of and distinct from the nidamental glands. The ovary contains ova in early stages of development (opaque or reticulated in appearance) in the proximal oviduct.	The lateral third arms (AIII) are more muscular than other arms and bend in strongly towards the mouth following fixation. The hectocotylus is obvious with an enlarged basal process, 3-4 enlarged arm suckers in the middle of the dorsal row, followed by 3-4 small suckers, then 4 large suckers before decreasing gradually in size towards the distal tips. The testis is smooth and translucent. The spermatophoric sac is visible but does not contain spermatophores.
IV	Advanced ova (clear amber yellow in appearance) are present in the distal oviduct of the ovary. Less advanced ova (with reticulated surface) are also present in the proximal oviduct. In fully mature individuals, the ovary may fill the posterior thus causing the mantle to bulge. The nidamental glands are displaced anteriorly by the ova. The accessory nidamental glands are mottled orange-red (caused by symbiotic bacteria) in fresh specimens. The *bursa copulatrix* may contain spermatangia if mating has occurred.	Hectocotylization is complete. The testis is large, opaque, and the surface is ridged with fine radiating lines. The spermatophoric sac, containing functional spermatophores, extends from the posterior mantle to the left side of the mantle cavity and opens into the 'penis'.

The fecundity of each animal was assessed from digital photographs of the gonads taken using a Nikon D40 digital camera. Specimens were placed under a dissecting photo-microscope (Novex-Holland, 65.560 RZT-SF) with scale and digital images captured. Captured images were acquired using analySIS® image software and the number of spermatophores and eggs were then counted. A gonado-somatic index (GSI) for females and a spermatophoric complex index (SCI) for males were calculated using the equation:

GSI or SCI= [gonad wt (g) / total wt – gonad wt (g)] x 100

The GSI or SCI expresses the weight of the gonad/spermatophoric complex and related tissues as a percentage of the total body weight minus the reproductive component (='residual body weight').

Statistical analyses

Following examination of the DML data for normality of distribution and homogeneity of variance, potential differences in DML between males and females from Y Foryd were tested using a One-way ANOVA, whilst differences in male and female DML in animals from Traeth Penrhos were examined using a Student t-test. The DML and Ww data of stage II to IV males and females from Y Foryd and Traeth Penrhos were tested for normality of distribution and homogeneity of variances. A one way ANOVA was undertaken on the large sample of data from the stage IV *Sepiola* from Y Foryd to determine whether there were significant differences in DML and Ww between males and females of the same reproductive stage. However, the less powerful Student t-test and Mood's Median tests were used to check if there

were any differences in DML and Ww of the stage II and III animals, where sample numbers were too small for ANOVA. Due to the small sample size of Stage III males from Traeth Penrhos (n= 2) statistical differences between the sexes at this site could not be established.

Following an examination of normality of distribution and homogeneity of variances, differences in gonado-somato indices were examined using a Mood's median test as tested pairs were neither normally distributed nor assumed to have equal variance. The DML and Ww data for male and female *S. atlantica* from Y Foryd and Traeth Penrhos were then examined for evidence of differential growth by testing each pair of size variables x and y for their fit to the allometric equation ($y = ax^b$) which, when logarithmically transformed, becomes ($\log 10y = \log_{10} a + b \log_{10} x$). The constants a (intercept) and b (slope) were estimated by regression analysis (see Richardson et al. 1995).

RESULTS

Distribution of *Sepiola atlantica* around the coast of Anglesey

Figure 1 shows the sites surveyed off the west and east coasts of Anglesey and within the Menai Strait. The north coast of Anglesey was not surveyed as this stretch of coastline is dominated by sea cliffs and small, rocky bays that are unsuitable for seine net surveys using light weight nets. Whilst not part of this study, B.W. Roberts (pers.comm.) reported capturing a single *S. atlantica* in 12 metres of water using a 2 m beam trawl in May 2007 off the coast of Penmon (east coast of Anglesey at the mouth of the Menai Strait). This solitary *Sepiola* was returned alive to the School of Ocean Sciences, Menai Bridge, for use in culture trials (see chapter 3). The GPS co-ordinates and level of wave exposure at each trawled site are given in Table 1 and these data show that *S. atlantica* was collected on a number of shores of varying wave exposure, from very sheltered to exposed shores, with most of the shores sampled being classified as either sheltered or semi-exposed according to Ballantine's biologically defined exposure scale.

Table 3 summarizes the abundance of *S. atlantica* around the coast of Anglesey between 2006 and 2008 and shows that in general, *Sepiola* are absent from the shallow subtidal zone until July, becoming highly abundant in July and August, with a consistent decline in the number of animals from September to October. After October *Sepiola* were absent from the shallow subtidal. The most *S. atlantica* collected in one haul of the seine net was 23 *Sepiola* caught in July 2006 from Trearddur Bay, whilst the largest mean number of *Sepiola*.haul^{-1} was 6.33 *Sepiola* from Y Foryd in July 2007 and 6.25 *Sepiola*.haul^{-1} from Traeth Bychan in July 2006. The largest number of *Sepiola* collected from four hauls was 42 *Sepiola* collected in July 2007 from Y Foryd, while 31 *Sepiola* were caught at this site the previous day.

Later in August 2006, 37 *Sepiola* were collected in four hauls at Y Foryd. Twenty one *S. atlantica* were collected from five hauls at Traeth Penrhos in July 2006 and a further 17 animals were caught in July 2007. A total of 24 *Sepiola* were collected from four hauls at Porth Tywyn mawr in August 2006. During the course of the study it was found that when the seine net was deployed when wind speeds approached, or exceeded 8 mph, no *Sepiola* were caught. During windy conditions waves were generated approaching 1m in height. Although *S. atlantica* were reported from Rhosneigr, none were collected when the site was visited in July 2006, although on this occasion sampling coincided with winds >8 mph and wave action.

Table 3. Summary of the numbers of *Sepiola atlantica* collected from various locations around the coast of Anglesey between 2006 & 2008. n/d = no data.

Year	Site	No. caught	No. of hauls	Max No. per haul	Mean No. per haul
2006					
June	Llanddona	0	4	0	0
	Y Foryd	0	10	0	0
July	Beaumaris banks	16	7	10	2.28
	Llanddona	2	4	1	0.5
	Porth Tywyn-mawr	12	4	5	3
	Rhosneigr*	0	3	Na	Na
	Traeth Bychan	25	4	9	6.25
	Traeth Llanddwyn	0	4	Na	Na
	Traeth Penrhos	30	9	6	3.33
	Trearddur Bay	23	1	23	23
August	Beaumaris banks	2	4	1	0.5
	Llanddona○	11	4	n/d	
	Llanfairfechan○	9	6	n/d	
	Porth Tywyn-mawr	39	8	8	4.87
	Traeth Penrhos*	3	7	1	0.42
	Y Foryd	45	8	16	5.62
September	Beaumaris banks**	0	4	Na	Na
	Llanddona○	7	3	6	
	Llanfairfechan○	1	4	1	
	Porth Tywyn-mawr	7	5	4	1.4
	Y Foryd	2	4	2	0.5
October	Beaumaris banks**	0	2	Na	Na
	Porth Tywyn-mawr◊	0	4	Na	Na
	Y Foryd	3	4	1	0.75
November	Y Foryd	0	4	Na	Na
December	Trearddur Bay	0	4	Na	Na
	Llanddona○	1	6	1	
2007					
January	Y Foryd	0	4	Na	Na
	Llanddona○	0	8	Na	Na
	Llanfairfechan○	0	8	Na	Na
February	Y Foryd	0	4	Na	Na
March	Traeth Penrhos◊	0	1	Na	Na
	Y Foryd	0	4	Na	Na

April	Y Foryd	1	8	1	0.12
May	Traeth Penrhos	0	4	Na	Na
	Y Foryd	0	4	Na	Na
June	Porth Tywyn-mawr	0	4	Na	Na
	Traeth Penrhos*◊	0	12	Na	Na
	Y Foryd	0	4	Na	Na
July	Traeth Bychan	0	2	Na	Na
	Traeth Penrhos	22	12	7	1.83
	Y Foryd	76	12	15	6.33
August	Traeth Penrhos◊	1	12	1	0.08
	Y Foryd	14	12	3	1.16
September	Y Foryd	0	4	Na	Na
	Traeth Penrhos	0	4	Na	Na
October	Traeth Penrhos	0	4	Na	Na
	Y Foryd	0	4	Na	Na
2008					
June	Portheilian	1	4	1	0.25
July	Traeth Penrhos	10	12	3	0.83
	Y Foryd	36	12	9	3
August	Traeth Penrhos*◊	1	12	1	0.08
	Y Foryd	9	12	4	0.75

Na = Not applicable, n/d = No data, * = Occasional wave action, ** = Strong currents on occasion, ◊ = Large quantities of algal material present on occasion, ○ = A subtidal population

Seasonal distribution of *S. atlantica* at Traeth Penrhos & Y Foryd

Figure 5 shows that there is a clear seasonal distribution of *S. atlantica* at Traeth Penrhos (exposed) and Y Foryd Bay (very sheltered) from 2006-2008. With the exception of July 2006 when sampling did not take place at Y Foryd Bay, *S. atlantica* were collected from July through September although a single juvenile *Sepiola* was collected in April 2007 during a period of unseasonably clement weather for April. While air temperatures were warm in April, this was not reflected in the

mean surface seawater temperature for April 2007 of 10°C, although the following year the mean seawater temperature for April 2008 was cooler at 8°C. Three *Sepiola* were collected in October 2006 yet *S. atlantica* were not present in October 2007 and 2008. In each of the three years when sampling took place, *S. atlantica* were only present during July and August at Traeth Penrhos. It is also evident from Figure 5 that *S. atlantica* were generally most abundant in the shallow subtidal zone at Y Foryd in July each year when seawater temperatures approached 17°C. In the August of 2006, 2007 and 2008, the number of animals caught decreased dramatically and were absent once temperatures dropped below 15°C. Once again, *S. atlantica* were most abundant in July at Traeth Penrhos when seawater temperatures approached 17°C. In August each year the number of *Sepiola* at Traeth Penrhos declined dramatically compared to the numbers of animals found in July. *Sepiola* on this exposed site appeared to leave the shallow subtidal zone slightly earlier when temperatures fell below 16°C.

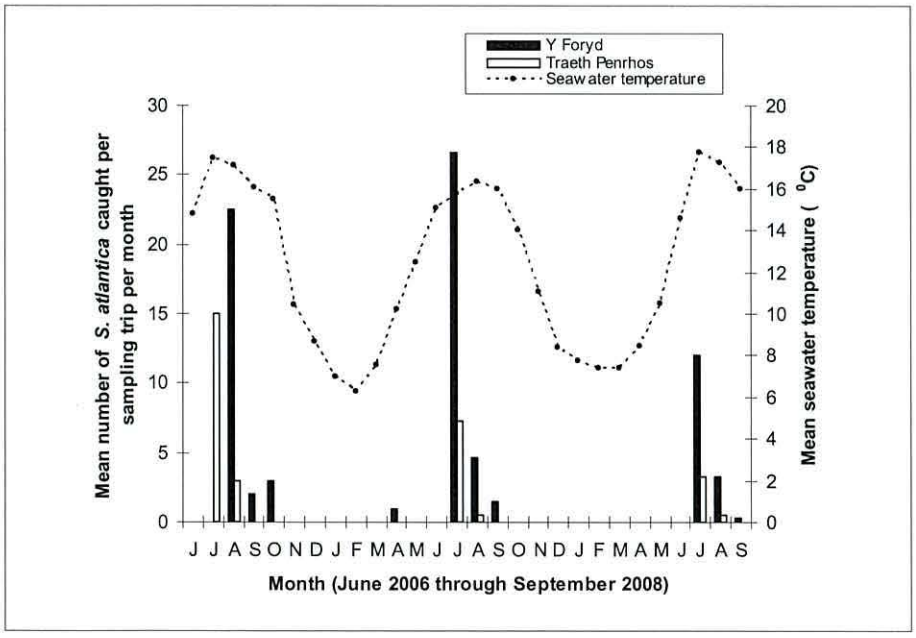


Fig. 5 The seasonal distribution of *S. atlantica* collected from Traeth Penrhos and Y Foryd bay, Anglesey between June 2006 and September 2008. N.B. Sampling was not undertaken during every month of the year (see Table.3).

Associated fauna

Data on the bycatch collected in the seine nets were recorded to investigate whether the presence of certain species in the shallow subtidal coincided with the appearance and abundance of *S. atlantica.* Tables 4a and 4b list the epibenthic and hyperbenthic macrofauna caught as bycatch during the seine net surveys at Y Foryd bay and Traeth Penrhos between 2006 and 2008. Those species listed as 'Very Common' were present in virtually every haul, 'Common' species were present in most hauls, while those species listed as 'Rare' were only present in a small number of hauls (<5). Species marked with an asterisk (*) denotes that when they were collected they were associated with a haul of macro-algae. The faunal community at Y Foryd bay was dominated by the sand eel, *Ammodytes tobianus*, followed by the brown shrimp, *Crangon crangon*, the sand goby, *Pomatoschistus minutus* and the greater sand eel, *Hyperoplus lanceolatus*. Numerous juvenile plaice, *Pleuronectes platessa*, were also commonly encountered as well as the sprat, *Sprattus sprattus* which on occasion was caught in large shoals of thousands of fish which filled the seine net. Owing to the presence of large number of sand eels at Y Foryd Bay the area is frequently visited by professional bait collectors and sport fishermen. By contrast, the faunal community at the exposed site, Traeth Penrhos, was dominated by an abundance of juvenile flatfish; *P. platessa* and turbot, Psetta *maxima*, while juvenile flounder, *Platichthys flesus* were also common. Other frequently, but less abundant species included *C. crangon* and the predatory lesser weaver fish, *Echiichthys vipera*, while there was an increase in the number of crustacean species which included a *Gammarus* sp. and *Synisoma lancifer* which were always encountered when storm driven rafts of loose macro-algae were caught in the seine net. The only other cephalopod species encountered during the seine net surveys was the squid *Alloteuthis subulata* which although rare at both sites, is a species that

spawns in nearby Caernarfon Bay (pers.obs). There was no apparent association between the abundance and presence of *S. atlantica* and species in the bycatch.

Table 4a. By-catch species observed during seine net surveys at Y Foryd between the months of June to September during 2006, 2007 & 2008 beach seine surveys.

Phylum (Class)	**Species**		
	Very common	**Common**	**Rare**
Cnidaria (Scyphomedusae)			*Chrysaora hysoscella*
Ctenophora (Tentaculata)		*Pleurobrachia pileus*	
Arthropoda (Eumalacostraca)	*Crangon crangon*	*Carcinus maenas*	*Neomysis integer* *Macropodia rostrata* *Pagurus bernhardus*
Mollusca (Cephalopoda)			*Alloteuthis subulata*
Chordata (Osteichthyes)	*Pomatoschistus minutus* *Ammodytes tobianus* *Hyperoplus lanceolatus*	*Sprattus sprattus* *Atherina presbyter* *Echiichthys vipera* *Pleuronectes platessa* juv.	*Salmo trutta* *Chelon labrosus* *Scomber scombrus* *Scopthalmus rhombus* *Eutrigla gurnardus* juv. *Platichthys flesus* *Syngnathus acus* *Agonus cataphractus* *Dicentrarchus labrax* *Spinachia spinachia*

Table 4b. By-catch species observed during seine net surveys at Traeth Penrhos between the months of June to September during 2006, 2007 & 2008 beach seine surveys. * denotes species collected in association with macro-algae.

Phylum (Class)	Species		
	Very common	**Common**	**Rare**
Cnidaria (Scyphomedusae)			*Chrysaora hysoscella* *Cyanea capillata*
Ctenophora (Tentaculata)			*Pleurobrachia pileus*
Arthropoda (Eumalacostraca)	*Crangon crangon*	*Diogenes pugilator* *Portumnus latipes* *Synisoma lancifer** *Gammarus sp.**	*Carcinus maenas* *Macropodia rostrata* *Palaemon serratus* *Liocarcinus depurator*
Mollusca (Cephalopoda)			*Alloteuthis subulata*
Chordata (Osteichthyes)	*Echiichthys vipera* *Psetta maximus* *Pleuronectes platessa* juv.	*Pomatoschistus minutus* *Platichthys flesus* *Entelurus aequoreus*	*Eutrigla gurnardus* juv. *Spinachia spinachia* *Trisopterus minutus* *Scomber scombrus* *Scopthalmus rhombus* *Solea solea* *Atherina presbyter* *Ammodytes tobianus* *Sprattus sprattus* *Syngnathus acus* *Nerophis lumbriciformis* *Pomatoschistus microps* *Labrus bergylta* juv. *Trigla lucerna* juv. *Chelon labrosus* *Gaidropsaurus vulgaris*

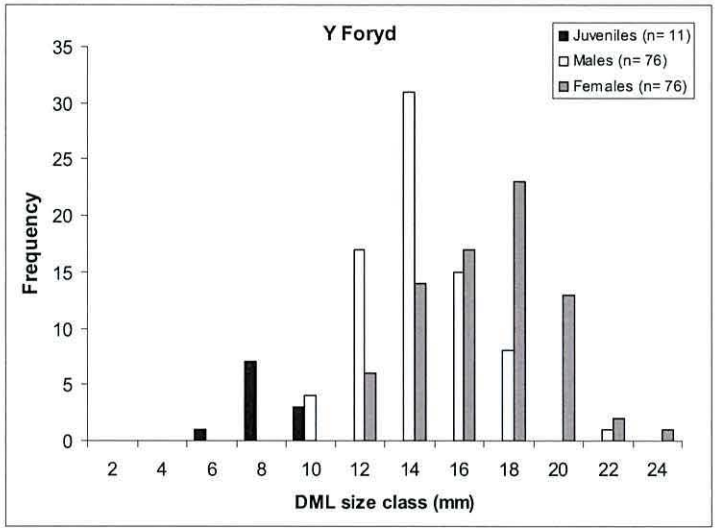
Population structure

Throughout the seine net survey conducted from beaches around the coast of Anglesey the largest *Sepiola* encountered was 28mm in DML, collected from Porth Tywyn Mawr in August 2006, while the smallest was 5 mm in DML, collected from Trearddur Bay in July 2006. A comparison of the seasonal distribution and population structure of *S. atlantica* from Y Foryd and Traeth Penrhos showed that two hundred and two *S. atlantica* were collected from Y Foryd between August 2006 and September 2008 (Figure 6a). The figure shows that *Sepiola* from this site ranged in size between 7 and 24 mm DML. Sixty-seven *S. atlantica* were collected from Traeth Penrhos between July 2006 and August 2008, with animals ranging in size from 7 to 20 mm DML (Figure 6b). The ratio of males:females of the *Sepiola* from Traeth Penrhos was 1.14:1 (n= 24:21), the remaining twenty two animals showed no signs of sexual differentiation and were classed as juveniles. The ratio of males to females examined from Y Foryd was 1:1 (n= 76:76) and fifty juvenile cuttlefish.

Figures 6a & b also shows the length-frequency distributions for juvenile, male and female *S. atlantica* collected from Y Foryd and Traeth Penrhos during the 2006-2008 seine net survey. From Figure 6a it is evident that juveniles from Y Foryd ranged in size between 7 and 11mm (mean = 9.33 ± 1.43mm SD), while males measured between 11 and 22 mm DML (mean = 15.20 ± 2.12mm SD) and females between 12 and 24 mm in DML (mean = 17.75 ± 2.55mm SD). A One-way ANOVA showed that Y Foryd females were significantly ($p<0.001$) larger than males. Figure 6b shows that juveniles from Traeth Penrhos ranged in size between 7 and 10 mm DML (mean = 10.28 ± 1.63 mm SD), while males ranged in size between 11 and 18 mm in DML (mean = 13.82 ± 1.91 mm SD), and females between 10 and 20 mm

DML (mean = 16.20 ± 2.66 mm SD). A Student t-test revealed that females were significantly (p= 0.001) larger than males.

a)



b)

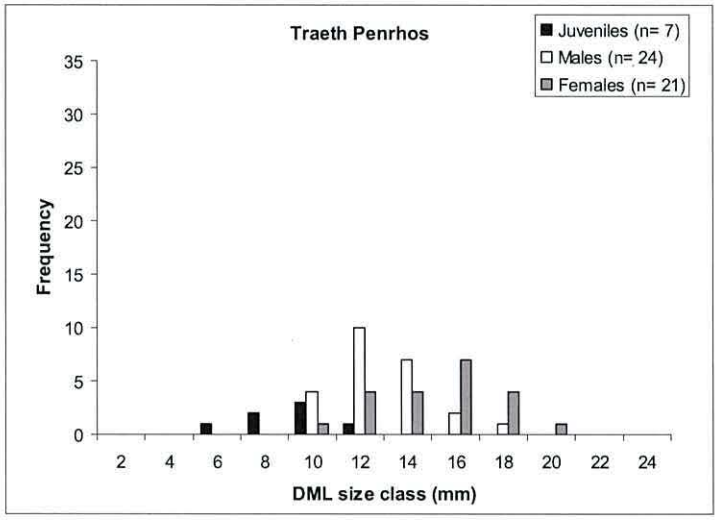


Fig. 6 Length-frequency distributions of juvenile, male and female *Sepiola atlantica* collected between 2006 & 2008 from a) Y Foryd and b) Traeth Penrhos.

Figure 7 shows the combined seasonal length-frequency distribution data for *S. atlantica* for the months of July, August, September and October between 2006 and 2008 collected from Y Foryd and Traeth Penrhos and illustrates the low abundance of

Sepiola at Traeth Penrhos between August & October. As only low numbers were collected it is difficult to draw any conclusions about changes in the population structure during these specific months. At Y Foryd, *Sepiola* had a low abundance during September and October. During all the four months when *Sepiola* were present at the two locations a wide size range of animals were present with representatives of the smallest and largest animals (in terms of DML) encountered during the course of the study. Figure 7 shows a bimodal distribution in the population at Y Foryd during the months of July and August, which may indicate the presence of two cohorts of *S. atlantica* from two different spawnings the previous year.

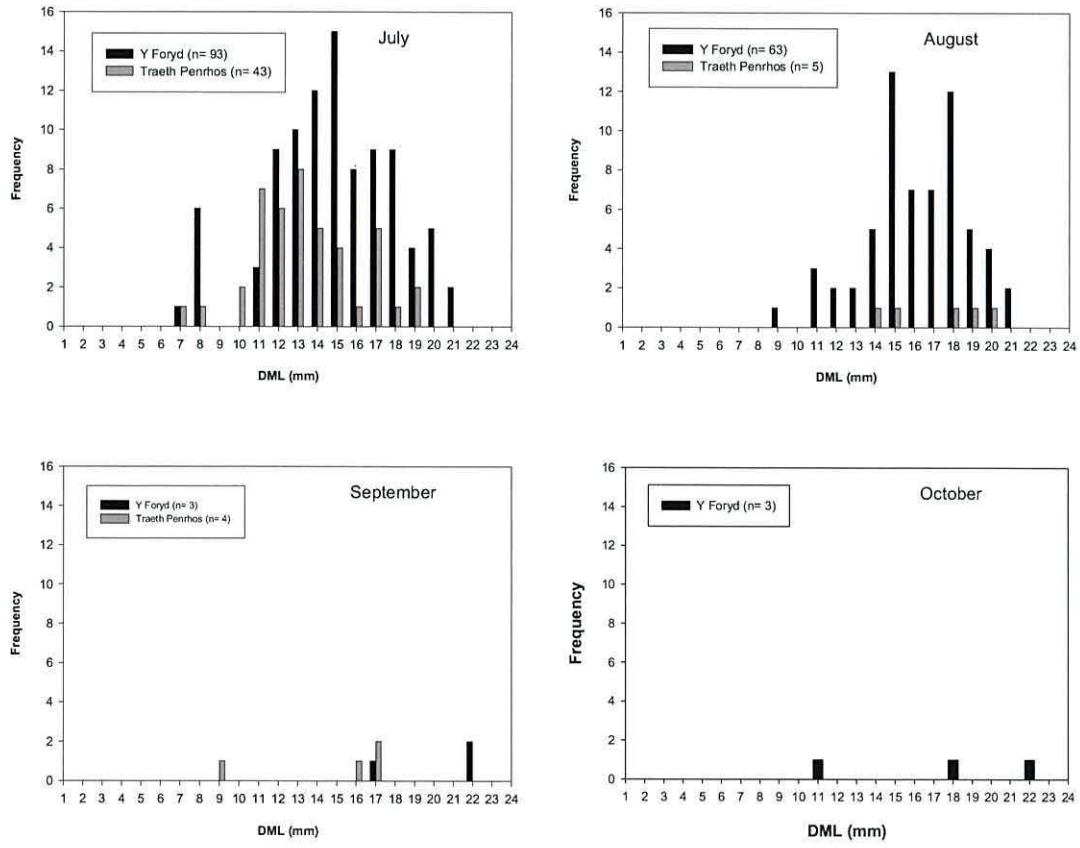


Fig. 7 Combined data from seine surveys conducted between July and October 2006 & 2007 to compare the monthly length-frequency distributions of *Sepiola atlantica* from Y Foryd and Traeth Penrhos.

Maturity

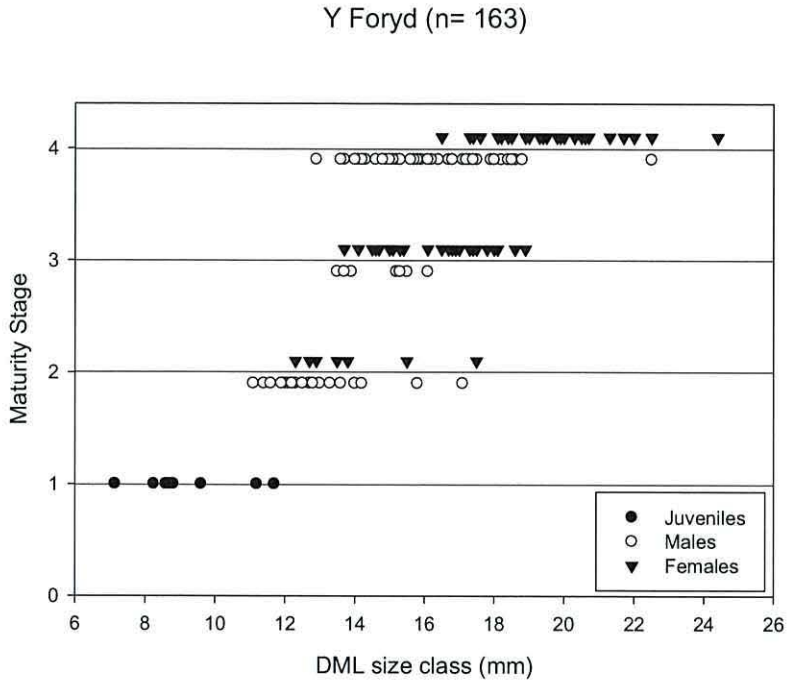
Examination of the relationship between maturity stage and body size in *S. atlantica* showed a general trend of increasing maturity stage with increasing body size (see Table 5, Figures 8a, b) at both sites. There was considerable overlap in the size ranges of animals from each of the four maturity stages, with the exception of stage III males and females from Traeth Penrhos, where the two males recorded were smaller in size than the smallest of the females collected. Table 5 suggests that stage II males from Y Foryd are smaller than stage II females from the same site, although a Mood's Median test showed that there was no significant difference (Chi-Square= 1.76 , $p>0.05$, df = 25). A Student t-test showed that stage III males from Y Foryd were significantly ($t = 2.58$, $p = 0.014$, df = 37) smaller in DML than stage III females, while a One-way ANOVA showed a significant difference ($F = 92.18$, $p = <0.001$, df = 86) in DML between stage IV males and females from Y Foryd. A comparison of male and female *S. atlantica* at each maturity stage from Traeth Penrhos showed that there was no significant difference ($t = -1.04$, $p>0.05$, df = 19) in DML between stage II male and female *Sepiola*, while a significant difference ($t = -3.72$, $p= 0.002$, df = 14) in DML was observed between stage IV males and females. A comparison of the wet weight (Ww) of males and females of each maturity stage showed that there was no significant difference ($p>0.05$) between the Ww of male and female from stage II or stage III from Y Foryd (Stage II; $t = -0.82$, df = 24, Stage III- $t = -1.61$ df = 37). However, a One-way ANOVA demonstrated that stage IV females were significantly ($F = 27.68$, $p = <0.001$, df = 86) heavier than males. Student t-tests showed that there were no significant differences ($t = -1.64$, $p>0.05$, df = 19) in Ww between stage II males and females from Traeth Penrhos as well as between stage IV males and females ($t = -1.02$, $p>0.05$, df = 14).

Stage I juveniles from Y Foryd had a mean DML of 9.33 mm ± 1.4 SD and a mean Ww of 0.39g ± 0.1 SD, while juvenile *Sepiola* from Traeth Penrhos appeared to be larger in size and heavier in weight (mean DML = 10.28 ± 1.6mm, mean Ww = 0.59 ± 0.07 g). Table 5 shows that at both sites, female *S. atlantica* had a greater DML and Ww than males, while in general, but not in all instances, male and female *S. atlantica* from Y Foryd were larger in DML and had a greater Ww than *S. atlantica* from Traeth Penrhos. Since there was a small (n= 21) sample size of *S. atlantica* collected from Traeth Penrhos it was not possible to directly compare the DML and Ww of *Sepiola* of each maturity stage and sex with data from those animals from Y Foryd using a Factorial ANOVA.

Table 5. Mean dorsal mantle length (DML) and Wet weight (Ww) at each maturity stage for juvenile, male and female *Sepiola atlantica* collected from Y Foryd and Traeth Penrhos. N/a = Not applicable. Asterisks denote the statistical tests used to determine significance. *One-way ANOVA. **Student t-test, ***=Mood's Median test.

DML Y Foryd					
Maturity stage	**N**	**Mean ♂ DML (mm) ± SD**	**N**	**Mean ♀ DML (mm) ± SD**	**Significant difference between ♂ & ♀**
II	19	12.97 ± 1.4	7	14.02 ± 1.8	Not significant***
III	8	14.81 ± 0.9	31	16.22 ± 1.4	Significant**
IV	49	16.13 ± 1.7	38	19.68 ± 1.6	Significant*
DML Traeth Penrhos					
II	14	12.70 ± 1.2	7	13.45 ± 2.1	Not significant**
III	2	14.35 ± .07	6	16.33 ± 1.2	N/a
IV	8	15.63 ± 1.7	8	18.50 ± 1.3	Significant**
Ww Y Foryd					
Maturity stage	**N**	**Mean ♂ Ww (g) ± SD**	**N**	**Mean ♀ Ww (g) ± SD**	**Significant difference between ♂ & ♀**
II	19	0.81 ± 0.1	7	0.88 ± 0.1	Not significant**
III	8	1.19 ± 0.2	6	1.38 ± 0.3	Not significant**
IV	49	1.58 ± 0.4	38	2.15 ± 0.5	Significant*
Ww Traeth Penrhos					
II	14	0.75 ± 0.1	7	0.93 ± 0.3	Not significant**
III	2	0.88 ± .09	6	1.33 ±0.1	N/a
IV	8	1.54 ± 0.6	8	1.84 ± 0.5	Not significant**

a)



b)

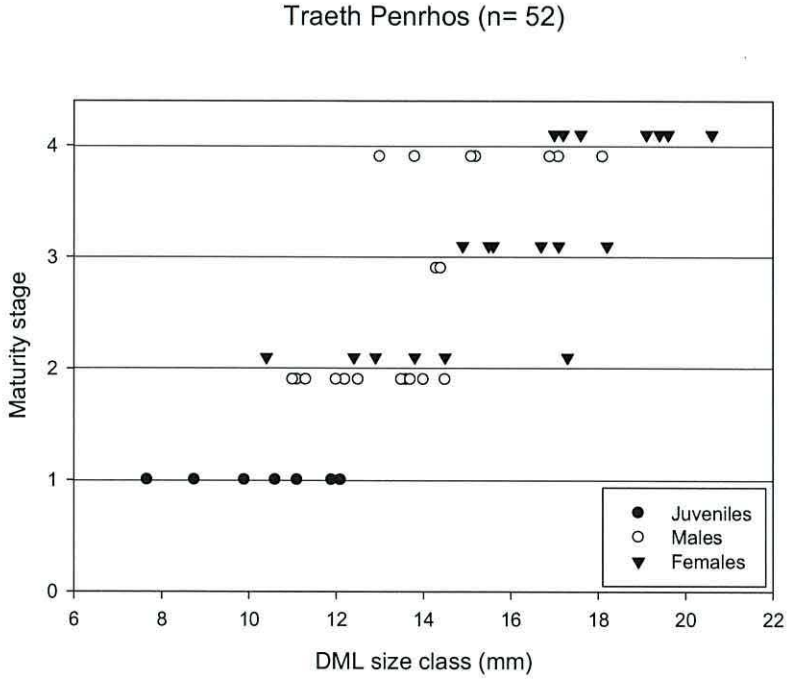


Fig. 8 The relationship between dorsal mantle length (DML) and maturity stage for *Sepiola atlantica* collected from a) Y Foryd and b) Traeth Penrhos. N.B. Data for males and females is slightly offset.

Figure 9a shows that 50% of male *S. atlantica* from Y Foryd were fully mature at 13.2 mm DML and 50% of females were fully mature between 16-17 mm. All males and females had reached sexual maturity by 18mm and 19.9 mm, respectively, indicating that males mature at a slightly smaller size than females. Similarly, Figure 9b indicates that male *Sepiola* from Traeth Penrhos matured at a smaller size than females, with 50% of males reaching full maturity at 14.2 mm DML and 50% of females fully mature at 15.7mm DML. All males and females from Traeth Penrhos were fully mature at 15.9 and 20mm DML, respectively.

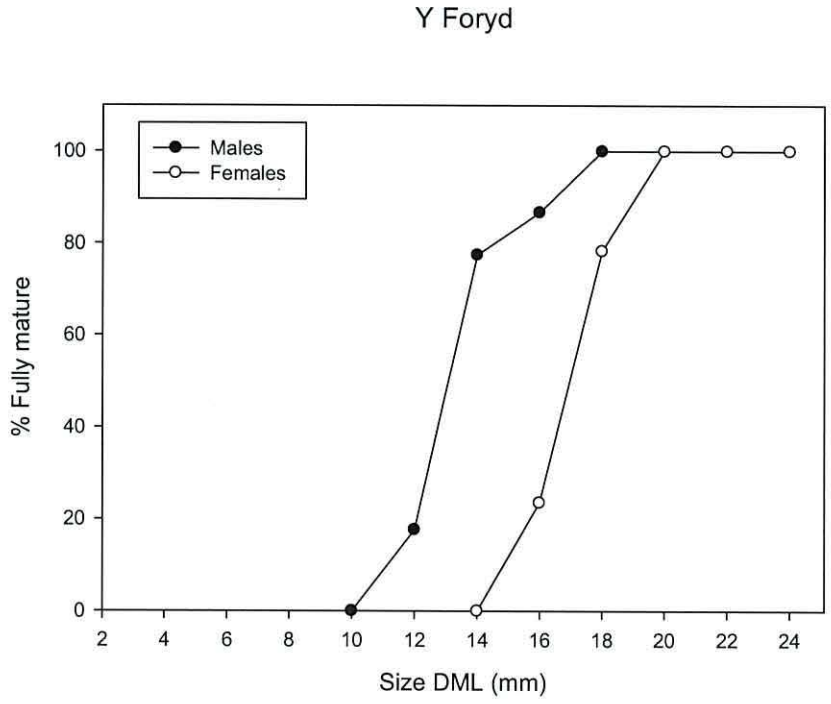


Fig. 9a Cumulative percentage of male and female *Sepiola atlantica* at full maturity (Stage IV) from Y Foryd.

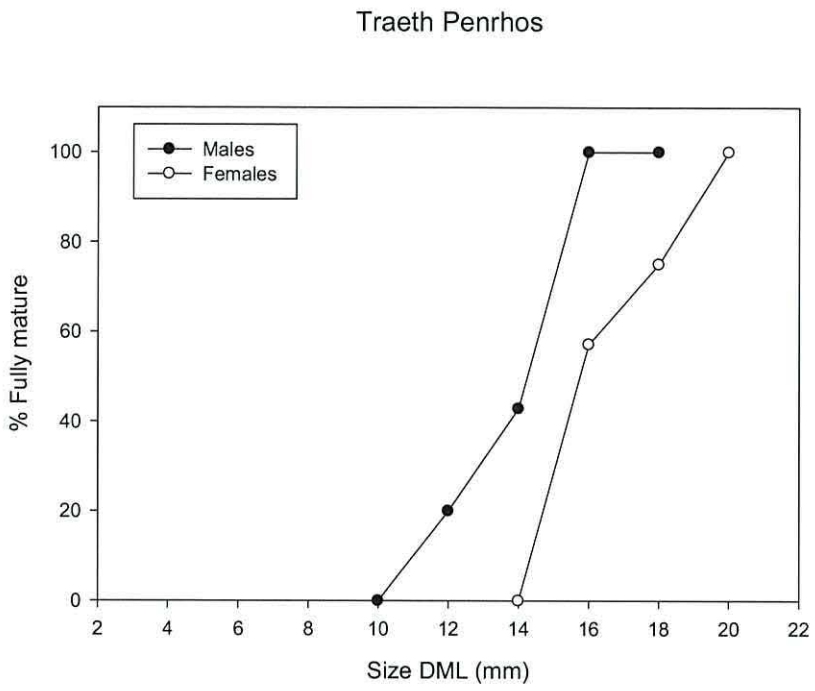


Fig. 9b Cumulative percentage of male and female *Sepiola atlantica* at full maturity (Stage IV) from Traeth Penrhos.

Table 6 gives details of the temporal variation in the number of *S. atlantica* from 10 of the 12 different locations around the coast of Anglesey and North Wales and the different stages of gonad development of animals from these sites. No *S. atlantica* were collected from Rhosneigr while animals from Traeth Llanddwyn were kept alive for culture experiments and were therefore excluded. The data show considerable variability in the number of little cuttlefish and the level of gonad development between locations. However, the low sample number collected from many of the sites makes it difficult to draw firm conclusions from the data. The data from Y Foryd suggest that stage I juveniles were present in low abundance throughout the summer months, stage II sub-adult and stage III *Sepiola* were only present in low abundance in July and August, whilst fully mature animals (stage IV) were present

throughout the season, being most numerous in July and August at which time they were the dominant group within the population. Fewer *S. atlantica* were collected from Traeth Penrhos, although the population structure appears to be similar in several respects to the population from Y Foryd. Stage I juveniles and stage II sub-adults were only present in July and September and in July almost half of the *S. atlantica* caught were stage II animals. Stage III *Sepiola* were present in low numbers at Traeth Penrhos during July and August only, while fully mature animals were found in comparatively low numbers in July, yet later they constituted a large proportion of the population in August and September.

Table 7 shows the monthly percentage occurrence of mated stage IV female *S. atlantica* collected from both study sites during the seine net survey. Despite the small sample size, it appears that between July & October, > 50% of stage IV females present at Y Foryd had previously mated as evident from the presence of spermatangia on the *bursa copulatrix*. Also there was a consistent monthly increase in the number of spermatangia observed on the *bursa copulatrix* of *S. atlantica* collected from Y Foryd. The mean number of spermatangia observed on the *bursa copulatrix* of Y Foryd females between 2006 and 2008 was 22.0 (± 13.83 SD), with a range of 2 to 48 spermatangia (n= 24). Interestingly only one mated female was recorded at Traeth Penrhos with 51 spermatangia on the *bursa copulatrix*.

Table 6. Temporal variation in the number of *Sepiola atlantica* from the different locations and different stages of gonad development. * denotes a subtidal population of *Sepiola atlantica*, n/d = no data.

			Stage I		
Sites	**J (%)**	**J (%)**	**A (%)**	**S (%)**	**O (%)**
Beaumaris banks	n/d	7 (50)	0	n/d	n/d
Llanddona	n/d	2 (100	n/d	n/d	n/d
Llanddona*	n/d	2 (100)	0	0	n/d
Llanfairfechan*	n/d	n/d	5 (55.5)	0	0
Portheilian	1 (100)	n/d	n/d	n/d	n/d
Porth Tywyn-mawr	n/d	9 (75)	0	n/d	n/d
Traeth Bychan	n/d	16 (76.1)	n/d	n/d	n/d
Traeth Penrhos	n/d	6 (13.9)	0	1 (25)	n/d
Trearddur Bay	n/d	13 (100)	n/d	n/d	n/d
Y Foryd	n/d	8 (8.6)	2 (3.1)	0	1 (25)
			Stage II		
Beaumaris banks	n/d	5 (35.7)	0	n/d	n/d
Llanddona	n/d	0	n/d	n/d	n/d
Llanddona*	n/d	n/d	1 (9.0)	2 (28.5)	n/d
Llanfairfechan*	n/d	n/d	2 (22.2)	1 (100)	0
Portheilian	n/d	n/d	n/d	n/d	n/d
Porth Tywyn-mawr	n/d	2 (16.6)	9 (23.6)	n/d	n/d
Traeth Bychan	n/d	2 (9.5)	n/d	n/d	n/d
Traeth Penrhos	n/d	20 (46.5)	0	1 (25)	n/d
Trearddur Bay	n/d	0	n/d	n/d	n/d
Y Foryd	n/d	17 (18.2)	9 (14.2)	0	0
			Stage III		
Beaumaris banks	n/d	2 (14.2)	0	n/d	n/d
Llanddona	n/d	0	n/d	n/d	n/d
Llanddona*	n/d	n/d	2 (18.1)	4 (57.1)	n/d
Llanfairfechan*	n/d	n/d	2 (22.2)	0	1 (100)
Portheilian	n/d	n/d	n/d	n/d	n/d
Porth Tywyn-mawr	n/d	0	7 (18.4)	n/d	n/d
Traeth Bychan	n/d	1 (4.7)	n/d	n/d	n/d
Traeth Penrhos	n/d	7 (16.2)	1 (20)	0	n/d
Trearddur Bay	n/d	0	n/d	n/d	n/d
Y Foryd	n/d	24 (25)	15 (23.8)	0	0
			Stage IV		
Beaumaris banks	n/d	0	2 (100)	n/d	n/d
Llanddona	n/d	0	n/d	n/d	n/d
Llanddona*	n/d	n/d	8 (72.7)	1 (14.2)	n/d
Llanfairfechan*	n/d	n/d	0	0	0
Portheilian	n/d	n/d	n/d	n/d	n/d
Porth Tywyn-mawr	n/d	1 (8.3)	22 (57.8)	n/d	n/d
Traeth Bychan	n/d	2 (9.5)	n/d	n/d	n/d
Traeth Penrhos	n/d	10 (23.2)	4 (80)	2 (50)	n/d
Trearddur Bay	n/d	0	n/d	n/d	n/d
Y Foryd	n/d	44 (47.3)	37 (58.7)	3 (100)	3 (75)

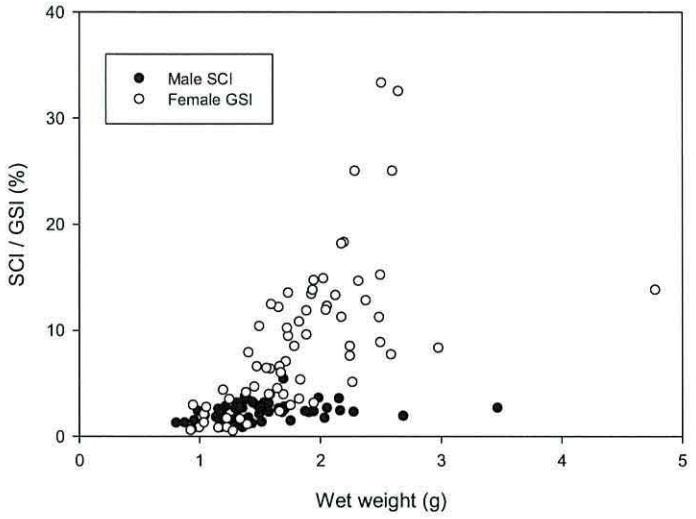
Table 7. Monthly percentage occurrence of mated sexually mature female *Sepiola atlantica* collected during beach seine surveys from July to October, 2006, 2007 and 2008 along with the mean number (± Standard Error) of spermatangia observed on the *bursa copulatrix* of each animal and (sample number). n/d = no data.

	Monthly % occurrence of mated stage IV females						
Site	**n**	**July**	**n**	**August**	**n**	**September**	**n**	**October**
Y Foryd	16	68.7	18	55.5	3	66.6	1	100
Traeth Penrhos	6	16.6	2	100	0	n/d	0	n/d
	Mean No. of spermatangia ± SE / Minimum & (Maximum)						
Site	**n**	**July**	**n**	**August**	**n**	**September**	**n**	**October**
Y Foryd	12	15.58 ± 3.85 / 2 (45)	10	26.30 ± 3.61 / 14 (48)	1	41	1	37
Traeth Penrhos	1	51	1	14	0	n/d	0	n/d

Figures 10a & 10b show the relationships between Ww and male SCI and female GSI of *S. atlantica* collected from Y Foryd and Traeth Penrhos. At both sites there was a high degree of variability in both SCI and GSI of animals of a similar Ww, with the female gonads constituting a far greater percentage of the total wet weight than the male gonads. There was no significant correlation ($p>0.05$) between Ww and SCI or GSI for males collected from either site, or females from Traeth Penrhos. However, a significant ($p<0.001$) Pearson's correlation coefficient of 0.62 between Ww and GSI for Y Foryd females was found although the analysis demonstrates only a weak relationship between the variables. No further comparisons between the variables were therefore undertaken. Data on the mean SCI and GSI of animals from both sites along with the range of observations made are shown in Table 8 and again illustrate the variability in the data although mean percentages for both SCI and GSI are similar at both sites. At both sites there was a significant difference in the total

percentage body weight occupied by the gonads of males and females (Y Foryd, p= <0.001, Chi-square = 40.97 ; Traeth Penrhos, p=0.001, Chi-square = 10.97).

a)



b)

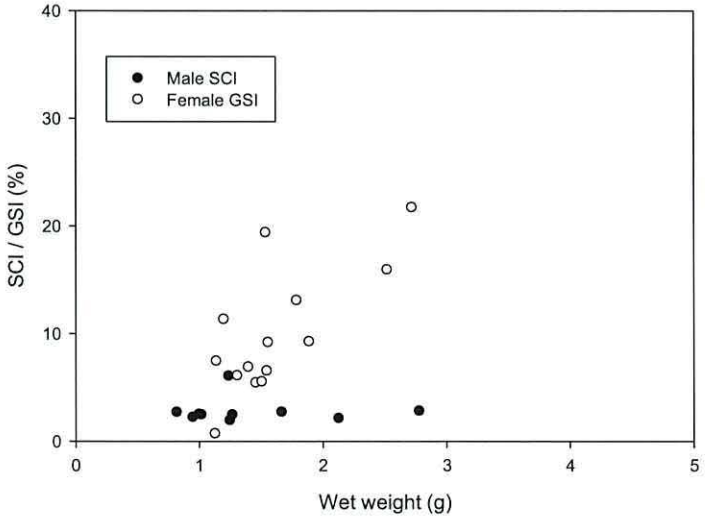


Fig. 10 The relationship between wet weight (Ww), spermatophoric complex index (SCI) and gonadosomatic index (GSI) for male and female *Sepiola atlantica* collected from a) Y Foryd and b) Traeth Penrhos between 2006 & 2008.

Table 8. A comparison of the spermatophoric complex indexes (SCI) and gonadosomatic indexes (GSI) of mature male and female *Sepiola atlantica* collected from Y Foryd and Traeth Penrhos between 2006 and 2008.

Site	Sex	N	Mean Ww (± SE) (g) of *Sepiola*	Range (g)	Mean SCI or GSI (± SE) (%)	Range (%) Min (Max)	Sig. diff in SCI or GSI (%) between ♂ & ♀
Y Foryd	♂	57	1.52 (± 0.05)	0.81 to 3.47	2.35 (± 0.11)	0.81 (5.39)	Significant
Y Foryd	♀	68	1.81 (± 0.07)	0.93 to 4.78	8.70 (± 0.85)	7.08 (33.29)	
Traeth Penrhos	♂	10	1.41 (± 0.19)	0.82 to 2.78	2.78 (± 0.37)	1.92 (6.04)	Significant
Traeth Penrhos	♀	14	1.62 (± 0.12)	1.13 to 2.72	9.88 (± 1.56)	5.84 (21.70)	

Figure 11 shows the relationships between Ww and the number of spermatophores and advanced ova present in *S. atlantica* collected from Y Foryd and Traeth Penrhos and highlights the variability in the data from *Sepiola* of similar weight. Although a significant correlation was found between Ww and the number of either spermatophores or advanced ova counted in *S. atlantica* from both sites, no Pearson's correlations coefficients were >0.8 (see Table 9) and therefore the relationships although significant ($p<0.001$), were deemed too weak for further analysis.

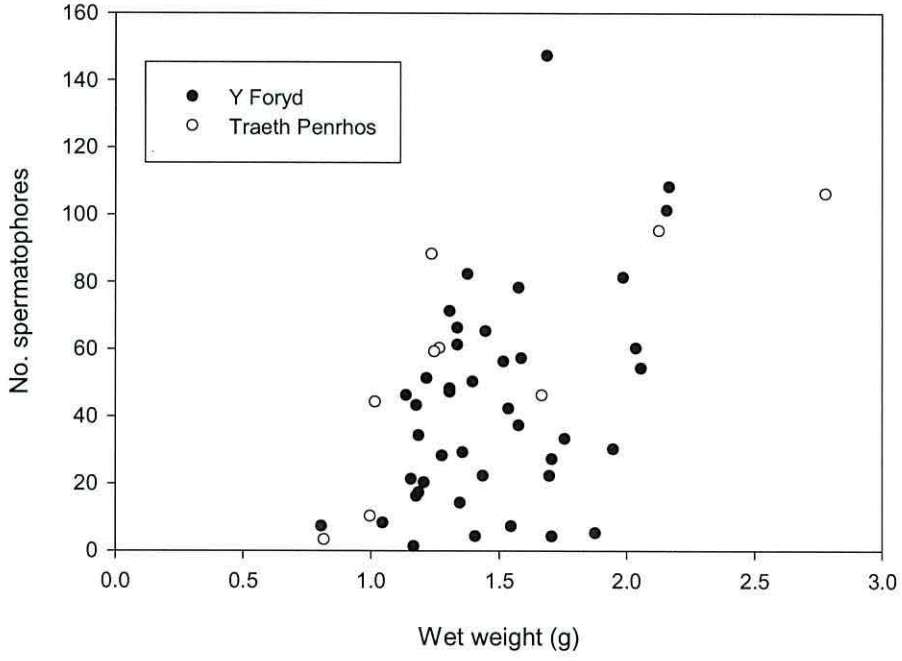


Fig. 11a The relationship between wet weight and number of spermatophores for male *Sepiola atlantica* collected from Y Foryd and Traeth Penrhos between 2006 and 2008.

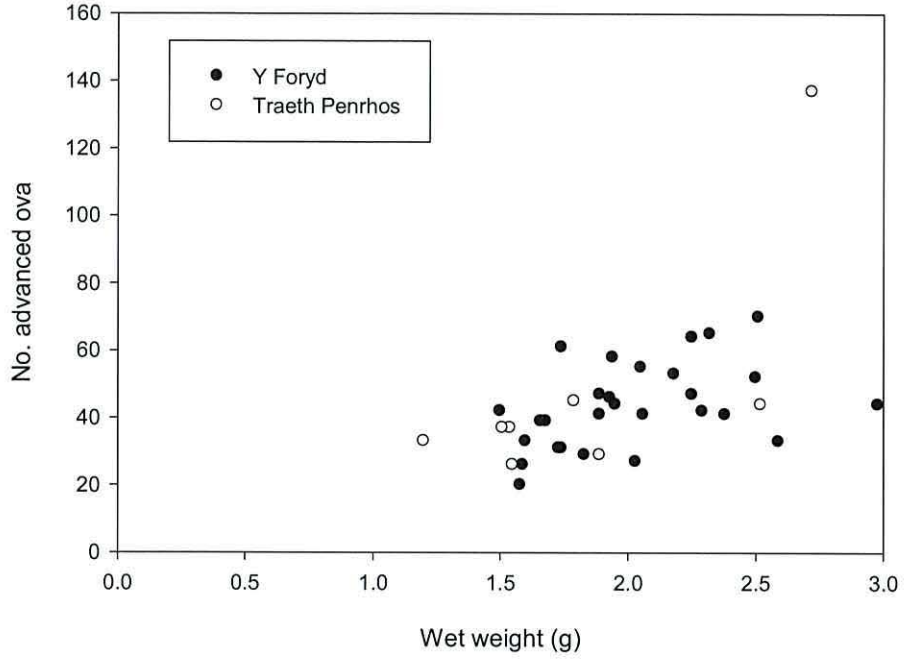


Fig. 11b The relationship between wet weight and number of advanced ova for female *Sepiola atlantica* collected from Y Foryd and Traeth Penrhos between 2006 and 2008.

Table 9. The relationship between Ww and Number of spermatophores or advanced ova found in *Sepiola atlantica* collected from Y Foryd and Traeth Penrhos between 2006 & 2008, with results of correlation analysis between Ww (g) and the number of either spermatophores or advanced ova. *= Significant at $p<0.05$.

Site	Sex	N	Mean Ww (± SE)	Mean No. spermatophores / advanced ova (± SE)	Range Min (Max)	Probability (Pearson's correlation coefficient)
Y Foryd	♂	42	1.48 (± 0.04)	42.86 (± 4.88)	1 (147)	0.005* (0.429)
Y Foryd	♀	28	2.02 (± 0.06)	43.61 (± 2.40)	20 (70)	0.022* (0.430)
Traeth Penrhos	♂	9	1.46 (± 0.21)	56.8 (± 11.90	3 (106)	0.012* (0.787)
Traeth Penrhos	♀	8	1.84 (± 0.18)	48.5 (± 12.9)	26 (137)	0.040* (0.730)

Morphometry of *Sepiola atlantica*

Of those *S. atlantica* collected from Y Foryd, the maximum female Ww recorded was 4.78g (DML of 24.4mm), while the maximum male Ww was 3.47g (DML of 22.5mm). The maximum Ww recorded of a Traeth Penrhos female was 2.72g (DML of 19.4mm) and the maximum Ww attained by a male *S. atlantica* was 2.78g (DML of 18.1mm). Figure 12a shows the curvilinear increase in wet weight with dorsal mantle length in juvenile, male and female *S. atlantica* collected from Y Foryd. Following log10 transformation, the good fit to a straight line model (no hint of curvilinearity in the residual plot), approximate normality in the residuals (Anderson-Darling= 0.671, p= 0.078) coupled with no significant differences in residual variance (Bartlett's statistic= 3.54, p= 0.170, R-sq= 94.01%) indicated that the slopes could be appropriately analysed by regression and ANOVA techniques, the results of which are shown in Table 10b.

Table 10. The analysis of the increase in Wet weight with Dorsal Mantle length in juvenile, male and female *Sepiola atlantica* collected from Y Foryd between 2006-2008.

(a) Slopes, intercepts (± SE) from regression analysis of the increase in Ww with increasing DML.

Sex	Slope (± SE) ($g.mm^{-1}$)	Intercept (SE range) (mg)
Juvenile	2.57 (± 0.28)	1.02 (0.65 - 1.59)
Males	2.46 (± 0.11)	1.59 (1.16 - 2.16)
Females	2.29 (± 0.11)	2.27 (1.66 - 3.09)
Pooled Mean	*2.44 (± 0.11)*	*1.54 (1.20 - 1.99)*

(b) Analysis of variance table with Log10 dorsal mantle length as a covariate, Seq= sequential, Adj= adjusted for entry order into the model. *= significant difference.

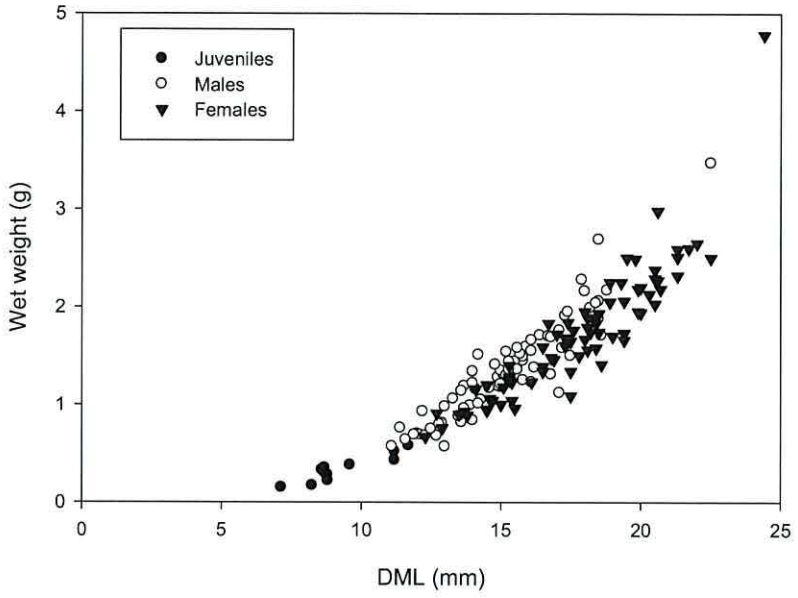
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Log10 Ww	2	4.9333	0.0054	0.0027	0.78	0.461
Log10 DML	1	3.5589	1.7747	1.7747	514.69	<0.001*
Interaction	2	0.0058	0.0058	0.0029	0.84	0.432
Error	157	0.5413	0.5413	0.0034		
Total	*162*	*9.0393*				

Figure 12b shows the increase in wet weight with increasing dorsal mantle length for juvenile, male and female *S. atlantica* collected from both Traeth Penrhos and Y Foryd. Table 11 summarises the relationships between Ww and DML of female and male *S. atlantica* from Y Foryd and Traeth Penrhos in terms of the constants b (slope) and a (intercept) of the allometric equation. Wet weight (Ww) was negatively allometric with respect to dorsal mantle length (DML) in Y Foryd males and females as well as in Traeth Penrhos females such that DML increased at a relatively faster rate than Ww. Wet weight was isometrically related to DML in Traeth Penrhos males showing that Ww increased at the same rate as DML. It is clear from these limited data that there are no consistent gender-related relationship between wet weight and size.

Table 11. Coefficient of allometry for wet weight (Ww) versus dorsal mantle length (DML) in male and female *Sepiola atlantica* from Y Foryd and Traeth Penrhos. Where a= intercept (± SE), b= slope of regression (± SE), r^2= regression coefficient, ns= not significant.

Y Foryd						
Dependent variable (log_{10}) y	**Independent variable (log_{10}) x**	**a**	**b**	**r^2**	**t**	**Relationship**
♂ Ww	♂ DML	-2.80 ± 0.13	2.46 ± 0.11	0.86	4.72	-ve Allometric
♀ Ww	♀ DML	-2.64 ± 0.12	2.29 ± 0.09	0.88	7.31	-ve Allometric
Traeth Penrhos						
♂ Ww	♂ DML	-3.22 ± 0.28	2.81 ± 0.24	0.84	0.76 ns	Isometric
♀ Ww	♀ DML	-2.42 ± 0.18	2.11 ± 0.15	0.90	5.84	-ve Allometric

a)



b)

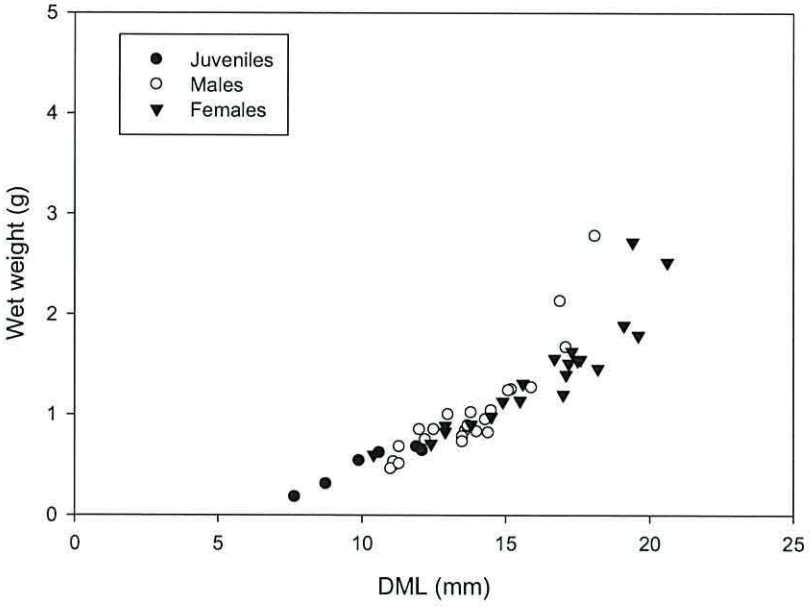


Fig. 12 The relationship between Wet weight (Ww) and dorsal mantle length (DML) of juvenile, male and female *Sepiola atlantica* collected from a) Y Foryd and b) Traeth Penrhos between 2006 and 2008.

DISCUSSION

While several researchers have studied aspects of the biology of *Sepiola atlantica* (Weill, 1927; Adam, 1934; Delamere & Duncan, 1977; Duncan & Pynsent, 1977; 1979; Jacob & Duncan, 1984; Willekens et al. 1984; Boucher-Rodini et al. 1995; Nishiguchi et al. 1998; 2004) the only previous study to examine the ecology and population biology of the species was by Yau & Boyle (1996) in Firemore Bay, Loch Ewe, Scotland. In the present study the distribution, population biology and reproductive cycle of *S. atlantica* from the shallow subtidal zone from two shores with contrasting exposure around the coast of Anglesey were examined.

Distribution (local & temporal)

Cephalopod populations are known to migrate over all geographic scales, whether they are large-scale (thousands of kilometres), meso-scale (hundreds of kilometres) or local or fine-scale movements of one or more kilometres (Boyle & Boletzky, 1996). According to Nesis (1985), squid undertake both horizontal and vertical migrations to areas that offer the optimum environmental conditions for spawning, embryonic development, juvenile and adult growth, with these migrations enhancing genetic exchange within a population (Boletzky, 1983). Migratory cycles are tuned to the reproductive cycle of the species often, but not always corresponding to seasonal cycles, with many neritic cephalopods migrating shoreward in the spring prior to spawning (Boyle & Boletzky, 1996). Boucaud-Camou & Boismery (1991) examined the migratory patterns of the cuttlefish *Sepia officinalis* in the English Channel and found that both mature and immature cuttlefish migrated inshore after the winter solstice and at the very latest around the spring equinox. The authors proposed that the seawater temperatures at depth where the animals over-wintered

were stable and it is the increasing day length that is the stimulus for migration. The autumn migration back to deep water is thought to be triggered by the first rapid fall in seawater temperature in shallow water rather than by the decreasing day length. However, light penetration to depths is limited so it is unlikely that the detection of day length at depth is the likely cause for stimulating migration from depths into shallow waters.

The wide distribution of *S. atlantica* on all shores visited around Anglesey implies that these sandy and muddy shores are suitable habitats for the species despite their varying degrees of wave exposure. It was apparent that even during periods of light wave action *S. atlantica* were absent from the shallow wave and swash zones at sites where they had once been abundant in the nets despite the continued presence of other frequently encountered species. Yau & Boyle (1996) suggested that populations of *S. atlantica* in coastal habitats could be eradicated during extreme weather events and rough seas. Boletzky (1984) noted that heavy swells generated during storms disturb inshore soft sediments, forcing the largest animals to migrate offshore in advance of inclement weather thus avoiding storms and only returning when conditions were once more favourable. While it appears that *S. atlantica* makes seasonal migrations into shallow water, this species has a wide bathymetric range so the sampling of only a narrow band of the coastal zone close to shore does not necessarily demonstrate that regular migrations occur (see also Boletzky, 1983). However, Boletzky (1983) has shown that regular migrations do not occur in all species, since representatives of each maturity stage of another sepiolid *S. robusta*, are encountered throughout the year in inshore populations within its range.

Seasonal distribution

In terms of the time spent in residence in the shallow sublittoral zone my observations show a distinct seasonal distribution of *S. atlantica* at both Y Foryd and Traeth Penrhos that appears to be related to seawater temperature. Various studies (e.g. O'Dor et al. 1982; Boyle et al. 1995; Forsythe et al. 2001) have shown that seawater temperature has a large influence on embryonic development, with embryos generally developing at a greater rate at elevated temperatures. Also a correlation has been established between spawning areas, the distribution of egg masses and specific temperature zones (Bower & Ichii, 2005). Further studies have linked environmental conditions during spawning and the subsequent hatching of cephalopods to recruitment success, hence influencing the population size the following season (Sakurai et al. 2000; Waluda et al. 2001; Waluda & Rodhouse, 2006). According to Semmens et al. (2007) sea surface temperature is the most commonly used parameter to assess the relationship between the distribution of cephalopods and environmental variables as it is an easily obtained proxy for oceanographic variability, although other variables such as rainfall, freshwater discharge from rivers, turbidity, solar flux, sea level pressure, wind speed and direction, sea level variability and salinity may also be important influences on both the distribution and migration of cephalopod species.

My findings differ from those made by Yau & Boyle (1996), who collected one hundred and sixty four *S. atlantica* (sampled between December and August) from December 1988 to July 1992 from Loch Ewe, where *S. atlantica* were present throughout the year. While they collected three animals in December 1988, at other times *S. atlantica* were only recorded in Loch Ewe between March & August, but since they did not normally sample between September and February animals may

have still been present. Around Anglesey *S. atlantica* were only present between July and October and were generally absent at other times of the year when sampling was undertaken. *Sepiola atlantica were* abundant between July and October at the sheltered Y Foryd although one specimen was unusually recorded in April 2007. Peak numbers were collected from Y Foryd in July and August whereas Yau & Boyle (1996) found *S. atlantica* to be most abundant in July. *Sepiola atlantica* arrive in the shallow sublittoral zone at Y Foryd and Traeth Penrhos once seawater temperatures reach 17°C. In a study of *S. atlantica* from the Flemish Banks (Oost Dyck, Buiten Ratel and Kwinte Bank) between 1981 & 1986 Maertens (1988) noted that they appeared seasonally in the spring and summer. Kristensen (1959) noted the presence of *S. atlantica* in coastal waters during the summer, whereas during the winter months they were commonly found 10 km from the coast. Beyst et al. (2001) collected juvenile *S. atlantica* in May in the surf zone off the Belgian coast although they were absent from the surf zone throughout the rest of the year. Lefkaditou & Kaspiris (2005), proposed that large (>22mm DML) *Sepietta oweniana* migrate from deep (400m) to shallower water (40m) off the north-eastern Greek coast between the early summer and the autumn in order to spawn, as is the case with the species in the northern Catalan Sea (Mangold-Wirz, 1963). Collins et al. (2002) investigated the distribution of cephalopods from plankton surveys around the British Isles and found that *S. atlantica* were present in all areas surveyed, with most individuals measuring 2-4mm DML and a few mature specimens (up to 20mm DML) in the autumn and winter. During an eight year study of the seasonal distribution of cephalopod species inhabiting the central and southern North Sea, De Heij & Baayen (2005) found moderate numbers of *S. atlantica* (mean of 49, range of 31 to 91) in February each year, regardless of depth (15-97m), seawater temperatures (between 1.3 and 9.4°C) or

salinity (between 28.4 & 35.5), while similar numbers (mean of 39) were collected between August and September in some years, whilst in other years the species was not collected in the sampling gear (range of 0 to 82). The authors proposed that between August and September two populations were present; one comprising large animals with a mean DML of 25mm that were found when seawater temperatures were between 9 and 12°C and another population comprising smaller animals with a mean DML of 19mm that occurred in warmer water at 21°C, although both populations occurred in seawater with a mean salinity of 34 (practical salinity scale).

From my observations and those in the published literature it is likely that adult *Sepiola atlantica* enter the shallow subtidal zone in the spring and summer for a number of reasons. Firstly, as demonstrated in my study prey species such as *C. crangon* are plentiful in the warm shallow waters around Anglesey. Food abundance together with warm seawater temperatures are the main factors controlling growth in cephalopods (Forsythe & Van Heukelem, 1987). Animals collected as juveniles from Trearddur Bay in early July 2006 grew rapidly in aquaria on a diet of *C. crangon* and *N. integer* (species found in abundance in Trearddur Bay). All these *S. atlantica* became sexually mature, although only one individual spawned in late September (see Chapter IV). Yau & Boyle (1996), found that captive *S. atlantica* readily attacked and consumed species that were associated with seine net catches of *S. atlantica* in the present study such as *N. integer*, *Crangon crangon*. However *S. atlantica* rejected other crustaceans encountered such as *Gammarus* sp., *Carcinus maenas* and *Liocarcinus depurator* along with the fish *Pleuronectes platessa*, and *Pomatoschistus minutus*. Only nine *S. atlantica* from Y Foryd and one animal from Traeth Penrhos caught in the seine nets showed signs of a full stomach.

Secondly, mass inshore migrations offer a good opportunity for mating, where there may be options for mate selection while the increased densities not found in deeper waters means that the chances of meeting potential mates are increased. Linley (2005) briefly examined the distribution of *S. atlantica* in the Menai Strait in the summer of 2005 and collected a pair of cuttlefish in a mating embrace. In 2008, I observed a mating pair of *S. atlantica* when removing animals from their transport box following collection, while *S. atlantica* were also observed to mate in aquaria during the summer (see Chapter 3). The inshore and near shore environment might provide suitable habitats to deposit eggs shortly after mating and fertilisation. However, despite extensive trawling and searching no egg masses were observed in the field. Data from Rosa et al. (2006) indicate that other members of the Sepiolidae, such as the larger *Rossia macrosoma*, are significantly more abundant in deep water between 300 and 600m in depth off the coast of Portugal and Cuccu et al. (2007), have shown that *Neorossia caroli* prefers to lay its egg masses in deep water off Sardinia rather than in shallow water and this may explain the scarcity of recorded egg masses in shallow water. The presence of animals of all maturity stages throughout the summer season around Anglesey implies that inshore migrations occur not only for reproduction. *Sepiola atlantica* were present at the sheltered site at Y Foryd for longer than at the more exposed Traeth Penrhos, possibly because storms and inclement weather were more frequent towards the end of the summer and as a result the Traeth Pehrhos population migrated offshore earlier than the population at Y Foryd.

Population structure

Yau & Boyle (1996) showed that peak recruitment of juvenile *S. atlantica* into the population at Firemore Bay was bimodal. Recruitment began in April and then again in July and August and coincided with a prolonged presence of mature animals in the bay and was indicative of an extended reproductive season. At Y Foryd, juveniles were recorded in July, August and October while their numbers peaked in July and at Traeth Penrhos juveniles were encountered in July and September, again with numbers peaking in July. Therefore recruitment into the Welsh populations at both sites appears to take place at a similar time to the Scottish Firemore Bay population. Interestingly a single juvenile *S. atlantica* was recorded at the Foryd in April, therefore it is possible that recruitment may take place in April, before the main population moves inshore to breed; presumably the April individual was the progeny from a late hatching the previous year. At Y Foryd and Traeth Penrhos mature specimens were collected throughout the season, which would support Yau & Boyle's theory of an extended reproductive season, with mature animals being most abundant in July and August when ambient seawater temperatures are at their peak.

Yau & Boyle (1996) found large differences in the population structure of *S. atlantica* from Firemore Bay between successive years and similar observations were made by De Heij and Baayen (2005) for *S. atlantica* in the central and southern North Sea. It is well known that cephalopod stocks fluctuate greatly from year to year (Pierce et al., 2008). The effects of seawater temperature, the availability of food and predation are the main factors that control the number of animals surviving to maturity. When there is no stabilising influence of older animals in the population the effect of these fluctuations is pronounced and is the primary cause of inter-annual variability in cephalopod biomass and numbers (Boyle & Rodhouse, 2005). Boyle &

Boletzky (1996) noted that a combination of cephalopod characteristics such as their short life span, rapid growth rates and their ability to reproduce at an early age were inherent problems when attempting to make generalisations about cephalopod populations, especially when individuals are highly mobile, are known to migrate and are able to avoid many types of sampling gear.

No estimates of *S. atlantica* density could reliably be made during the present study since it was difficult to estimate the sampling efficiency of the net and net avoidance as well as determining the area the seine net covered. Yau & Boyle (1996), however, estimated an overall density of 1.73 *Sepiola*.1000m^2 in the shallow waters of Loch Ewe and suggested that the distribution of this species on the sea floor was patchy, although they admitted that their proposed density was likely to be a gross underestimation of the actual density of *S. atlantica*. Beyst et al. (2001), recorded an average density of 1 *Sepiola*.100m^2 in the surf zone off the coast of Belgium and both Russell (1922) and Stephen (1944) reported high densities of *S. atlantica* in the northern North Sea yet no quantitative data were presented with which to compare with Yau & Boyle's (1996) estimates. Collins et al. (2001) examined the distribution of deep-water cephalopods in the north-east Atlantic and found *S. atlantica* at densities of ~116 *Sepiola*.km^2 at a depth of 150m.

From a total of 173 animals, Yau & Boyle (1996) found that *S. atlantica* from Loch Ewe ranged in size from 3 to 21mm DML. Of the 409 *Sepiola* collected during the course of the present study the smallest animal was 5mm in DML, close to that collected from Loch Ewe, while the largest had a DML of 28 mm, considerably greater than the size of the little cuttlefish collected from Loch Ewe. In fact, a total of 9 animals >22 mm DML was collected from around the coast of Anglesey; De Heij & Baayen (2005) recorded *S. atlantica* up to 31mm DML. The size range of animals

from Y Foryd and Traeth Penrhos were both similar to each other and indeed to those found in Loch Ewe.

Sex Ratios

According to Mangold-Wirz (1963), females are generally more numerous than males within cephalopod populations. The male: female ratio of 1.4:1 for *S. atlantica* in Loch Ewe (Yau & Boyle 1996) is similar to the ratios determined in this study 1.14:1 for Traeth Penrhos and 1:1 for Y Foryd therefore it could be assumed that *Sepiola* populations are approximately equally divided between males and females. De Heij and Baayen (2005) similarly found male and female *S. atlantica* to be present in approximately equal numbers in the central and southern North Sea. Zumholz & Frandsen (2006) determined a male: female ratio of 1:0.91 (n=70) in *Rossia moelleri* from depths of between 182-528m off the west coast of Greenland. Rosa et al. (2006), determined an approximately equal sex ratio of 1.02:1 (n= 923) in *Rossia macrosoma* collected off the coast of Portugal and observed that the male: female ratios were similar regardless of depth. Orsi-Relini & Bertuletti (1989) found a male: female ratio of 1: 0.7 (n= 119) in *Sepiola intermedia* and 1:1.4 in both *Rondeletiola minor* (n= 117) and *Sepietta obscura* (n= 71) in the Ligurian Sea, while Jereb et al., (1997) noted that male *Sepietta oweniana* and *R. minor* from the Strait of Sicily outnumbered their female conspecifics by 1.41:1 (n= 1,840) and 1.31:1 (n= 111) respectively.

Yau & Boyle (1996) estimated the mean size of male *S. atlantica* to be 15.36mm and females to be 15.78 mm in Loch Ewe although there were no significant differences between the mean size (DML) of males and females. In this study, both Y Foryd and Traeth Penrhos females were found to be significantly larger

than males. The mean DML of males was 15.20 mm and 17.75 mm for females from Y Foryd, and from Traeth Penrhos males were 13.82mm and females 16.20mm. Juvenile *S. atlantica* from Loch Ewe ranged in size between 4 and 10 mm DML (Yau & Boyle 1996) and were similar to the 5-11mm size range found during the present study. In the current study and in those conducted by Yau & Boyle (1996) and Collins et al., (2001), no *S. atlantica* <4.0mm DML were captured probably because they were too small to be retained in the fishing gears used. It is interesting to note that the maximum size of *S. atlantica* collected in plankton tows from Loch Ewe by Yau (1994) was also 4.0mm DML (minimum of 1.8 mm) and compares favourably with 4mm, the mean size at settlement (see Chapter 3).

Fecundity

Fecundity in female cephalopods is generally estimated by counting the number of advanced ova present in the ovary of wild specimens, or by counting the number of eggs laid by females in captivity. However, conditions in captivity are often different to those in the wild since counts of ova in wild-caught specimens may give an underestimation of fecundity as spawning may have already been initiated prior to capture (Mangold-Wirz, 1963). Nevertheless, counts of advanced ova in species that are known to migrate to specific spawning grounds may give a realistic estimate of female fecundity providing that the female has just arrived at the spawning ground as long as the species only spawns once. Estimations of fecundity in *S. atlantica* based on the five egg masses spawned in captivity can be made from my observations of egg laying by *S. atlantica* held in captivity (see chapter 3, Table 1). Under laboratory conditions between 8 and 161 eggs were laid in captivity although these numbers varied considerably from the mean numbers of advanced ova (Y Foryd

= 43 (range = 20-70) and Traeth Penrhos = 48 (26-137) observed in wild caught female *S. atlantica* collected from both these sites. When the range in numbers of advanced ova are taken into consideration these estimates appear to be comparable. Estimates of sepiolid fecundity based on observations made during other studies where egg masses were spawned in captivity are similar to my own estimates and are discussed further in chapter 3, while Table 12 shows estimates of both female and male fecundity along with potential fecundity (the sum of total oocyte numbers in the ovary and egg numbers in the female oviduct) in wild-caught sepiolids. Yau & Boyle (1996), examined 24 female *S. atlantica* from Firemore Bay where the numbers of advanced ova ranged between 42 and 126 (mean = 68.6 ± 3.8), and these estimates are not dissimilar to my findings of 20-137 advanced ova found in *S. atlantica* from Y Foryd and Traeth Penrhos. The ova recorded by Yau & Boyle (1996), measured between 1.0 and 4.0 mm in diameter.

Fecundity in male cephalopods is estimated from the number of spermatophores present in the spermatophoric (Needham's sac). In the present study the mean number of spermatophores observed in male *S. atlantica* from Y Foryd and Traeth Penrhos was 42 and 56 spermatophores, respectively, estimates considerably lower than those of Yau & Boyle (1996). The range in number of spermatophores in *S. atlantica* from both sites combined i.e. 1-147 (n=51) is considerably narrower than the range in number of spermatophores found in males from Firemore Bay. It is possible that the fecundity of males in the present study is lower as my observations were made on two populations that appear to make seasonal migrations to the study sites and therefore could have already mated a number of times during their migration into shallower water prior to capture. Hence the number of spermatophores could have been underestimated. The fecundity of the female *S. atlantica* in the Scottish

population was not different from the Anglesey populations. It, therefore, seems likely that the male *S. atlantica* from Loch Ewe have more spermatophores than those in *S. atlantica* from the coastal waters of Anglesey.

Table 12. The reported fecundity of selected female and male sepiolid species. *= potential fecundity.

Females			
Species	**Number of advanced ova**	**Mean**	**Reference**
Sepiola atlantica	42-126	68	Yau & Boyle, 1996
Sepiola rondeleti	80-120		Mangold-Wirz, 1963
Sepiola robusta	117-245*	159*	Salman & Önsoy, 2004
Sepiola steenstrupiana	163-191*	177*	Salman & Önsoy, 2004
Sepiola intermedia	111-407*	231*	Salman & Önsoy, 2004
Sepietta oweniana	150-200		Mangold-Wirz, 1963
Sepietta oweniana	30-103		Bergström & Summers, 1983
Sepietta oweniana	58-236		Salman, 1988
Semirossia patagonica	218*		Önsoy et al, 2008
Rossia macrosoma	382-837*		Laptikhovsky et al. 2008
Rossia moelleri	200-385*		Laptikhovsky et al. 2008
Rossia pacifica	355-1246*		Laptikhovsky et al. 2008
Neorossia caroli caroli	551-609*		Laptikhovsky et al. 2008
Neorossia caroli jeannae	215-825*		Laptikhovsky et al. 2008
Neorossia caroli	24-611		Cuccu et al. 2007
Males			
Species	**Number of spermatophores**	**Mean**	**Reference**
Sepiola atlantica	59-338		Yau & Boyle, 1996
Sepiola robusta	109-386	231	Salman & Önsoy, 2004
Sepiola intermedia	98-217	156	Salman & Önsoy, 2004
Sepietta oweniana	87-824	320	Salman, 1998
Sepietta oweniana	55		Mangold-Wirz, 1963
Euprymna tasmanica	15-106		Norman & Lu, 1997
Rossia macrosoma	85		Mangold-Wirz, 1963
Rossia moelleri	19-56	34	Hoving et al. 2009
Neorossia caroli	40		Cuccu et al. 2007
Semirossia patagonica	13-229		Önsoy et al. 2008
Heteroteuthis dispar	5		Hoving et al. 2008
Stoloteuthis leucoptera	>40		Orsi Relini & Massi, 1991

Gonadosomatic Index

Salman & Önsoy (2004) determined gonadosomatic index values for male and female Sepiolids from the Aegean Sea and estimated the SCI or GSI to be 0.75 to 8.53 % (mean of 4.85 %) and 1.03 to 2.53 % in male and female *S. intermedia*, 12.8 to 22.3 % (mean of 17.5 %) and 0.55 to 1.89 % (mean of 1.67 %) in *S. robusta* and 8.3 to 10.1 % (mean of 9.21 %) and 0.22 to 2.23 % (mean of 1.32 %) in *S. steenstrupiana,* respectively. Salman (1998) studied changes in the GSI of male and female *S. oweniana* in the Aegean Sea and found that the species had a bimodal reproductive pattern. The first peak in female GSI being between April and May where an average GSI of 6.1 % was determined and a second, smaller peak in November with a mean GSI of 4.6 %. However, males were found to have homogenous GSI values throughout the year. Önsoy et al. (2008) reported a GSI for mature female *S. patagonica* of between 13.0 & 29.4 % (mean= 19.5 %) and an SCI of between 3.1 & 14.9 % (mean= 6.7 %) in mature males. Laptikhovsky et al. (2008) found GSI values for female deep sea and polar Rossinae of 6.1 to 20.2% (mean= 10.6) e.g. in *R. macrosoma*, 13.8 to 45.9% (mean= 28.5), in *R. moelleri*, 7 to 9% in *R. pacifica*, 3.5% in *N. caroli caroli* and 10.4 to 10.7% in *N. caroli jeannae.*

Unlike my own findings, Yau & Boyle (1996) reported a significant linear correlation between female gonad development (GSI) and male spermatophoric complex index (SCI) for *S. atlantica* from Loch Ewe. It is possible that a significant linear relationship was not established in my study as the animals that were examined were of similar size in terms of DML, and some may have recently spawned and therefore the GSI would have been lower compared with animals of a similar size that had not yet spawned and were gravid. Yau & Boyle (1996) found that the ovary of fully mature females accounted for as much as 22% of the residual body weight,

while in males the spermatophoric sac complex and testis accounted for only ~7.8% of residual body weight. Similarly, in the present study it was found that the ovary of fully mature females accounted for up to 33% (mean of 8.7%) and 21% (mean of 9.8%) of the residual body weight at Y Foryd and Traeth Penrhos, respectively. In males from Y Foryd the spermatophoric sac complex and testis accounted for up to 5% (mean of 2.35%) of the residual body weight, while a maximum SCI of 6% (mean of 2.78%) was determined for mature males from Traeth Penrhos, similar to the Firemore Bay population. Both my findings and those of Yau & Boyle (1996) are comparable to those made on other members of the Sepiolinae and Rossinae.

Copulation

The implantation of spermatangia is a commonly used sperm transfer strategy in male oceanic and deepwater cephalopods (Nesis, 1995). While the mechanism responsible for the implantation of spermatangia is not clear, Hoving et al. (2009) suggest that the implantation mechanism is likely to be a combination of both a mechanical means, such as the presence of the oral spike in the spermatangia of the Japanese common squid, *Todarodes pacificus,* or the oral tubular extension in the spermatangia of *Octopoteuthis sicula* which forcefully pierces the mantle tissue, or through chemical means such as the secretion of cement bodies secreted orally from the spermatangium of *R. moelleri* aids implantation through lysis of the surrounding tissue. Hoving & Laptikhovsky (2007) found that the spermatophores of the squid *Moroteuthis ingens* are able to autonomously implant into muscle tissue. In the Sepiolinae, spermatangia are stored on the *bursa copulatrix* located near the female genital opening (Jereb & Roper, 2005) yet in the Rossinae they may also be implanted near the oviducal opening, the oviduct, the head and mantle, or stored in a ridged area

near the oviduct (Hoving et al. 2008) while in the Heteroteuthinae they may be implanted in the region of the head and arms (Villanueva & Sanchez, 1993). Hoving et al. (2009) found that male *R. moelleri* needed to deposit spermatophores on the female 10-30 seconds following spermatophore release from the Needham's sac otherwise implantation would not take place, in which case spermatophore transfer was rendered useless. The authors proposed that this time constraint along with the possibility that the female may struggle during mating may explain why spermatangia are sometimes found in different regions of the female's body. Önsoy et al. (2008) found that mated female *S. patagonica* had between 2 and 19 (mean= 9) spermatangia within the mantle cavity. Spermatangia were generally found close to the oviducal gland opening while on one occasion 3 spermatangia were found on the surface of the viscero-pericardial coelom which covers the ovary. Hoving et al. (2009) recorded between 6 and 21 (mean= 14 ± 5) spermatangia.female^{-1}, implanted on the left hand side of the head, neck and anterior mantle region of *R. moelleri*, the same side as the opening to the distal oviduct, whilst Cuccu et al. (2007) recorded between 5 and 24 spermatangia implanted on the inner left hand side of the ventral mantle surface (the area that overlaps the oviduct) in 38 female *N. caroli*.

In the present study spermatangia were observed on the *bursa copulatrix* of *S. atlantica*, as is the case in other members of the Sepiolinae. The mean number and range of spermatangia implanted in Y Foryd females was slightly higher than in other bobtail squid, however the mean number of spermatangia observed was approximately half that of the mean number of spermatophores present in males from this site, while the range was considerably smaller than the range of spermatophores found in the Anglesey Y Foryd males, suggesting that males are promiscuous, mating with several females. According to Yau (1994), spermatangia may be stored on the

bursa copulatrix for up to one month in *S. atlantica*. Önsoy et al. (2008) observed that the number of spermatangia in mated females was approximately ten times lower than the number of spermatophores found in mature males and that it is likely to be considerably lower than the total spermatophore production during ontogenesis. Önsoy et al. (2008) also noted that male *S. patagonica* with full spermatophoric sacs also had large, functioning testes while the production of spermatophores was ongoing indicating a protracting promiscuous copulation. The fact that male *R. moelleri* have more spermatophores in the Needham's sac than females have implanted spermatangia may indicate that not every spermatophore that is transferred to a female is in fact implanted (Hoving et al. 2009) These authors observed that in species where spermatangia are deposited on the *bursa copulatrix* or similar seminal receptacles there is a risk of poor attachment leading to the loss of sperm, unlike *Heteroteuthis dispar* which has an internal seminal receptacle for spermatophores and hence attachment is unnecessary. It is also highly likely that males mate with more than one female since promiscuity amongst males and females and the multiple paternities of spawned eggs have previously been documented in squid (Hanlon et al. 1997; Shaw & Sauer, 2004).

In conclusion, the results in this chapter have demonstrated that the little cuttlefish, *S. atlantica*, has a seasonal distribution in the shallow subtidal zone around the coast of Anglesey where it is likely to be an important prey species as well as a predator of coastal invertebrates. Information collated from the study indicates that animals of all sizes and maturity stages congregate in the shallow waters around Anglesey between July and October when environmental conditions are favourable for enhanced growth and maturation and where the high numbers of animals in the shallow subtidal zone enhances opportunities for mating and genetic exchange. From

observations and from anecdotal evidence it appears that *S. atlantica* mate while in the shallow subtidal zone of Y Foryd and possibly spawn in the area soon afterwards. No egg masses were, however, found at low water of spring tides or in the shallow subtidal perhaps suggesting that *S. atlantica*, having mated in shallow water, spawns in deeper water offshore. The study has highlighted the importance of Y Foryd as an area of scientific and conservation importance to *S. atlantica* and the area is therefore worthy of conservation. Y Foryd is visited regularly by sand eel fishers who inadvertently remove *S. atlantica* from the area on a daily basis along with their catches of sand eels. Some form of fishery regulation is probably necessary and appropriate to conserve *S. atlantica*.

REFERENCES

Adam, W. 1934. Notes on the cephalopods: 4. The variation of the radula of *Sepiola atlantica* d'Orbigny 1839. Bulletin of the Royal Museum of Natural History of Belgium. 10 (24), 1-4

Al-Rashada, Y.N.A. 2009. Population dynamics of Plaice *Pleuronectes platessa* L. in the inshore waters of North West Wales and Eastern Anglesey. Unpublished PhD, Bangor University, 355pp

Amaratunga, T. 1987. Population Biology. In: Boyle, P.R. (eds.). Cephalopod Life Cycles, Volume II. Academic Press, London. 239-252

Ballantine, W.J. 1961. A biologically-defined exposure scale for the comparative description of rocky shores. Field Studies. 1 (3), 1-19

Bello, G. 1986. Catalogo dei molluschi cefalopodi viventi nel Mediterraneo. Bollettino Malacologico. 22 (9-12), 197-214

Bello, G. 1992. Addenda al catalogo dei molluschi cefalopodi viventi nel Mediterraneo. Bolletino Malacologico. 28 (1-4), 93-95

Bergström, B. & Summers, W.C. 1983. *Sepietta oweniana* in: Cephalopod Life Cycles Vol. I Species Accounts. Academic Press, London. 75-91

Beyst, B., Buysse, D., Dewicke, A. & Mees, J. 2001. Surf zone hyperbenthos of Belgian sandy beaches: Seasonal patterns. Estuarine, Coastal and Shelf Science. 53, 877-895

Boletzky, S.v. 1983. *Sepiola robusta* in: Cephalopod Life Cycles Vol I Species Accounts. Academic Press, London. 53-67

Boletzky, S.v., Boletzky, M.v., Frösch, D. & Gätzi, V. 1971. Laboratory rearing of Sepiolinae (Mollusca: Cephalopoda). Marine Biology. 8, 82-87

Boucaud-Camou, E. & Boismery, J. 1991. The migrations of the cuttlefish (*Sepia officinalis*) in the English Channel. In The Cuttlefish (ed. E. Boucaud), pp. 179-189. Caen: Univ. Caen Documentation Centre

Boucher-Rodini, R., Martin, M. & Peduzzi, R. 1995. Preliminary results of a comparative study on antigenicity of Cephalopod eye-lens proteins: the sepiolids. Bulletin de l'institut océanographique. 16, 104

Bower, J.R. & Ichii, T. 2005. The red flying squid (*Ommastrephes bartramii*): a review of recent research and the fishery in Japan. Fisheries Research. 76, 39-55

Boyle, P.R. 1990. Cephalopod biology in the fisheries context. Fisheries Research (Amsterdam). 8, 303-321

Boyle, P.R. & Ngoile, M.A.K. 1993. Assessment of maturity state and seasonality of reproduction of *Loligo forbesi* (Cephalopoda: Loliginidae) from Scottish waters. In: Recent advances in cephalopod fisheries biology (ed. T. Okutani et al). Tokai University Press, Tokyo. pp 37-48

Boyle, P.R., Pierce, G.J. & Hastie, L.C. 1995. Flexible reproductive strategies in the squid *Loligo forbesi*. Marine Biology. 121, 501-508

Boyle, P.R. & Boletzky, S.v. 1996. Cephalopod populations: definition and dynamics. Philosophical Transactions of the Royal Society of London. Series B. 351, 985-1002

Boyle, P.R. & Rodhouse, P. 2005. Life cycle in: Cephalopods: ecology and fisheries. Blackwell, Publishing, Oxford. pp 80-100

Brazier, P., Holt, R., Murray, W. & Nichols, D. 1999. Marine Nature Conservation Review – Sector 10; Cardigan Bay and North Wales: area summaries (Connor, D. (eds)). Joint Nature Conservation Committee. pp 129-190

Collins, M.A., Yau, C., Allcock, L. & Thurston, M.H. 2001. Distribution of deep-water benthic and bentho-pelagic cephalopods from the north-east Atlantic. Journal of the Marine Biological Association of the United Kingdom. 81, 105-117

Collins, M.A., Yau, C., Boyle, P.R., Friese, D. & Piatkowski, U. 2002. Distribution of cephalopods from plankton surveys around the British Isles. Bulletin of Marine Science. 71 (1), 239-254

Cuccu, D., Mereu, M., Cannas, R., Follesa, M.C., Cau, A. & Jereb, P. 2007. Egg clutch, sperm reservoirs and fecundity of *Neorossia caroli* (Cephalopoda: Sepiolidae) from the southern Sardinian sea (western Mediterranean). Journal of the Marine Biological Association of the United Kingdom. 87, 971-976

De Heij, A. & Baayen, R.P. 2005. Seasonal distribution of cephalopod species living in the central and southern North Sea. Basteria. 69 (4-6), 91-119

Delamere, N.A. & Duncan, G. 1977. A comparison of ion concentrations, potentials and conductances of amphibian, bovine and cephalopod lenses. The Journal of Physiology. 272, 167-186

De Pierrepont, J.F., Dubois, B., Desormonts, S., Santos, M.B. & Robin, J.B. 2005. Stomach contents of English Channel cetaceans stranded on the coast of Normandy. Journal of the Marine Biological Association of the United Kingdom. 85, 1539-1546

Duncan, G. & Pynsent, P.B. 1977. Intracellular and extracellular photoreceptor potentials in the retina of the cephalopod *Sepiola atlantica.* The Journal of Physiology. 267 (1), 37-38

Duncan, G. & Pynsent, P.B. 1979. An analysis of the wave forms of photoreceptor potentials in the retina of the cephalopod *Sepiola atlantica.* The Journal of Physiology. 288,171-188

Forsythe, J.W. & Van Heukelem, W.F. 1987. Growth, In: Cephalopod Life Cycles Vol. II. pp 135-156

Forsythe, J.W., Walsh, L.S., Turk, P.E. & Lee, P.G. 2001. Impact of temperature on juvenile growth and age at first egg laying of the Pacific reef squid *Sepioteuthis lessoniana* reared in captivity. Marine Biology. 138, 103-112

Gibson, R.N., Robb, L., Burrows, M.T. & Ansell, A.D. 1996. Tidal, diel and longer term changes in the distributions of fishes on a Scottish sandy beach. Marine Ecology Progress Series. 130, 1-17

Grimpe, G. 1925. Zur kenntnis der cephalopodenfauna der Nordsee. Wissenschaftliche Meeresuntersuchungen. 16, 1-122

Hanlon, R.T., Claes, M.F., Ashcraft, S.E. & Dunlap, P.V. 1997. Laboratory culture of the sepiolid squid *Euprymna scolopes*: a model system for bacteria-animal symbiosis. Biological Bulletin. 192, 364-374

Hanlon, R.T., Maxwell, M.R. & Shashar, N. 1997. Behavioural dynamics that would lead to multiple paternity within egg capsules of the squid *Loligo pealei.* Biological Bulletin. 193, 212-214

Hoving, H.J.T. & Laptikhovsky, V. 2007. Getting under the skin: autonomous implantation of squid spermatophores. Biological Bulletin. 212, 177-179

Hoving, H.J.T., Laptikhovsky, V., Piatkowski, U. & Önsoy, B. 2008. Reproduction in *Heteroteuthis dispar* (Rüppell, 1844) (Mollusca: Cephalopoda): a sepiolid reproductive adaptation to an oceanic lifestyle. Marine Biology. 154, 219-230.

Hoving, H.J.T., Nauwelaerts, S., Van Genne, B., Stamhuis, E.J. & Zumholz, K. 2009 Spermatophore implantation in *Rossia moelleri* Steenstrup, 1856 (Sepiolidae: Cephalopoda). Journal of Experimental Marine Biology and Ecology. 372, 75-81

Jacob, T. J.C. & Duncan, G. 1984. A comparative study of the membrane permeability properties of amphibian and cephalopod mollusc lenses. Journal of Comparative Physiology B. 154, 333-341

Jereb, P., Mazzola, A. & Di Stefano, M. 1997. Sepiolinae (Mollusca: Cephalopoda) from the Strait of Sicily. Scientia Marina. 61 (4), 459-470

Jereb, P. & Roper, C.F.E (eds). 2005. Cephalopods of the world: An annotated and illustrated catalogue of cephalopod species known to date. Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae). FAO Species Catalogue for Fishery Purposes. No. 4, Vol. 1. Rome, 262 pp

Laptikhovsky, V.V., Nigmatullin, Ch.M., Hoving, H.J.T., Onsoy, B., Salman, A., Zumholz, K. & Shevstov, G.A. 2008. Reproductive strategies in female polar and deep-sea bobtail squid genera *Rossia* and *Neorossia* (Cephalopoda: Sepiolidae). Polar Biology. 31, 1499-1507

Lefkaditou, E. & Kaspiris, P. 2005. Distribution and abundance of Sepiolids (Mollusca: Cephalopoda) off the north-eastern Greek coasts. Belgian Journal of Zoology. 135 (2), 199-204

Linley, T. 2005. Potential of sediment in *Sepia officinalis* (L.) culture and comparison of its behaviour with the more sediment dependent *Sepiola atlantica* (Orbigny, 1839) (Mollusca: Cephalopoda). M.Sc thesis, University of Wales, Bangor. pp 6-52

Lordan, C., Warnes, S., Cross, T.F. & Burnell, G.M. 2001. The distribution and abundance of cephalopod species caught during demersal trawl surveys west of Ireland and in the Celtic Sea. Irish Fisheries Investigations No.8. Marine Fisheries Services Division, Marine Institute, Abbotstown, Sungboro Road, Dublin 15. 26pp

Lyle, J.M. 1983. Food and feeding habits of the lesser spotted dogfish, *Scyliorhinus canicula* (L.), in Isle of Man waters. Journal of Fish Biology. 23, 725-737.

Maertens, D. 1988. The western sand extraction area on the Belgian continental shelf. ICES report C.M. 1988/E:2. 42pp

Mangold-Wirz, K. 1963. Biologie des céphalopodes benthiques et nectoniques de la Mer Catalane. Vie et Milieu, 13, Supplement, 1-286

Mangold, K. & Boletzky, S.v. 1988. Mediterranean cephalopod fauna. In Clarke, M.R. & Trueman, E.R. (eds). The Mollusca, Vol. 12. Paleonotology and Neontology of Cephalopods. Academic Press, London. pp 315-330

Nabhitabhata, J., Nilaphat, P., Promboon, P. & Jaroongpattananon, C. 2005. Life cycle of cultured bobtail squid, *Euprymna hyllebergi* Nateewathana, 1997. Phuket Marine Biological Centre Research Bulletin. 66, 351-365

Naef, A. 1916. Ueber neue Sepioliden aus dem Golf von Neapel. Pubblicazioni della Stazione Zoologica di Napoli. 1, 1-10

Nesis, K.N. 1985. Oceanic cephalopods: Distribution, life forms, evolution. Nauka Press, Moscow. 286 pp

Nesis, K.N. 1995. Mating, spawning and death in oceanic cephalopods: a review. Ruthenica. 6 (1), 23-64

Nishiguchi, M.K., Ruby, E.G. & McFall-Ngai, M.J. 1998. Competitive dominance among strains of luminous bacteria provides an unusual form of evidence for parallel evolution in Sepiolid squid-*vibrio* symbioses. Applied and Environmental Microbiology. 64 (9), 3209-3213)

Nishiguchi, M.K., Lopez, J.E. & Boletzky, S.v. 2004. Enlightenment of old ideas from new investigations: more questions regarding the evolution of bacteriogenic light organs in squids. Evolution & Development. 6 (1), 41-49

Norman, M. & Lu, C.C. 1997. Redescription of the southern dumpling squid *Euprymna tasmanica* and a revision of the genus *Euprymna* (Cephalopoda: Sepiolidae). Journal of the Marine Biological Association of the United Kingdom. 77, 1109-1137

O'Dor, R.K., Balch, N., Foy, E.A., Hirtle, R.W.M., Johnston, D.A. & Amaratunga, T. 1982. Embryonic development of the squid, *Illex illecebrosus*, and effect of temperature on development rates. Journal of Northwest Atlantic Fishery Science. 3, 41-45

Önsoy, B., Laptikhovsky, V. & Salman, A. 2008. Reproductive biology of the Patagonian bobtail squid, *Semirossia patagonica* (Sepiolidae: Rossiinae) in the south-west Atlantic. Journal of the Marine Biological Association of the United Kingdom. 88 (5), 1019-1023

Orsi Relini, L. & Bertuletti, M. 1989. Sepiolinae (Mollusca, Cephalopoda) from the Ligurian Sea. Vie Milieu. 39 (3/4), 183-190

Orsi Relini, L. & Massi, D. 1991. The butterfly squid *Stoloteuthis leucoptera* in the Mediterranean. Journal of the Marine Biological Association of the United Kingdom. 71, 47-51

Patterson, K.R. 1985. The trophic ecology of whiting (*Merlangius merlangus*) in the Irish Sea and its significance to the Manx herring stock. Journal du Conseil International Pour l'Exploration de la Mer. 42, 152-161

Pearson, T.H. 1968. The feeding biology of sea-bird species breeding on the Farne Islands, Northumberland. The Journal of Animal Ecology. 37 (3), 521-552

Pecl, G. 2001. Flexible reproductive strategies in tropical and temperate *Sepioteuthis* squids. Marine Biology. 138, 93-101

Pierce, G.J., Valavanis, V.D., Guerra, A., Jereb, P., Orsi-Relini, L., Bellido, J.M., Katara, I., Piatkowski, U., Pereira, J., Balguerias, E., Sobrino, I., Lefkaditou, E., Wang, J., Santurum, M., Boyle, P.R., Hastie, L.C., MacLeod, C.D., Smith, J.M., Viana, M., González, A.F. & Zuur, A.F. 2008. A review of cephalopod-environment interactions in European Seas. Hydrobiologia. 612, 49-70

Quetglas, A., Carbonell, A. & Sánchez, P. 2000. Demersal continental shelf and upper slope cephalopod assemblages from the Balearic Sea (North-Western Mediterranean). Biological aspects of some deep-sea species. Estuarine, Coastal and Shelf Science. 50, 739-749

Richardson, C.A., Seed, R., Brotohadikusumo, N.A. & Owen, R. 1995. Age, growth and allometric relationships in *Septifer virgatus* (Bivalvia: Mytilidae). Asian Marine Biology. 12, 39-52

Rocha, F., Guerra, A. & Gonzalez, A.F. 2001. A review of reproductive strategies in cephalopods. Biological Reviews. 76, 291-304

Rosa, R., Pereira, J., Costa, P.R. & Nunes, M.L. 2006. Distribution, abundance, biology and biochemistry of the stout bobtail squid *Rossia macrosoma* from the Portuguese coast. Marine Biology Research. 2, 14-23

Russell, E.S. 1922. Report on the Cephalopoda collected by the research steamer 'Goldseeker', 1903-1908. Scientific Investigations. Fishery Board of Scotland. 3, 1-45

Sakurai, Y., Kiyofuji, H., Saitoh, S., Goto, T. & Hiyama, Y. 2000. Changes in inferred spawning areas of *Todarodes pacificus* (Cephalopoda: Ommastrephidae) due to changing environmental conditions. ICES Journal of Marine Science. 57, 24-30

Salman, A. 1998. Reproductiver biology of *Sepietta oweniana* (Pfeffer, 1908) (Sepiolidae: Cephalopoda) in the Aegean Sea. Scientia Marina. 62 (4), 379-383

Salman, A. & Önsoy, B. 2004. Analysis of fecundity of some bobtail squid of the genus *Sepiola* (Cephalopoda: Sepiolida) in the Aegean Sea (eastern Mediterranean). Journal of the Marine Biological Association of the United Kingdom. 84, 781-782

Semmens, J.M., Pecl, G.T., Gillanders, B.M., Waluda, C.M., Shea, E.K., Jouffre, D., Ichii, T., Zumholz, K., Katugin, O.N., Leporatti, S.C. & Shaw, P.W. 2007. Approaches to resolving cephalopod movement and migration patterns. Reviews in Fish Biology and Fisheries. 17, 401-423

Shaw, P.W. & Sauer, W.H.H. 2004. Multiple paternity and complex fertilisation dynamics in the squid *Loligo vulgaris reynaudii*. Marine Ecology Progress Series. 270, 173-179

Silva, M.A. 1999. Diet of common dolphins, *Delphinus delphis*, off the Portuguese continental coast. Journal of the Marine Biological Association of the United Kingdom. 79, 531-540

Smale, M.J. 1996. Cephalopods as Prey. Philosophical Transcrriptions of the Royal Society, London. 351, 1067-1081

Stephen, A.C. 1944. The Cephalopoda of Scottish and adjacent waters. Transactions of the Royal Society of Edinburgh. 61, 247-270

Summers, W.C. 1985. Ecological implications of life stage timing determined from the cultivation of *Rossia pacifica* (Mollusca: Cephalopoda). Vie Milieu. 35 (3/4), 249-254

Ünsal, İ., Ünsal, N., Erk, M. H. & Kanansakal, H. 1999. Demersal cephalopods from the Sea of Marmara with remarks on some ecological characteristics. Acta Adriatica. 40 (1), 105-110

Villanueva, R. 1992. Deep-sea cephalopods of the north-western Mediterranean: indications of up-slope ontogenetic migration in two bathybenthic species. Journal of Zoology, London. 227, 267-276

Villanueva, R. & Sanchez, P. 1993. Cephalopods of the Benguela current off Namibia: new additions and considerations on the genus *Lycoteuthis*. Journal of Natural History. 27 (1), 15-46

Volpi, C., Borri, M. & Boletzky, S.v. 1995. Mediterranean Sepiolidae: an introduction. Bulletin de L'Institut Océanographique, Monaco. 16, 7-14

Waluda, C.M. & Rodhouse, P.G. 2006. Remotely sensed mesoscale oceanography of the Central Eastern Pacific and recruitment variability in *Dosidicus gigas*. Marine Ecology Progress Series. 310, 25-32

Waluda, C.M., Rodhouse, P.G., Podestá, G.P., Trathan, P.N. & Pierce, G.J. 2001. Surface oceanography of the inferred hatching grounds of *Illex argentinus* (Cephalopoda: Ommastrephidae) and influences on recruitment variability. Marine Biology. 139, 671-679

Weill, R. 1927. Recherche sur la structure, la valeur systématique et le fonctionnement du spermatophore de '*Sepiola atlantica*' d'Orbigny. Bulletin Biologique de France et de Belgique. 62, 59-92

Willekens, B., Vrensen, G., Jacob, T. & Duncan, G. 1984. The ultrastructure of the lens of the cephalopod *Sepiola*: a scanning electron microscopic study. Tissue Cell. 16(6), 941-950

Würtz, M., Matricardi, G. & Repetto, N. 1995. Sepiolidae (Mollusca: Cephalopoda) from the lower Tyrrhenian Sea, central Mediterranean. In S.v. Boletzky (ed) Mediterranean Sepiolidae les sépioles de Méditerranée. Monaco Bulletin de L' Institut Océanographique. 16 (special), 35-39

Yau, C. 1994. The ecology and ontogeny of cephalopod juveniles in Scottish waters. PhD thesis, University of Aberdeen, Scotland

Yau, C. & Boyle, P.R. 1996. Ecology of *Sepiola atlantica* (Mollusca: Cephalopoda) in the shallow sublittoral zone. Journal of the Marine Biological Association of the United Kingdom. 76, 733-748

Zumholz, K. & Frandsen, R.P. 2006. New information on the life history of cephalopods off west Greenland. Polar Biology. 29, 169-178

CHAPTER 3

Laboratory culture of the little cuttlefish, *Sepiola atlantica* (Cephalopoda; Sepiolidae)

ABSTRACT

Live *Sepiola atlantica* were collected from around the coast of Anglesey and transported to the School of Ocean Sciences where they were maintained in aquaria with flowing seawater at ambient seawater temperature (~17°C). Pairs of *S. atlantica* mated in the "male parallel position" for between 21 and 77 minutes. Over a period of several weeks following mating five female *S. atlantica* laid egg masses of between 8 and 161 eggs onto the sides of their aquaria. Embryonic development took 33 days at 15.4°C and the hatching phase lasted for 23 days, with a mean hatching success of 32%. Hatchlings emerged from the eggs at a mean dorsal mantle length (DML) of 1.91 mm and entered a pelagic paralarval phase lasting 6 days. Ten to twenty days post hatching the hatchling internal yolk sac became exhausted, during a period when the hatchlings were fed *ad libitum* on wild-caught zooplankton at a density of ~90 organisms. L^{-1} or with enriched adult *Artemia* (density 50. L^{-1}). All the hatchlings fed *Artemia* were dead by day 38 while ~38% of the *S. atlantica* fed on zooplankton survived to this point, with the remaining juveniles reared to adulthood on a diet of *Crangon crangon*. These laboratory-reared juveniles matured and mated but no eggs were laid by the females which died 230-250 days post-hatching and 10 to 19 days post-mating, at a DML of between 21.7 and 23.2 mm, while the smaller males died 265 to 293 days post-hatching (17.4 to 21.4 mm DML). These data confirm that *S. atlantica* has a similar life cycle to other cultured sepioids, although more experimentation is required in order to routinely rear *S. atlantica* through consecutive generations in the laboratory.

INTRODUCTION

For over forty years researchers have attempted to culture and rear sepiolids in the laboratory in order to study their life history and to provide a regular supply of laboratory reared animals for experimental purposes. Members of the family of sepioids are characteristically small, have a short life cycle and generally adapt well to life in captivity, making them excellent candidates for laboratory studies (Boletzky et al. 1971; Summers, 1985).

Choe & Oshima (1963) and Choe (1966) reared *Euprymna berryi* for two months and Boletzky et al., (1971) reared five species of sepiolids (*Sepiola rondeleti*, *S. robusta*, *S. ligulata*, *Sepietta neglecta* and *S. obscura*) in the laboratory from eggs through to the adult with the three *Sepiola* species maturing and going on to spawn. Boletzky (1975) later reared and cultured *S. affinis* and *Rossia macrosoma* for 240 days (Boletzky & Boletzky, 1973; Boletzky, 1994) and then followed the embryonic development of these two species. Summers (1985) and Summers and Colvin (1989) cultured and reared *R. pacifica* in the laboratory for one and a half generations during a study of the diet and behaviour of the species. Summers and Bergström (1981) and later Bergström and Summers (1983) reared a second generation of *Sepietta oweniana*, while Steer et al. (2004) cultured the dumpling squid *Euprymna tasmanica* and investigated the role of temperature and maternal ration in embryo survival. Nabhitabhata et al. (2005) studied the life cycle of *E. hyllebergi* over three consecutive generations as part of a feasibility study into the small-scale production of the squid as an ornamental species. *Euprymna scolopes* was reared for 202 days by Arnold et al. (1972) and 120 days by Singley (1983) who noted that skin abrasions

developed as a result of the squid jetting into the sides of their small aquaria and this resulted in high mortalities of laboratory maintained squid. Hanlon et al. (1997) successfully reared the paralarvae of *E. scolopes* and completed the life cycle of the species, identifying three key factors for their successful laboratory culture. They observed that (i) the eggs should be maintained under optimum conditions so that competent hatchlings develop, (ii) water quality should be good (i.e. nitrogen compounds should be close to zero, while seawater temperature, pH and salinity should be kept as stable as possible) and lighting regimes must be tailored to the requirements of the particular species being cultured (for example, low levels of illumination were found to enhance feeding in *E. scolopes*) and (iii) the correct type and quantity of prey species must be offered.

The symbiotic relationship between the Hawaiian bobtail squid *Euprymna scolopes* and the bioluminescent bacterium *Vibrio fischeri* has been used extensively as an experimental model in cell and molecular biology (Wei & Young, 1989; McFall-Ngai & Ruby, 1991; Ruby & McFall Ngai, 1992) and therefore any improvement in the culture methodology of the squid host will be of benefit to these studies. It is broadly considered that bioluminescent bacteria such as *V. fischeri* form pathogenic and cooperative relationships with marine organisms, causing disease in invertebrates (Ruby & Lee, 1998). Therefore, a greater understanding of the relationship between pathogenic bacteria and their host organisms will be of benefit to biotechnological and biomedical research (Ruby, 1999). Like *E. scolopes*, all members of the genus *Sepiola* possess a light organ that is home to at least one of two species of luminous *V. fischeri* or *V. logei* bacteria (Nishiguchi, 2000).

The term 'paralarva' was introduced by Young & Harman (1988) to describe the pelagic, post-hatching phase of the cephalopod life cycle, that was distinctly different from that of the adult conspecifics. Several researchers have noted that high mortalities occur during the culture of cephalopod paralarvae through their critical pelagic life cycle (Mangold & Boletzky, 1973; Hanlon et al. 1979; Forsythe & Hanlon, 1985). The presence of an internal yolk sac offers embryonic paralarvae a source of energy, while in post-embryonic juveniles, energy is obtained following prey capture (Boletzky, 2003). The provision of appropriate nutrition to stimulate feeding behaviour is critical for cephalopod paralarvae survival (Iglesias et al. 2006). High mortality of paralarvae is believed to result from the provision of an inadequate rearing environment where feeding is not promoted or the paralarvae are starved due to an inappropriate supply of food (Hanlon, 1987; Vidal et al. 2002). According to Vidal et al. (2002) the function of the internal larval yolk sac is to provide the developing paralarvae with an energy source while the hatchlings are learning to hunt. As with all stages in the cephalopod life cycle, paralarvae are carnivorous, predominantly feeding on crustaceans, molluscs and fishes (Nixon, 1987) and a number of researchers have successfully reared cephalopod paralarvae using a variety of live prey including zooplankton (Hanlon et al. 1979), palaemonids and mysids (Walsh et al. 2002), *Artemia* (Iglesias et al. 2006), crab zoeae (Villanueva, 1994) and fish (LaRoe, 1971). According to Navarro and Villanueva (2000), young cephalopods require prey items that are both high in polyunsaturated fatty acids (PUFA) and decosahexaenoic acid (DHA).

The development of an appropriate culture methodology and the advancement of suitable husbandry techniques for the captive maintenance of the little cuttlefish, *Sepiola atlantica*, would allow more extensive research aimed at understanding its biology,

physiology and behaviour throughout its life cycle, as well as providing animals for display in public aquaria. A study of the life cycle and husbandry requirements of *S. atlantica* was undertaken with the objective of developing a suitable methodology for the successful laboratory culture of the species. This chapter reports on the first laboratory culture of *Sepiola atlantica* including data on aquarium husbandry, its behaviour, a description of the egg mass, embryonic development, the paralarval phase, juvenile growth and age at sexual maturity.

METHODS

Brood stock collection

Between June 2006 and August 2008, 73 *Sepiola atlantica* (2006 = 13, 2007 = 38, 2008 = 22) were collected using a beach seine net (20 x 2.2 m, diameter of mesh at cod end = 5 mm) from the shallow subtidal zone of shores around the coast of Anglesey, North Wales (UK), i.e. primarily from a sheltered shore in Y Foryd Bay (Menai Strait), and a wave-exposed shore at Traeth Penrhos (SW Anglesey). The soft mesh paneling of the net allowed specimens to be collected in good physical condition, unlike specimens caught by beam trawl which often succumb to physical damage and rarely last for more than 2-4 weeks in captivity (Yau & Boyle, 1996). Upon capture, S. *atlantica* were placed in polythene bags (31 x 39 cm) filled with 3L of seawater, containing a 3-4cm bed of fine sand from the collection site and placed in the dark in small (18 x 12 x 17cm) insulated boxes to reduce temperature fluctuations during transport (up to two hours) from the collection site to the School of Ocean Sciences, Menai Bridge (UK). The presence of sand offered the *S. atlantica* the opportunity to burrow immediately following capture and during transit, thus reducing the stress to the cuttlefish. Upon return to the School of Ocean Sciences (SOS) the little cuttlefish were acclimated to their new aquarium conditions by floating the open plastic bags in large aquarium tanks, with partial water changes (~ 20%) every 20 minutes. The entire process of acclimation was carried out under subdued lighting (~55 lux) for a minimum of one hour before release into the holding tanks.

Aquarium Husbandry

The *S. atlantica* were sexed and a pair of *Sepiola* (1♂ & 1♀) were maintained in each of 15 rectangular, black plastic crates (39 x 28 x 22cm = ~21 L. (See Figure 1) situated on a seawater table with a flow of seawater through each tank (flow rate = ~0.32 $L.min^{-1}$). Each tank was provided with seawater at ambient temperature, aerated and a submersible circulation pump (Aquarium Systems, Micro-Jet MC320) ensured continuous circulation. A 3-4cm deep bed of fine sand from the collection sites was placed on each aquarium base, whilst each tank was covered with a wooden lid to minimize disturbance caused by passing research workers. All animals collected in 2006 and 2007 and the laboratory reared animals mated in 2008, (along with two wild collected females), were kept in constant darkness, whilst field collected animals in 2008 were kept on a 12h L:12h D cycle. *Sepiola atlantica* were fed *ad libitum* on the mysid *Neomysis integer* and the shrimp *Crangon crangon* collected from the Cefni estuary (Malltraeth) and Red Wharf Bay (Traeth Coch), respectively; the prey species were kept in a healthy condition by daily feeding with mussels, *Mytilus edulis* collected from Brynsiencyn. Water flow into the aquaria was checked daily to ensure uniform flow rates, with salinity, pH, ammonia, nitrite and nitrate recorded weekly. Seawater temperature was recorded hourly using a data logger. A refractometer, a hand held pH probe and standard laboratory test kits were used to monitor water quality.

Fig. 1 PVC crates used to house adult *S. atlantica*.

Mating behaviour

In 2006, *S. atlantica* were opportunistically observed mating in a laboratory aquarium following the removal of a tank lid during routine husbandry work. To further understand the process of mate selection and mating, during the following summers (2007 and 2008), thirteen pairs of *Sepiola* were filmed in darkness in the process of mating using a digital video camera (SONY Handycam DCR-HC96E) with a built in infra red light. Observations of *S. atlantica* were made in black, PVC, 6L circular tanks containing a 3 cm depth bed of fine sand with a flow through of seawater (flow rate = 0.32 $L.min^{-1}$). On each occasion, one male and one female *S. atlantica* were placed in the tank, separated by a mesh divider for one hour prior to removal of the divider. Footage of the mating behaviour was downloaded directly to a PC using 'Scenalayzer' time lapse photography software and

the resulting footage viewed and examined using Windows media player. Following mating the males and females were removed and returned to their own tanks.

Egg, paralarva and juvenile husbandry

Egg masses were photographed *in situ* using a Nikon D40 camera and the number of eggs counted. The diameter of the eggs were measured using analySIS® image analysis software. Three eggs were removed at random from the egg mass every third day for periodic measurement, placed in a Petri dish containing seawater and the eggs photographed using a photo microscope (Novex-Holland, 65.560 RZT-SF) and the images of the eggs measured using the analySIS software. The outer opaque chorion was carefully removed using forceps and each egg re-photographed to document the stage of embryonic development, while the behaviour of the embryo was also noted. These eggs were then transferred to a rearing cylinder (11 x 9cm, 150μm mesh base) (Figure 2) suspended within the tank in which they were laid. This procedure continued until the physical disturbance of continually removing individual eggs from the egg mass caused premature hatching (evident from the presence of yolk sacs left within the egg cases or still attached to the hatchlings), which coincided with the time of first hatching of animals from any undisturbed eggs.

The aquaria containing the *in situ* eggs masses were inspected twice daily (~ 10 am & ~5 pm) by torch light and the number of hatchlings within the tank recorded. The hatchlings were carefully removed with a turkey baster (25ml pipette) to avoid damage that might have been caused if a net was used to capture the paralarvae and they were aerially exposed during transfer and then placed at random in one of six circular rearing tanks (3

tanks per treatment) at a density of ~1 paralarva.L^{-1}. The rearing tanks (see Figure 3) were round black plastic bowls (diameter 31cm, depth 13cm, volume = ~6L) and supplied with a flow through of ambient temperature seawater (flow rate = 10ml.min^{-1}). Each tank had a central drilled stand pipe (1cm diameter) covered with 150μm mesh that permitted the retention of the paralarvae and food items whilst allowing the overflow of water from the tank. Each tank was vigorously aerated, as dissolved oxygen concentrations close to saturation are known to enhance feeding (Choe & Ohshima, 1963) and a 3mm bed of fine sand was placed on the base of the tank to allow burrowing. Tanks were covered with a thin black polythene sheet to reduce overhead illumination and any disturbance from passing research workers. Low light levels are known to enhance feeding in *Euprymna scolopes* (Hanlon et al. 1997). During the course of the rearing experiment the paralarvae had reduced in number so that by day 80 the surviving *Sepiola* could be separated and assigned individually to tanks identical to those in which they had previously been reared. Having outgrown the rearing tanks and attained sexual maturity (day 208), the *Sepiola* were transferred to large rectangular tanks identical to those in which the brood stock were maintained.

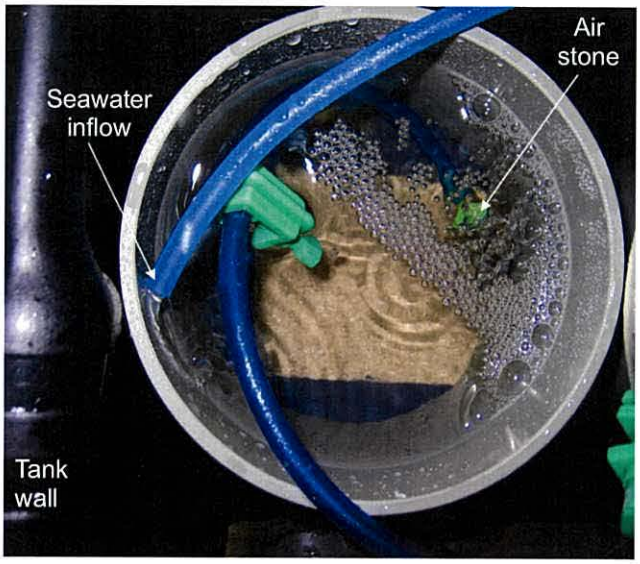


Fig. 2 Mesh-based PVC cylinder used to maintain the eggs of *S. atlantica* following removal of the chorion.

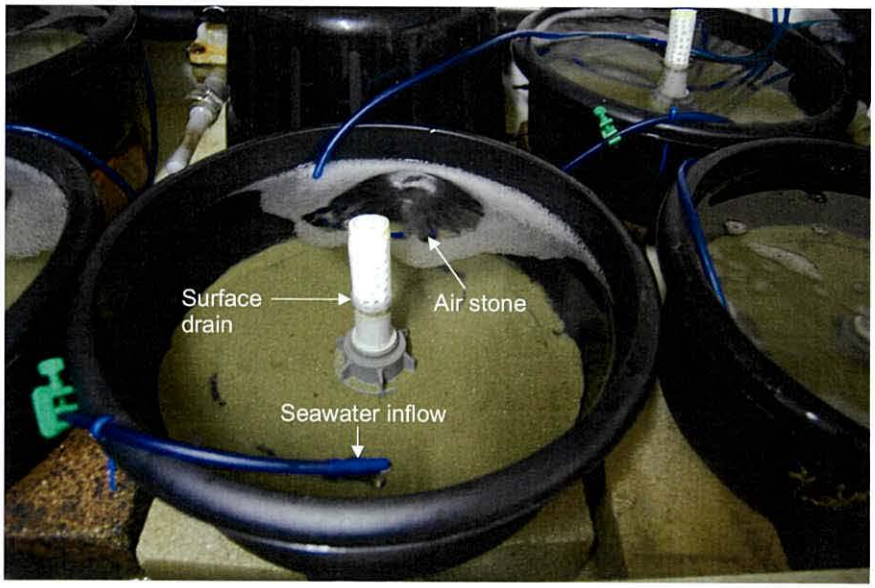


Fig.3 PVC bowls used to rear *S. atlantica* through the paralarval phase to sexual maturity.

Diet

Paralarvae were fed *ad libitum* on one of two diets: 1) a natural zooplankton mix (density of ~90.L^{-1}) or 2) with enriched adult *Artemia* sp. (density of ~50.L^{-1}). These diets were chosen because of their ease of culture, size and availability. Zooplankton was collected using a standard zooplankton tow net (420µm diameter mesh, net opening diameter = 52 cm, net length = 150 cm) deployed every third day from Menai Bridge Pier in the Menai Strait. The collection bottle containing the zooplankton was emptied into a 4L black PVC bucket. The sample was allowed to settle, and a light was placed above the bucket in order to attract the plankton to the surface where they were siphoned off. Predatory chaetognaths (*Sagitta* sp.) and leptomedusae (*Phialidium* sp.) were removed by using a 1 mm pipette as it was feared they may attack or consume the *Sepiola* paralarvae. Zooplankton were transferred to a 20L aerated cylinder (35cm diameter x 15cm depth) and maintained with 80% daily water changes and the daily addition of 500 ml of each of the micro-algae *Pavlova lutheri* and *Rhinomonas reticulata* cultured at densities of (~6,000 cells.μl^{-1} & 1,000 cells.μl^{-1}, respectively). The most abundant species in the plankton tows were the copepods *Temora longicornis*, *Paracalanus parvus*, *Pseudocalanus elongatus*, *Centropages typicus*, *Calanus finmarchicus* and *Oithona nana* and juveniles of the malacostracan crustaceans *Crangon crangon*, *Corophium volutator*, *Neomysis integer*, *Inachus dorsettensis* (megalopa) and *Parathemista* sp.

Artemia was cultured from cysts using the standard methodology of Lavens & Sorgeloos, (1996). Twenty four hours prior to being fed to the *Sepiola*, one week old *Artemia* sp (Gold label, Vinh Châu, Vietnam) (size 2-3mm total length) were enriched

using Algamac 2000 (Bio-Marine Brand) according to the manufacturer's specifications. This product was chosen as it was readily available and is high (>24%) in DHA which is of primary importance to the larval and post-larval development of aquatic organisms. *Artemia* sp were harvested using a 250μm sieve, rinsed in filtered seawater and fed *ad libitum* to the *S. atlantica.*

Following several days where little predation of zooplankton was observed, about day 60, small *C. crangon* (total length 4-5 mm) and *C. volutator* (total length 3-4 mm) were offered and readily consumed by the juvenile *S. atlantica.* These crustacean species were selected as they are known to be readily consumed by adult *S. atlantica* (Yau & Boyle, 1996). *Crangon crangon* was selected for the remainder of the trial as this species is abundant locally and progressively larger shrimp could be provided to the *Sepiola* as they grew, while *C. volutator* only attains a relatively small size (~10 mm total length) compared with that of *C. crangon* (~90 mm total length). By day 80 *S. atlantica* were being fed exclusively on *C. crangon.*

Survival & growth

The incidence of dead *S. atlantica* was monitored daily and mortality estimates were determined from animals that had died of natural causes only and which were found on the floor of the black circular aquaria. For the first 40 days of the culture trial one randomly selected animal from each circular black aquarium was euthanized in ice water every fifth day for the measurement of Wet Weight (Ww) & dorsal mantle length (DML). A digital image of the paralarva/juvenile was taken for later measurement of DML (to the nearest 0.01 mm) and the blotted (tissue paper) flesh of each animal weighed (Ww) (to the

nearest 0.001g) on a top loading balance (Ohaus Analytical Plus) (see Summers 1985). As the experiment progressed mortality increased, so the time between measurements was extended to every tenth day in order to ensure there were sufficient animals to prolong the growth experiment. By the end of the experiment only five *S. atlantica* remained alive. Once mating of these animals had occurred, the females were no longer selected for measurement as Hanlon et al. (1997) suggested that disturbance following mating may affect egg production and delay spawning.

The relationship between DML and experimental time, after first checking for normality of distribution and homogeneity of variances, was investigated. A General Linear Model was undertaken to determine whether growth rates, increase in DML varied significantly amongst the five little cuttlefish that were successfully reared to adulthood. Attempts were made to compare the growth rates among the five animals following the onset of sexual maturation unfortunately the data were not normally distributed and variances were unequal so that further statistical analysis was not possible.

RESULTS

Water quality during larval rearing

The mean seawater temperature in the circular aquaria was 17.34°C ± 0.36 SD (range = 16.3 to 17.9°C) during the mating trials. Table 1 indicates the range of seawater temperatures experienced during embryonic development in the eggs and paralarval growth. Throughout the juvenile growth experiment, ammonia, nitrite and nitrate was not detected and remained within the recommended levels proposed by Hanlon et al., (1997), (i.e. ammonia and nitrite = 0.10 $mg.L^{-1}$, nitrate = 20 $mg.L^{-1}$)), while the pH ranged between 8.30 & 8.90 (mean = 8.55 ± 0.16 SD) and salinity between 31 & 35 (mean = 32).

Mating behaviour

In 2006, one pair of *S. atlantica* was observed mating *in situ*, while three pairs were observed mating in 2007. Male and female *S. atlantica* engaged in a mating embrace known as the male parallel position (Figure 4) as described by Hanlon & Messenger (1996) for between 20 and 77 minutes (mean = 51.8 minutes). From thirteen video observations mating was on average initiated 26 minutes (± 32.1 SD, range = 1 to 83 minutes) following removal of the tank divider, with the male and female mating on average for 64 minutes (± 9.77 SD, range = 49-77 minutes). Upon completion of mating the males usually tended not to mate again. However, one pair displayed multiple mating, initially mating for 49 minutes, again two hours later for 64 minutes and four and a half hours later for 58 minutes, while another pair mated for 77 minutes and then separated for eight minutes before mating for a further 7 minutes. A third pair mated for 68 minutes after which point the female inked on two occasions and died, lying lifeless on the tank floor. The male remained in a

mating embrace with the dead female for a further 117 minutes before releasing the female, without paying any further attention to the corpse. Mating only took place in darkness and no courtship behaviour was observed at any time. Males choosing to 'pounce' on the females from behind, while mating generally occurred on the sandy bottom. At the point of separating the *S. atlantica* inked eight out of the thirteen times mating was filmed. The remaining pairs of mating *S. atlantica* were separated on the assumption that mating had already occurred; however, none of the females of these pairs went on to lay eggs.

Fig. 4 *Sepiola atlantica* in a mating embrace known as the male parallel position. The male is positioned below the female.

On occasion, following copulation, the ventral surface of the mantle of the female appeared to be 'rolled' down slightly, with the orange coloured accessory nidamental glands clearly visible. This presumably resulted from deposition, by the male, of spermatophores on the *bursa copulatrix*. Following copulation females were observed to

have pale coloured wounds on their mantles (2 to 11 days later), wounds that had presumably been inflicted by the male as a result of post coital bullying, with the captive female unable to escape the attentions of the male. An examination of stage IV females collected in August 2006 and 2007 from Traeth Penrhos (Anglesey) and Foryd Bay (Menai Strait) revealed that the females displayed spermatangia on the *bursa copulatrix* indicating that 63% (n=30) had already mated (see chapter 2). In 2008, ten females, including three laboratory reared adult animals were allowed to mate with laboratory reared males in the laboratory but none of these females subsequently laid eggs in captivity. This included two wild collected females that were allowed to mate with the two laboratory reared males, unfortunately the females died without spawning.

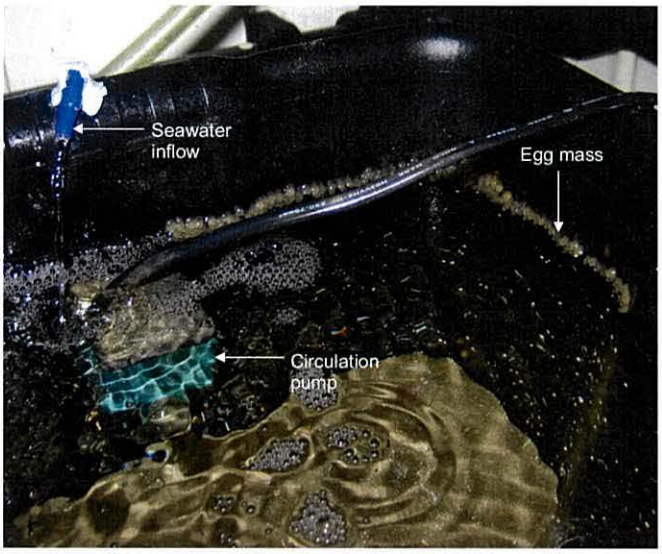
Spawning & hatching success

In 2006 and 2007, five females laid eggs at night on the black circular aquaria walls at the air-water interface. Eggs were laid in the corner of the tank directly above the air stone where the water was vigorously agitated and well aerated (Figure 5). The number of eggs laid was variable e.g. between 8 and 161 were laid 17 to 83 days post-capture, and the females went onto survive for between 1 and 8 days post egg laying (see Table 1). Lemon-shaped, initially cream coloured eggs were laid in a single layer, the eggs subsequently turning golden-brown in colour during development. On one occasion early one evening during an episode of egg laying a female *S. atlantica* was observed clinging to the aquarium wall with its arms spread outwards and egg laying was interrupted. The startled *S. atlantica* swam around the tank, investigating numerous areas of the aquarium walls with its arms but did not lay anymore eggs (see Table 1). In 2008, no eggs were laid by either the

laboratory reared, or the wild collected females. During some of the initial experiments in 2006 the hatched paralarvae were frequently lost or died soon after hatching. For example in 2006 a small egg mass laid by one *S. atlantica* (individual no. 1) was left completely undisturbed (see Table 1). The hatchlings emerged at night and disappeared down the overflow pipe and were lost. On another occasion, thirty eight days post-spawning, a lone hatchling emerged from an egg mass laid by another *Sepiola* (individual no. 2) To avoid the loss of the newly hatched paralarvae from the spawning tank the egg mass was carefully removed from the aquarium wall and the eggs placed individually into separate rearing chambers. However, disturbance of the egg masses caused the hatchlings to emerge 15 minutes following transfer into the rearing chambers. Of the 19 hatchlings that emerged, all survived to day seven after which mortality increased with 100% mortality attained by day 14. The 11 dead hatchlings (11 to 14 days old) measured 2.06 mm ± 0.28 SD (range = 1.62 to 2.52 mm) and had a mean Ww of 4.88 mg ± 1.03 (range = 3.05 to 6.35). The following year (2007), the egg mass (132 eggs) laid by *Sepiola* (individual no. 3) initially appeared to be developing normally, however, by day 18 only twenty well developed embryos, with silver coloured eyes, remained alive, and by day 21 those embryos had died. The rapid mortality of the entire egg mass was probably due to an overnight mechanical failure in the air supply. Small egg masses laid by *S. atlantica* No. 4 also failed to develop for unknown reasons. Examination of five dead females showed that the ovaries were not completely spent after spawning. Of the 5 females that spawned in captivity *S. atlantica* No. 5 laid the most eggs (see Table 1). During development mean egg diameter increased from an initial 2.48 mm ± 0.05 SD, to a maximum of 3.37 mm ± 0.09 SD on day 26, decreasing in size to 2.54 mm ± 0.13 at first hatching, with a mean diameter of 3.13 mm ±

0.18 at last hatching. The process of paralarval hatching from all the eggs lasted 23 days, at a mean temperature of 14.35°C ± 0.33 SD, with a hatching success of 32%, while a peak in hatching rate (i.e. 12 hatchlings) occurred fourteen days from the start of hatching (Figure 6). On hatching, the paralarvae had a mean DML of 1.91 mm ± 0.26 (SD), n = 52.

a)



b)

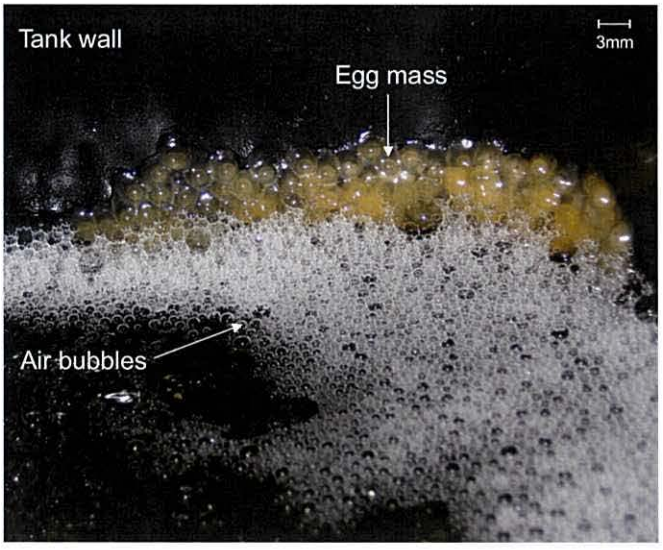


Fig. 5 a) *Sepiola atlantica* eggs laid in the corner of a tank at the air-water interface b) the aerated eggs of *S. atlantica.*

Table 1. Brood female origin, size of egg mass, temperature during embryonic development, length of development, time to first hatching and death post spawning of five wild caught female *Sepiola atlantica*. NV: Not viable, *: *Sepiola* disturbed during spawning

	***Sepiola atlantica* number**				
	1	2	3	4	5
Collection site	Trearddur Bay	Porth Tywyn-mawr	Traeth Llanddwyn	Y Foryd Bay (A)	Y Foryd Bay (B)
Level of exposure	Exposed	Exposed	Exposed	Sheltered	Sheltered
Date of collection of adults	06.07.06	11.09.06	09.05.07	24.08.07	28.08.07
Days in captivity prior to spawning	83	17	24	33	20
Mean developmental temperature (^{0}C) ± SD / Maximum and (minimum)	15.77 ± 1.20 / 18.3 (13.3)	15.19 ± 1.60 / 18.3 (11.9)	16.58 ± 0.74 / 18.2 (15.0)	15.46 ± 0.80 / 17.4 (13.6)	15.29 ± 0.96 / 17.7 (13.6)
Number of eggs laid	12 *	123	132	8	161
Number of days before 1st eggs hatched	39	44	NV	NV	33
Death post-spawning in aquaria (Days)	3	8	1	1	3

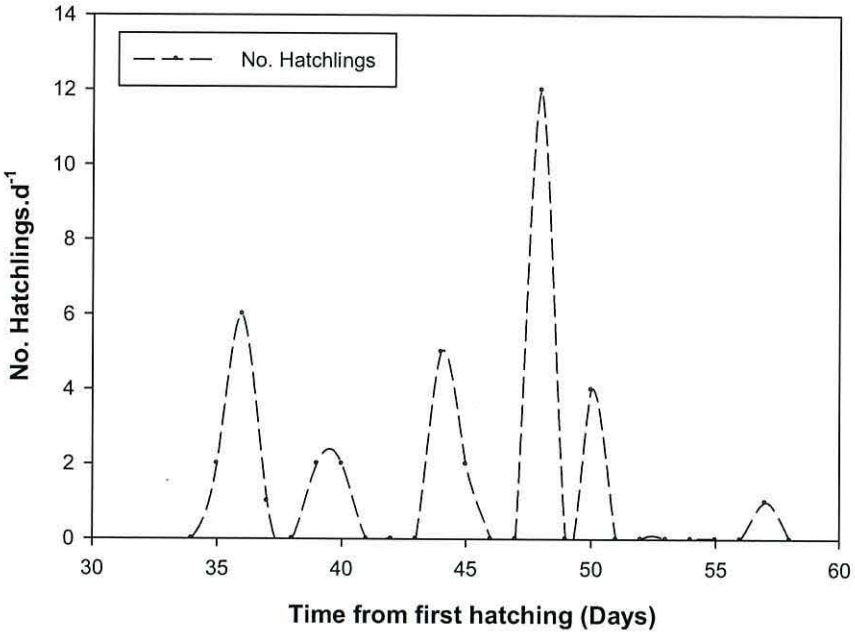


Fig. 6 The number of *S. atlantica* hatchlings recorded over a 23 day period at 14°C.

Embryonic development

The following description of embryonic development comes from microscopical observations of the batch of eggs laid by *Sepiola* No. 5. Figure 7 A (Day 9) shows that the developing embryo is not visible when viewed under a dissecting microscope. By day 14, however, the embryo is clearly visible (Figure 7 B), with orange pigmented eyes and early development of the arms. By day 17 (Figure 7 C), the embryos started to rotate within the egg capsules, whilst the eyes increased in size and the eye lenses could be seen. Internal organs were also visible within the mantle, while externally, fins started to develop on the mantle. Figure 7 D (Day 20) shows suckers on the arms, with the appearance of red eyes. On day 23 orange coloured chromatophores appeared on the dorsal surface of the head, while the embryo and yolk sac occupied the entire volume of the egg. By day 26,

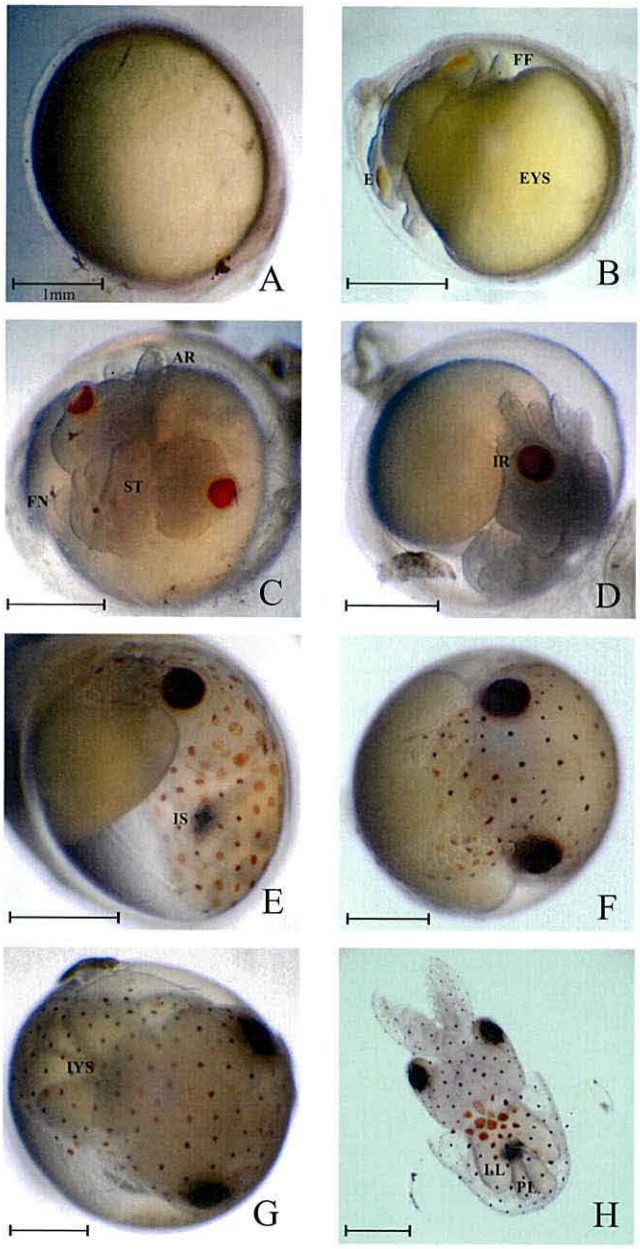


Fig. 7 Developmental stages of laboratory reared *S. atlantica* at 14°C. A) day 9 B) day 14 C) day 17 D) day 20 E) day 29 F) day 32 G) day 34; H) hatchling. Where E= eyes; FF= funnel folds; EYS= external yolk sac; AR= arms; FN= fins; ST= statocysts; IR= iridophores; IS= ink sac; IYS= internal yolk sac, Ll= lateral lobe of inner yolk sac; PL= posterior lobe of inner yolk sac. Scale bars = 1mm.

chromatophores covered the head and both dorsal and ventral surfaces of the mantle. The eyes were blood red in colour and the yolk envelope was observed to pulsate. Figure 7 E (Day 29) shows the onset of ink secretion, thus the ink sac is clearly visible within the mantle cavity. Brown coloured chromatophores were also visible on both the head and mantle of the embryo. By day 32 (Figure 7 F) orange, brown and black chromatophores were visible on the head and mantle. The eyes were black in colour and were well developed, while some movement was observed in the arms. By day 34 (Figure 7 G) the fins were well developed and moving freely and a greater degree of control was seen in the chromatophores as they rhythmically expanded and contracted. Figure 7 H shows the posterior lobes (Pl) of the inner yolk sac along with the lateral lobes (Ll) of the inner yolk sac through the mantle of an *S. atlantica* hatchling. A prematurely hatched *Sepiola* paralarva with attached external yolk sac can be seen in Figure 8.

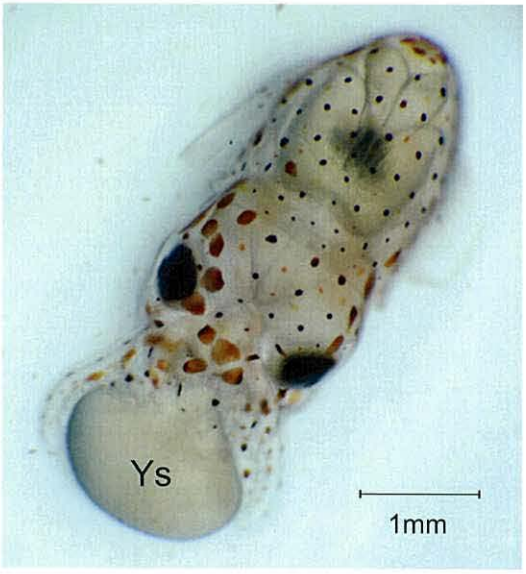


Fig. 8 Premature *S. atlantica* hatchling with external yolk sac still attached.

Hatching & feeding behaviour

During examination of the embryos under a dissecting microscope four hatchlings emerged after 2-3 minutes following their removal from the aquarium wall as a result of disturbance. On each occasion, the paralarvae hatched distal end first (Figure 9). With the mantle free from the egg, each paralarva rapidly expanded and contracted its mantle several times until the paralarva was free from the egg case, the whole process taking ~15secs. Hatched paralarvae constantly swam in the water column for six days and were never observed feeding. From day 6 onwards, however, the hatchlings adopted a habit similar to their adult conspecifics i.e. they were observed hunting and capturing prey and began to adopt a more epibenthic lifestyle settling on the aquarium floor. From these observations it is assumed that the paralarval phase (at ~2.4 mm DML) of *S. atlantica* is a pelagic one and lasts for six days during which time they are unable to feed independently, instead deriving their nutrition from the energy reserves held in their internal yolk sac. After six days, the hatchlings obtained their nutrition from the prey they hunted and captured (Figure 10). By settling on tank bottom, partially buried in sand and adopting a benthic lifestyle similar to that of the adults the *S. atlantica* can now be classified as juveniles (see the classification in Young & Harman, 1988). From observations of the animals under the dissecting microscope it was evident that the internal yolk sac was entirely exhausted 10-20 days post-hatching and that the complete burying of *S. atlantica* juveniles was widespread by day 30.

Fig. 9 A) *S. atlantica* hatchling B) *S. atlantica* emerging from an egg distal end first C) *S. atlantica* within the egg prior to hatching.

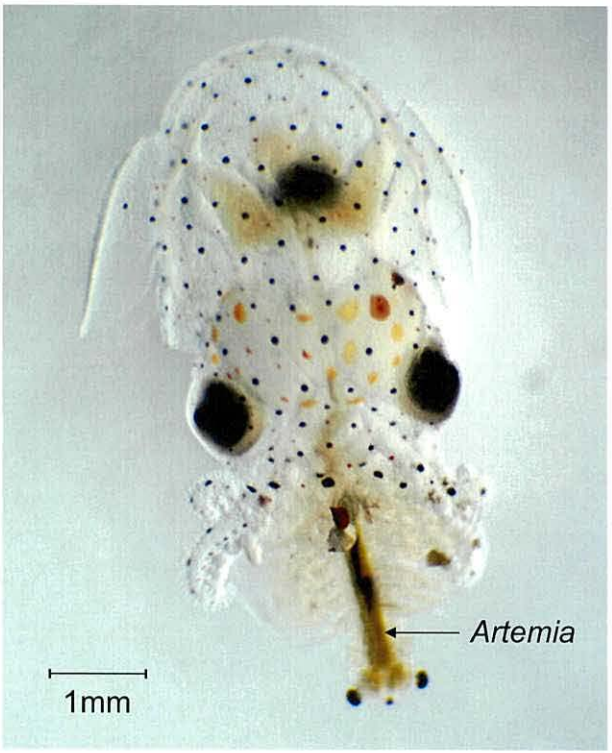


Fig. 10 Juvenile *S. atlantica* consuming its *Artemia* prey.

Post-hatching juvenile *S. atlantica* showed less interest in the zooplankton diet (day 50) and by day 54 they fed and readily captured ~4-5mm shrimp, *C. crangon*. Juvenile *S. atlantica* made several approaches towards the shrimp, swimming head down in mid water or at the surface, with their arms forming a cone shape before extending their tentacles to seize the prey in a similar manner to that described for *Euprymna berryi* by Choe (1966) and *S. robusta* by Boletzky (1983). The *S. atlantica* were often observed making 3-4 approaches before striking and on occasion were observed to pin the shrimp to the aquarium wall, with the shrimp escaping several seconds before the *S. atlantica* was aware that it had lost its prey. On one occasion a juvenile *S. atlantica* was seen to leave its resting position buried in the sand on the tank floor before attacking the prey from below. Once the *Crangon* was captured the *S. atlantica* remained head down in the water column whilst consuming the prey (Figure 11). When the amphipod *C. volutator* was offered as prey the little cuttlefish were seen to attack and easily capture the slow moving shrimp within seconds of their introduction into the rearing aquaria.

Survival

Survival of the *S. atlantica* paralarvae and juveniles reared on a diet of either mixed zooplankton or enriched *Artemia* sp. nauplii or adults can be seen in Figure 12. Over the first ten days, survival was greater in those groups of *S. atlantica* reared in 2006 on enriched adult *Artemia*, nauplii but survival declined rapidly until all the parlarvae/juveniles were dead by day 14. In the following year (2007) the survival of *S. atlantica* fed *Artemia* gradually declined slowly so that by day 38 there was 100% mortality. Survival on a zooplankton mixture in 2007 was generally better so that by day thirty eight ~38% of the

little cuttlefish were still alive. By day 40, survival of these animals had declined to ~25 % although they survived until the following year before finally succumbing following mating (i.e. by day 293). Overall, 9.6 % of the hatchlings from the three rearing experiments in 2006 and 2007 survived to adulthood. Whilst no little cuttlefish laid eggs in 2008, despite having mated successfully, the three laboratory-reared female *S. atlantica* died 230-250 days after hatching at a DML of between 21.7 and 23.2 mm and 10 to 19 days after mating. Males outlived the females dying between 265 and 293 days post-hatching (DML 17.4 to 21.4mm). Neither of the two wild caught females that mated successfully with the laboratory-reared males went on to spawn, dying 8 and 51 days post-mating, respectively.

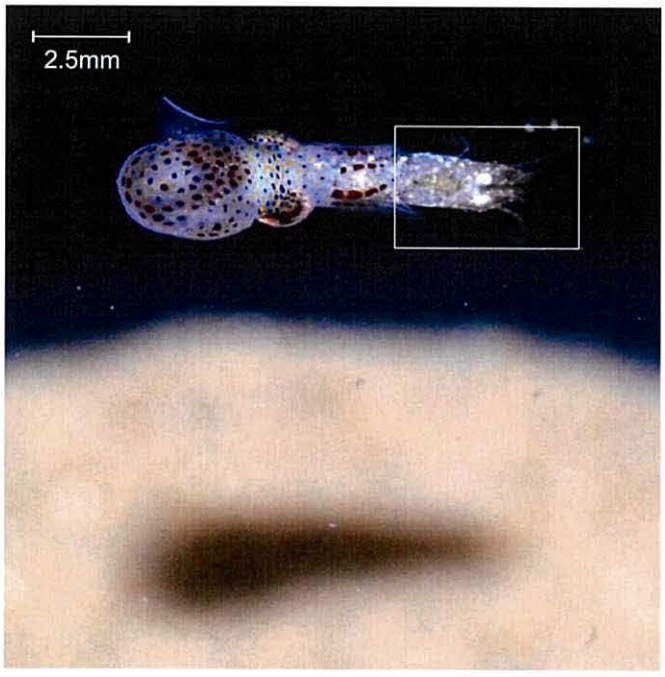


Fig. 11 Juvenile *S. atlantica* consuming *Crangon crangon* prey (object within the white box).

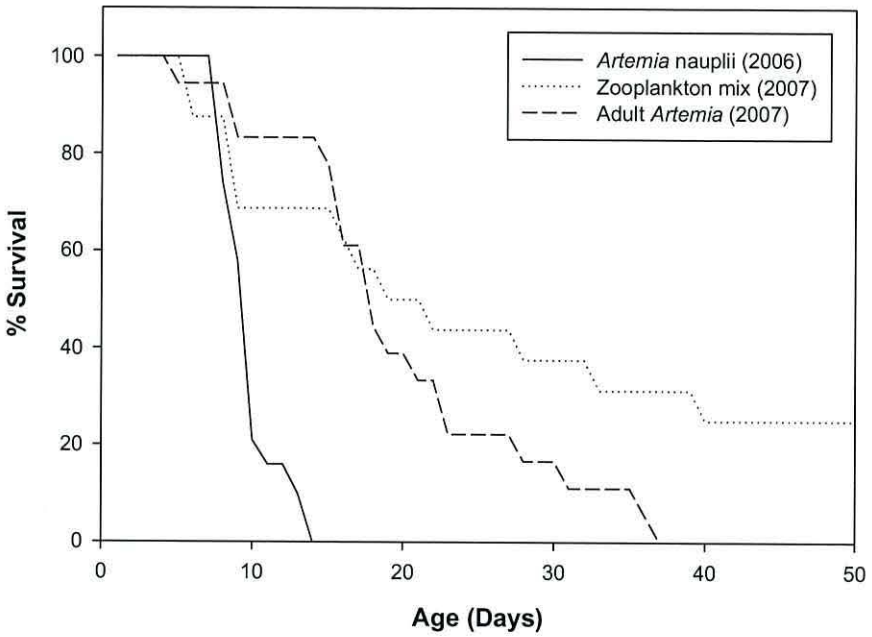


Fig. 12 Comparison of the survival of *Sepiola atlantica* paralarvae and juveniles reared on a diet of *Artemia* nauplii, adult *Artemia* or a zooplankton mix over a 50 day period at ambient seawater temperatures in 2006 and 2007.

Growth

Growth in a mixed sex population of laboratory-reared *S. atlantica* appears to have two phases. From Figure 13 growth in DML during the first 120 days was relatively slow, increasing thereafter to day 210 after which time growth levelled off. During the 120 day period a mean growth rate of 0.05mm.d^{-1} was recorded for the mixed sex population (0.043mm.d^{-1} in males, 0.055mm.d^{-1} in females). A combined growth rate for the population over 210 days gave a mean daily growth rate of 0.08 mm.d^{-1} (males = 0.07mm.d^{-1}, females = 0.09mm.d^{-1}). The growth of the female little cuttlefish slowed markedly following mating and although the females died off after this point the growth

rate of the remaining live male *Sepiola* was 0.04mm.d^{-1} from day 210 until the completion of the experiment. There was large variability in the size of individuals between days 200-260 although increase in size was largely independent of seawater temperature; little cuttlefish grew at a similar rate at 10°C as they did at 15°C. No significant difference in growth rate ($F = 1.34$, $p = 0.274$, R-Sq = 96.43 %) was observed between the five little cuttlefish (2 males and 3 females) between days 90 and 180 when they became sexually mature (see Figure 14A). Whilst it was not possible to test for statistical differences in growth rate amongst the five animals post-sexual maturation until death, from Figure 14B there is a suggestion that females grew faster than males once they were sexually mature. Although the data are limited there appears to be less variability in size amongst the females than between the males.

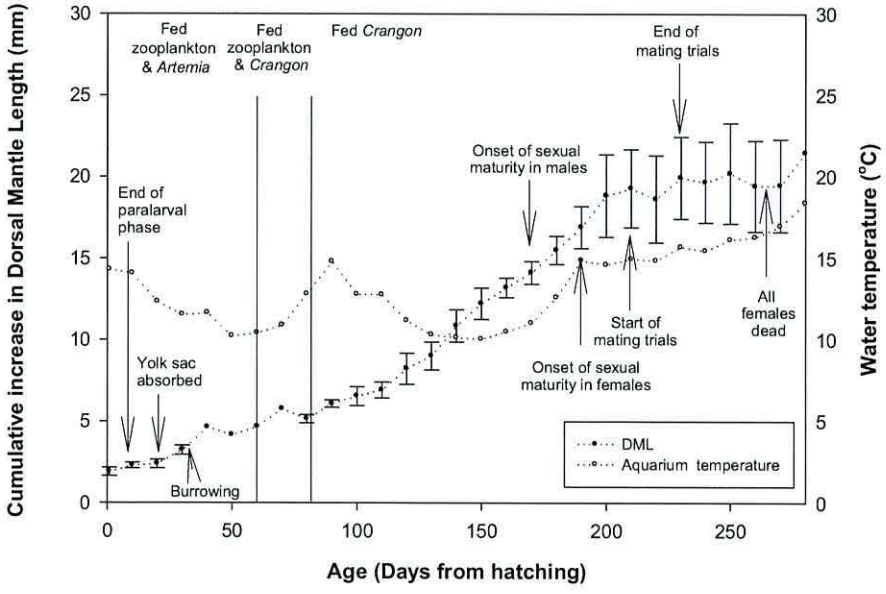
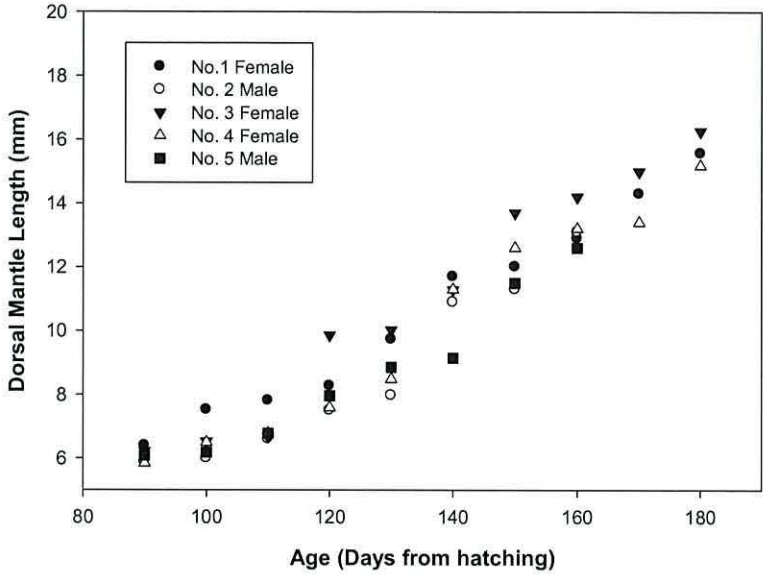


Fig. 13 A comparison of the cumulative increase in Dorsal Mantle Length (DML) of a mixed sex population (n = 5) of laboratory reared *Sepiola atlantica* and ambient seawater temperature. Key phases in the life cycle are indicated. Data points without error bars are from one observation only.

a)



b)

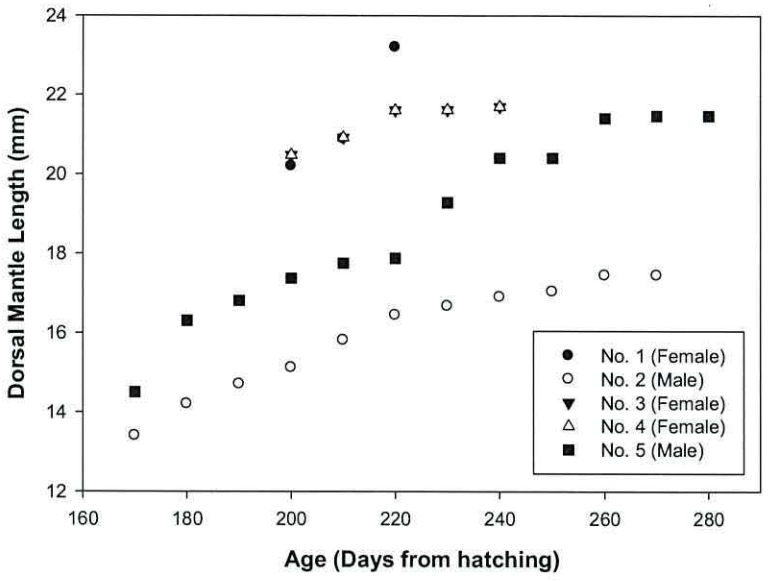


Fig. 14 Increase in dorsal mantle length (DML) of male and female *Sepiola atlantica* reared individually in the laboratory a) at 12°C to the onset of sexual maturity and b) at 15°C from the onset of sexual maturity to death.

DISCUSSION

Until now there have been no studies dedicated to the aquarium husbandry, culture and rearing of *S. atlantica*, yet a number of authors have described methodologies for the successful culture and rearing of members of the Sepiolidae (e.g. Choe & Oshima,1963; Choe, 1966; Boletzky et al. 1971; Arnold et al. 1972; Boletzky & Boletzky, 1973; Boletzky, 1975; Summers and Bergström, 1981; Bergström & Summers, 1983; Singley, 1983; Summers, 1985; Summers & Colvin, 1989; Hanlon, 1991; Boletzky, 1994; Hanlon et al. 1997; Steer et al. 2004, & Nabhitabhata et al. 2005). The present study has demonstrated that *S. atlantica* can readily adapt to and survive under laboratory conditions.

Mating behaviour

In agreement with observations of other Sepiolidae (Hanlon et al. 1997; Claes & Dunlap, 2000) mating in laboratory maintained *S. atlantica* only occurred at night, or in the dark, suggesting that the same behaviour probably occurs in wild conspecifics. This may be a strategy employed to avoid predation as captive *S. atlantica* were observed to position themselves on the aquarium floor, or hover in the water column whilst mating for more than an hour, which during daylight hours would leave them vulnerable to predation. My observations and those of Yau (1994) indicate no courtship behaviour prior to mating in *S. atlantica,* as is the case with *E. scolopes* (Singley, 1983) and *E. hyllebergi* (Nabhitabhata et al. 2005), with the male 'pouncing' on the female from behind and assuming the male parallel position as in *R. pacifica* & *E. hyllebergi* (Brocco, 1971; Nabhitabhata et al. 2005). In the present study mating was observed to last for between 49 and 77 minutes whilst Yau (1994), on one occasion observed mating for >30 minutes and Hanlon et al. (1997)

recorded mating embraces in *E. scolopes* lasting between 30 and 50 minutes (average 35 minutes) and 7-10 minutes in *E. hyllebergi* (Nabhitabhata et al. 2005). The observed inking on separation of the mating embrace by *S. atlantica* may occur to distract the male whilst the female retreats.

Spawning

Hanlon et al. (1997) proposed that optimal conditions for brood stock management must be attained and maintained in order for 'normal' reproductive behaviour and high fecundity to occur. Whilst five batches of eggs were laid by the females over the course of my study it was not possible to purposefully trigger spawning despite maintaining excellent water quality, providing an abundant and enriched food source, providing a number of spawning substrata and the manipulation of lighting and temperature regimes. It is likely therefore that spawning is triggered by one or a number of environmental factors that were not always present here in the relevant quantities. The data, however, suggest that *S. atlantica* always spawned during periods of rising seawater temperature as Ambrose (1988) has similarly noted for many cephalopod species.

Boletzky (1994) hypothesized that spawning sites used by Sepiolids should be in areas with strong water flow allowing for oxygenation of the egg mass, yet should be protected from wave action. Spawning usually takes place on coarse substrata, with the egg mass camouflaged by the female with fragments of shell, sand grains and spicules from sponges (Okutani & Sasaki, 2007). Sepiolid eggs have been recorded from inside empty bivalve shells, on ascidians, corals, sea grasses, stones, man made objects, sub-littoral algae and coral gravel (Choe; 1966; Boletzky et al. 1971; Boletzky, 1983; Bergstrom &

Summers, 1983; Deickert & Bello, 2005; Nabhitabhata et al. 2005). Yau & Boyle (1996) suggested that *S. atlantica* are likely to lay their eggs in and on empty bivalve and gastropod shells, kelp holdfasts and sponges. In the current study female *S. atlantica* laid their eggs masses on the aquaria tank walls, close to the corner of the tank at the air/water interface where aeration and turbulence was at a maximum, ensuring that the eggs were bathed in oxygen saturated seawater that would allow normal development. According to Boletzky (1974), *S. atlantica* deposit their eggs on aquaria walls when there is a lack of suitable spawning substrata. Although despite the presence of substrata that wild Sepiolids are known to deposit their eggs upon, Boletzky (1983) and later Boletzky et al. (1971) noted egg masses of *S. robusta* and *Sepietta obscura* were deposited on aquaria tank walls, close to the surface and sometimes in the tank corners. Summers (1985), Summers & Colvin (1989), and Anderson (1991) found eggs of *Rossia pacifica* on aquaria walls as well as on bricks, PVC pipe, empty shells, sponges and on overhanging rocks while Bergström & Summers (1983) found the eggs of *Sepietta oweniana* high up on tank walls in the corner of the aquarium. Singley (1983) recorded that the eggs of captive *Euprymna scolopes* were deposited on tank walls, small rocks and coral rubble where eggs were generally laid at the coral/sand interface, at the base of coral fragments but also within the fingers of the coral. The eggs of *E. hyllebergi* were deposited on coral, on the underside of PVC pipe dens and on tank walls ~100-150 mm off the tank floor (Nabhitabhata et al. 2005). From my own observations it appears that *S. atlantica* spawns in the early evening or at night. Such behaviour has been observed in *S. robusta* (Boletzky, 1983), *Euprymna scolopes* (Singley, 1983; Hanlon et al. 1997) and *Sepietta obscura* (Boletzky et al. 1971),

while *E. hyllebergi* spawns at dawn (Nabhitabhata et al. 2005). It is likely that these spawning behaviours occur during the hours of darkness as a means of predator avoidance.

Description of egg masses

Five batches of between 12 and 161 eggs were laid by *S. atlantica*, similar in number to the size of the egg masses of other wild and laboratory maintained Sepiolids. Egg masses of *E. scolopes* numbered between 12 and 310 eggs (Singley, 1983; Claes & Dunlap, 2000), Steer et al. (2004) noted 3 to 107 eggs laid by *Euprymna tasmanica*, 25 to 50 eggs were laid by *Rossia pacifica* (Summers, 1985; Summers & Colvin, 1989; Anderson, 1991), 13 eggs were laid in one mass by *Rossia mollicella* (Okutani & Sasaki, 2007) and again by *Neorossia caroli* (Cuccu et al. 2007), whilst between 2 and 176 eggs were laid by *Sepietta oweniana* (Bergstrom & Summers, 1983; Bello & Deickert, 2003; Deickert & Bello, 2005). According to Nabhitabhata et al. (2005), the number of eggs spawned by captive *E. hyllebergi* ranged between 108 and 464. In this study, female *S. atlantica* died between 1 and 8 days post-spawning, similar to laboratory-held *R. pacifica*, *Sepietta obscura*, *S. oweniana*, *E. scolopes; S. robusta* and *E. hyllebegi* (Anderson, 1991; Summers, 1985; Bergstrom & Summers, 1983; Singley, 1983; Boletzky, 1983 & Nabhitabhata et al. 2005).

The eggs of *S. atlantica* are a similar lemon shape to those of *S. oweniana* (Bergstrom & Summers, 1983) and as with *Sepiola robusta* and *S. oweniana* (Boletzky, 1983; Deickert & Bello, 2005) they are laid in a single layer, with each egg measuring approximately 2.5mm in diameter, increasing to ~3.1mm when the paralarvae are close to hatching. According to Boletzky et al. (1971), egg diameter changes during the course of

embryonic development, initially decreasing in size during the early stages of development as a result of shrinking of the jelly layers surrounding the chorion, later increasing due to swelling of the chorion and attaining a maximum size shortly before hatching. A similar pattern of development was seen in *S. atlantica*. Just after spawning the eggs of *S. robusta* are between 3.5 to 4.5mm in diameter, shrinking to a diameter of 3 to 3.5mm at developmental stage X (Naef, 1928), increasing to between 4.5 and 5mm in diameter at the time of hatching (Boletzky, 1983). The eggs of *S. atlantica* are similar in size to those of *S. robusta*, *S. oweniana* (2.7mm increasing to 5.0mm), *E. scolopes* (4.0 mm) and *E. hyllebergi* (4.15 x 3.34mm) (Boletzky, 1983; Bergstrom & Summers, 1983; Singley, 1983, & Nibhatbhata et al. 2005), but considerably smaller in diameter than those of *E. berryi* (6.3mm) and members of the subfamily Rossinae: *R. pacifica* (8-9mm), *Neorossia caroli* (9.0-9.3mm) *Rossia mollicella* (10.2mm) and *Rossia macrosoma* (6.5mm) (Choe, 1966; Summers & Colvin, 1989; Cuccu et al. 2007 & Okutani & Sasaki, 2007; Boletzky & Boletzky, 1973). According to Steer et al. (2004), female nutrition prior to spawning is of importance since *E. tasmanica* maintained on a low food ration produced significantly fewer (~60% less) and smaller eggs per clutch than those females maintained on a high ration, yet there is no evidence to suggest that seawater temperature or female size affects egg size or reproductive output.

Duration of development & hatching success

Sepiola atlantica developed over 33 days at a mean seawater temperature of 15.4°C before the first hatchlings appeared, in contrast to observations made by Batt (pers.comm)

who recorded a developmental period of 71 days at temperatures of between 18 and 20°C. This difference in development times seems inexplicable especially as those eggs spawned at the higher temperature would be expected to hatch sooner. According to Boletzky (1974) the length of embryonic development is a function of seawater temperature while Boyle & Rodhouse (2005) noted that temperature has a significant effect on the duration of embryonic development, with high temperatures reducing developmental times. Hatching in cephalopods is generally delayed when temperatures close to the lower temperature range of the species are experienced (Boletzky, 1987 in Boletzky, 1994) whilst at the upper end of the temperature range hatching takes place at the earliest opportunity (Boletzky, 1994). Short developmental times of 12-18 days at 27.5°C were recorded in *E. hyllebergi* (Nabhitabhata et al. 2005), 21 days at 23°C in *E. scolopes* (Claes & Dunlap, 2000), 20 days in *E. berryi* maintained at 23.5 to 24°C (Choe, 1966), ~40 days at 18°C in *E. tasmanica* which was more than three times faster than when reared at 11°C (Steer et al., 2004) and 1 to 1.5 months in *S. affinis* maintained at 20°C (Boletzky, 1974). Considerably longer developmental times of 2.5 months were recorded in *S. robusta* maintained at 13°C by Boletzky (1994), 2 to 2.5 months in *S. affinis* at 15°C (Boletzky, 1974), >3 months at 15 °C in *R. macrosoma* (Boletzky & Boletzky, 1973), 2 months at 13°C, 2.5 months at 10°C and 5.5 months at 7°C in *S. oweniana* (Bergström & Summers, 1983). Summers and Colvin (1989) reported developmental times in *R. pacifica* of 4.5 months at 6 to 12°C, whilst some viable eggs remained unhatched for 9 months when maintained at <7°C. Boyle and Rodhouse (2005) suggested that the interaction between spawning and temperature would affect the length of development which will have a knock on effect on the environmental conditions experienced by the hatchlings, affecting hatchling growth and survival.

A hatching success of 32% recorded in this study is considerably lower than 70-90% in *E. scolopes* found by Claes & Dunlap (2000) and >80 % recorded by Choe (1966) in *E. berryi* and 82-100 % in *E. hyllebergi* (Nabhitabhata et al. 2005). Hanlon et al. (1997) observed that any physical disturbance to the egg masses, such as checking the eggs, should be kept to an absolute minimum and that seawater temperature, pH, salinity, nitrogen levels and lighting regimes should remain constant. According to Choe & Ohshima (1963) it is vital that the eggs are maintained in fresh seawater. In the current study although the environmental parameters such as water chemistry and lighting levels were kept constant, the observed low hatching successes could be a result of unintentional physical disturbance of the egg mass when removing eggs for observations of embryonic development, while dramatic changes in seawater temperature could explain the failure of individual little cuttlefish to lay their eggs during the laboratory spawnings. According to Steer et al. (2004) differences in embryo mortality and reproductive output are largely attributable to maternal ration. A reduction in lipid intake increases mortality as embryos have insufficient lipid reserves required to complete embryogenesis. However, in my study, as the *S. atlantica* broodstock were fed to excess on shrimp that were themselves fed daily on mussel flesh it seems unlikely that embryo mortality was affected by female ration.

Hatchling size

Sepiola atlantica hatchlings had a mean DML of 1.91mm, similar to the mean DML of 2.2mm in *S. robusta* (Boletzky, 1983), 2.5mm for *S. oweniana* (Bergström & Summers, 1983), 1.5mm to 1.9mm in *E. scolopes* (Hanlon et al. 1997; Singley, 1983) and 2.20mm in *E. hyllebergi* (Nabhitabhata et al. 2005). In *E. tasmanica*, hatchling size was found to be

affected by female ration. Females maintained on a high ration produced significantly larger offspring than those females maintained on a low ration (Steer et al. 2004), and maternal temperature appears to have an impact on the size of offspring due to the interaction between temperature and food availability (McKee & Ebert, 1996). Hatchling size is a good indicator of hatchling competency and has serious implications for hatchling survival (Pepin, 1989; Chambers & Trippel, 1997). In comparison to small hatchlings, large hatchlings are thought to be less vulnerable to predation and starvation as a result of superior swimming abilities and predation competency (Pepin, 1989; Steer et al. 2003). Therefore, female ration is critical as it not only affects embryogenesis, but has serious implications for hatchling survival (Steer et al. 2004).

Paralarval phase

From observations of the *S. atlantica* hatchlings it is suggested that the term 'paralarva' be applied to individuals older than 6 days when they are around ~2.4mm DML (see Young & Harman's 1988 definition of a paralarvae). This is considerably shorter than the 30 days recorded in *E. scolopes* (Hanlon et al. 1997) whilst Boletzky (1983) found that *S. robusta* has a large inner yolk sac that is absorbed within the first week of life, with young feeding on mysids within 1 to 2 days following hatching. The internal yolk sac was visible for the first three days in *E. hyllebergi*, with a planktonic phase lasting 6-8 hours (Nabhitabhata et al. 2005), whilst one day old *S. oweniana* attacked crustaceans (Bergström & Summers, 1983; Bergström, 1985). Anderson (1991) found that hatchling *R. pacifica* immediately assumed a benthic lifestyle akin to their adult conspecifics and fed shortly afterwards. These observations indicate that the paralarval phase of the species is shorter

than, or comparable to that of *S. atlantica*. In young cephalopods, active feeding is always initiated before the internal yolk sac is exhausted (Boletzky, 1987). In *S. atlantica* the inner yolk sac is absorbed between 4-14 days after the juveniles were seen to actively hunt and consume their prey.

Juvenile diet & survival

In 2006 paralarvae hatched prematurely in a plastic bag following physical disturbance to an egg mass during transfer of eggs from one tank to another. According to Boletzky (1994) premature hatching occurs prior to the complete exhaustion of the outer yolk sac. Hatchlings either retained the external yolk sac briefly following hatching or dropped the yolk sac prior to hatching and therefore did not receive all the nutrients that they would have benefited from under normal hatching conditions. Hanlon (1990) states that premature hatching can occur following mechanical disturbance of the egg mass or if parameters such as temperature, pH, salinity, nitrogenous waste, lighting levels and light cycles are not kept constant. If premature hatching does occur, mass mortality will often result within days of hatching. Therefore it is apparent in my study that at day 38 the embryos inside the eggs were too far along the developmental process and should not in hind sight been moved and when they were moved they then subsequently only survived to day 14. Under normal developmental conditions *S. atlantica* embryos were close to hatching once the arms and eyes were well developed, good control of the chromatophores was exhibited, and movement of the arms became frequent.

In 2007, survival of juvenile *S. atlantica* juveniles fed on a diet of enriched *Artemia* was poor, although survival was greater when they were reared on a diet of mixed

zooplankton. Similar findings were found by Singley (1983) who demonstrated that *E. scolopes* fed entirely on *Artemia* suffered high mortality rates and exhibited poor growth. A variety of diets have been used to rear Sepiolinae with varying degrees of success. These include grass shrimp (Claes & Dunlap, 2000), mysids (Choe, 1966; Boletzky et al. 1971; Bergström & Summers, 1983; Boletzky, 1983; Singley, 1983; Hanlon et al. 1997; Nabhitabhata et al. 2005), *Artemia* sp. (Singley, 1983; Anderson, 1991), zooplankton (Hanlon et al. 1997), fish (Hanlon et al. 1997; Nabhitabhata et al. 2005), shrimp/prawns (Choe, 1966; Summers & Colvin, 1989; Hanlon et al. 1997; Nabhitabhata et al. 2005), copepods (Bergström & Summers, 1983) and amphipods (Bergström & Summers, 1983). When various diets were provided in abundance, Hanlon et al. (1997) recorded 100% mortality by day 8 in *E. scolopes* fed on a zooplankton mix as well as in a control group that were unfed. Survival rates of 10 % were achieved by feeding *Euprymna* on postlarval mysids, ~16 % survival on a diet of various post-larval shrimp, whilst ~13 % of those reared on a combination of post-larval mysids, fishes, zooplankton and adult mysids survived to settlement. In my study after day 40, survival leveled off suggesting that the first 40 days are a critical time in the culture of *S. atlantica*.

Bergström & Summers (1983), Singley (1983), Nabhitabhata et al. (2005) observed cannibalistic tendencies in young *S. oweniana*, *E. scolopes* and *E. hyllebergi*, respectively, although no cannibalism was observed in this study suggesting that the diets offered were of an appropriate size and at a suitable prey density. Bergström (1985) suggested that in *S. oweniana* prey capture is initially indiscriminate, with young squid attacking any moving object of an appropriate size. However, Bergström & Summers (1983) found that young *S. oweniana* prefered prey (*Praunus* sp.) with a body length from half, to two-thirds that of

the squid itself. Whilst mysids similar to their own size were occasionally captured, their preferred diet was that of smaller mysids, large copepods and amphipods. While feeding preference was not studied during my work, juvenile *S. atlantica* appeared to show increased activity levels whenever the amphipod, *C. saltator*, was introduced into the rearing tanks compared to the addition of *C. crangon* or zooplankton. Hanlon et al. (1997) suggest that the increased motion and swimming activity of the prey when added to a rearing tank is enough of a stimulus to promote an attack and therefore they recommend that feeding should take place several times a day to ensure the prey are in optimal condition.

Growth

Cephalopods are generally short lived, with high, often variable growth rates (Mangold, 1983), and their growth is influenced by a number of biotic and abiotic factors such as seawater temperature, diet, age, gender and maturity (Forsythe, 1984, 1993; Forsythe & Van Heukelem, 1987, Forsythe & Hanlon, 1988). Variations in the growth of individuals can influence a population in a number of ways such as its population size and age structure, reproductive dynamics, and hatchling survival, which in turn affect the abundance of the species (Leporati, 2007).

Growth studies of captive cephalopods have shown a two-phase growth pattern, generally comprising a short exponential stage of high growth, with a subsequent decline in growth rate at a point when energy is diverted from growth into reproduction (Mangold, 1983); *S. atlantica* similarly conforms to this pattern of body growth. Boletzky (1983) reported a mean growth rate of $0.1 mm.d^{-1}$ (DML) in *S. robusta* reared at 20°C, followed by

a general decrease in growth accompanied by increased variability in the growth of individuals. Boletzky et al. (1971) reported similar growth rates in *Sepiola* and *Sepietta* of 2.5mm.month^{-1} (DML) during the first 5 months in *Sepiola* and 3 months in *Sepietta*, while the growth rate later increased to 5mm.month^{-1} in *Sepietta*. They concluded that growth rate was not dependant on seawater temperature since *S. obscura* grew fastest during periods with the lowest temperatures. Summers (1985) and Summers & Colvin (1989) observed that growth rates did not remain constant throughout the life cycle of *R. pacifica*, with growth rates of 0.034mm.d^{-1} during the first 6 months, increasing to 0.065mm.d^{-1} and 0.25mm.d^{-1} for males and females, respectively, thereafter. Bergström & Summers (1983) recorded a mean growth rate of 4.8mm.month^{-1} in *S. oweniana*, with females growing significantly faster than males (5.3mm.month^{-1} in females, 4.2mm.month^{-1} in males) during the first 6 months of life. Nabhitabhata et al. (2005) recorded an instantaneous growth rate (IGR) of 4.83 % in DML in *E. hyllebergi* of between 10 and 20 days post hatching, with an average IGR of 2.41 % DML from hatching to day 100. Growth in *E. hyllebergi* displayed two phases, the first being from hatching to day 30, and then from day 30 to 122. Food conversion efficiency in *E. hyllebergi* cultured over three consecutive generations ranged between 37.22 and 50.8 %. According to Summers & Colvin (1989) growth in *R. pacifica* ceases 2 to 10 weeks before death. In *S. atlantica* feeding and growth reduced considerably in both males and females once mating had occurred.

Sexual maturation & length of life cycle

Sexual maturation in cephalopods is controlled by a number of environmental cues such as light and seawater temperature (Boletzky 1973), with no single factor governing the

onset of sexual maturation in *S. oweniana* (Mangold et al. 1975; Mangold-Wirz, 1963). Sexual maturation of captive *S. affinis* is unaffected by the absence of a day-night cycle (i.e. constant illumination); however, sexual maturation is delayed by low food ration (Boletzky 1975).

In the present study, the small sample of five *S. atlantica* that survived to adulthood sexually matured ~7 months after hatching (~15mm DML in males, ~17mm in females). Yau (1994) demonstrated that male *S. atlantica* from a Scottish wild population attained sexual maturity earlier, and at a slightly smaller size (12.8mm DML) than females (15.0mm DML). Whilst none of the laboratory-reared animals spawned, it can be estimated that the life cycle of *S. atlantica* is completed within 8 months, whilst the longest lived animal survived for almost 10 months. These observations are in agreement with a longevity of <12 months proposed by Yau (1994), and similar to that of the 6 to 9 months in Sepiolinae proposed by Boletzky (1974), yet shorter than the 18 months recorded in *R. pacifica* by Summers & Colvin (1989). As with other members of the family Sepiolinae, female *S. atlantica* died shortly after spawning followed by the males several weeks later (Boletzky, 1971; 1983). Female *S. robusta* become sexually mature by 17mm DML, with a complete life cycle of 5.5 months, while males are smaller in length on maturation (Boletzky, 1983). Hanlon et al. (1997) found that laboratory reared *E. scolopes* reached adult size within 2 months, with the entire life cycle (egg to egg) complete in 80 days and the longest lived animal attaining an age of 139 days although Singley (1983) found that *E. scolopes* reach sexual maturity when 4 to 6 months old, with a life span of between 7 to 10 months. Male and female *S. robusta* usually die within a few months of reaching sexual maturity, with females dying days after spawning, followed closely by males who are generally 6-8

months old at death (Boletzky, 1983). According to Nabhitabhata et al. (2005) mating in *E. hyllebergi* occurred as early as 66 days after hatching, with an average life span of 98 days. Boletzky et al. (1971) found that male *Sepietta neglecta*, *S. obscura* and *Sepiola* sp. reached adult size between 110 and 190 days old, with *S. rondeleti* spawning ~140 days post hatching, ~170 days in *S. robusta* and ~180-220 days in *Sepiola* sp, males survive the females by a few weeks.

In conclusion, *S. atlantica* appears to have a similar life cycle to a number of other sepioids that have previously been cultured in the laboratory through consecutive generations. The data in this chapter demonstrated that *S. atlantica* can be cultured successfully in the laboratory although the data generated were limited due to the low number of paralarvae that were successfully reared. While it was not achieved here, it seems entirely possible that a second captive generation of *S. atlantica* could be reared in the future, and therefore more experimentation is required. The environmental cues that trigger spawning in this species remain unclear, while a greater understanding of the nutritional requirements of paralarvae and juveniles would increase survival of laboratory reared paralarvae and juveniles through the critical initial phase of the life cycle.

REFERENCES

Ambrose, R.F. 1988. Population dynamics of *Octopus bimaculatus*: influence of life history patterns, synchronous reproduction and recruitment. Malacologia. 29, 23- 39.

Anderson, R.C. 1991. Aquarium husbandry of the sepiolid squid *Rossia pacifica*. AAZPA Annual Conference Proceedings. 206-211

Arnold, J.M., Singley, C.T. & Williams-Arnold, L.D. 1972. Embryonic development and post-hatching survival of the Sepiolid squid *Euprymna scolopes* under laboratory conditions. The Veliger, 14 (4), 361-364

Bello, G. 1986. Catalogo dei Molluschi Cefalopodi viventi nel Mediterraneo. Bolletino Malacologico. 22 (9-12), 197-214

Bello, G. 1992. Addenda al catalogo dei molluschi cefalopodi viventi nel Mediterraneo. Bolletino Malacologico. 28 (1-4), 93-95

Bello, G. & Deickert, A. 2003. Multiple spawning and spawning batch size in *Sepietta oweniana* (Cephalopoda: Sepiolidae). Cahiers de Biologie Marine. 44, 307-314

Bergström, B.I. 1985. Aspects of natural foraging by *Sepietta oweniana* (Mollusca, Cephalopoda). Ophelia. 24 (1), 65-74

Bergström, B. & Summers, W.C. 1983. *Sepietta oweniana* In: Boyle, P.R (eds.) Cephalopod Life Cycles Vol. I Species Accounts. Academic Press, London. pp 75-91

Boletzky, S.v. 1974. Élevage des céphalopods en aquarium. Vie et Milieu. 24, 309-340

Boletzky, S.v. 1975. The reproductive cycle of Sepiolidae (Mollusca, Cephalopoda). Publicazzioni della Stazione Zoologica di Napoli. VIII European Marine Biology Symposium, Sorrento (Naples) 39, 84-95

Boletzky, S.v. 1983. *Sepiola robusta* in Cephalopod Life Cycles Vol I. Species Accounts. Academic Press, London. 53-67

Boletzky, S.v. 1987. Juvenile Behaviour In: Boyle, P.R. (eds.). Cephalopod Life Cycles Vol. II Academic Press, London. pp 45-60

Boletzky, S.v. 1994. Embryonic development of cephalopods at low temperatures. Antarctic Science. 6 (2), 139-142

Boletzky, S.v. 2003. Biology of early life stages in cephalopod molluscs. Advances in Marine Biology. 44, 143-203

Boletzky, S.v., Boletzky, M.v., Frösch, D. & Gätzi, V. 1971. Laboratory rearing of Sepiolinae (Mollusca: Cephalopoda). Marine Biology. 8, 82-87

Boletzky, S.v. & Boletzky, M.V.v. 1973. Observations on the embryonic and early post-embryonic development of *Rossia macrosoma* (Mollusca, Cephalopoda). Helgoländer wiss Meeresunters. 25, 135-161

Boyle, P.R. & Rodhouse, P. 2005. Cephalopods: ecology and fisheries. Blackwell publishing, Oxford. 452 pp

Brocco, S.L. 1971. Aspects of the biology of the Sepiolid squid *Rossia pacifica* Berry. Victoria: MS Thesis, University of Victoria

Chambers, R.C. & Tippel, E.A. 1997. Early Life History and Recruitment in Fish Populations. Chapman & Hall, London

Choe, S. 1966. On the eggs, rearing, habits of the fry, and growth of some cephalopoda. Bulletin of Marine Science. 16 (2), 330-348

Choe, S. & Ohshima, Y. 1963. Rearing of cuttlefishes and squids. Nature. 197, 307.
Claes, M.F. & Dunlap, P.V. 2000. Aposymbiotic culture of the Sepiolid squid *Euprymna scolopes*: Role of the symbiotic bacterium Vibrio fischeri I host animal growth, development, and light organ morphogenesis. Journal of Experimental Zoology. 286, 280-296
Collins, M.A., Yau, C., Boyle, P.R., Friese, D. & Piatkowski, U. 2002. Distribution of cephalopods from plankton surveys around the British Isles. Bulletin of Marine Science. 71 (1), 239-254
Cuccu, D., Mereu, M., Cannas, R., Follesa, M.C., Cau, A. & Jereb, P. 2007. Egg clutch, sperm reservoirs and fecundity of *Neorossia caroli* (Cephalopoda: Sepiolidae) from the southern Sardinian sea (western Mediterranean). Journal of the Marine Biological Association of the United Kingdom. 87, 971-976
Deickert, A. & Bello, G. 2005. Egg masses of *Sepietta oweniana* (Cephalopoda: Sepiolidae) collected in the Catalan Sea. Scientia Marina. 69 (2), 205-209
De Pierrepont, J.F., Dubois, B., Desormonts, S. Santos, M.B. & Robin, J.P. 2005. Stomach contents of English Channel cetaceans stranded on the coast of Normandy. Journal of the Marine Biological Association of the United Kingdom. 85, 1539-1546
Duncan, G. & Pynsent, P.B. 1977. Intracellular and extracellular photoreceptor potentials in the retina of the cephalopod *Sepiola atlantica*. Journal of Physiology. 267, 37-38
Duncan, G. & Pynsent, P.B. 1979. An analysis of the wave forms of photoreceptor potentials in the retina of the cephalopod *Sepiola atlantica*. Journal of Physiology. 288, 171-188
Forsythe, J.W. 2004. *Octopus joubini* (Mollusca: Cephalopoda): a detailed study of growth through the full life cycle in a closed seawater system. Journal of Zoology (London). 202, 393-417
Forsythe, J.W. 1993. A working hypothesis of how seasonal temperature change may impact the field growth of young cephalopods. In: Okutani, T. O'Dor, R.K., Kubodera, T. (eds). Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo. pp 133-143
Forsythe, J.W. & Hanlon, R.T. 1988. Effect of temperature on laboratory growth, reproduction and life span of *Octopus bimaculoides*. Marine Biology. 98, 369-379
Forsythe, J.W. & Van Heukelem, W.F. 1987. Growth. In: Boyle, P.R. (ed). Cephalopod Life Cycles, Vol 2. Academic Press, London. pp 135-155
Grimpe, G. 1925. Zur Kenntnis der Cephalopodenfauna der Nordsee. Wissenschaftliche Meeresuntersuchungen. 16, 1-22
Hanlon, R.T. 1987. Mariculture. In: Boyle, P.R. (eds.). Cephalopod Life Cycles, Volume II. Academic Press, London. pp 291-305
Hanlon, R.T. 1990. Maintenance, rearing and culture of teuthoid and sepioid squids. pp 35-62. In: Gilbert, D.L., Adelman, W.J. & Arnold, J.M. (eds.), Squid as experimental animals. Plenum Press, New York
Hanlon, R.T. & Messenger, J.B. 1996. Cephalopod Behaviour. Cambridge University Press, Cambridge. pp 97-99
Hanlon, R.T., Claes, M.F., Ashcraft, S.E. & Dunlap, P.V. 1997. Laboratory culture of the sepiolid squid *Euprymna scolopes*: a model system for bacteria-animal symbiosis. Biological Bulletin. 192, 364-374

Herring, P.J., Clarke, M.R., Boletzky, S.v. & Ryan, K.P. 1981. The light organs of *Sepiola atlantica* and *Spirula spirula* (Mollusca: Cephalopoda): Bacterial and intrinsic systems in the order Sepioidea. Journal of the Marine Biological Association of the United Kingdom. 61, 901-916

Iglesias, J., Fuentes, L., Sánchez, J., Otero, J.J., Moxica, C. & Lago, M.J. 2006. First feeding of *Octopus vulgaris* Cuvier, 1797 paralarvae using *Artemia*: Effect of prey size, prey density and feeding frequency. Aquaculture. 261, 817-822

Jereb, P. & Roper, C.F.E. (eds.). 2005. Cephalopods of the world: An annotated and illustrated catalogue of cephalopod species known to date. Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae). FAO species catalogue for fishery purposes No. 4, Vol. I. 262 pp

Jones, B.W. & Nishiguchi, M.K. 2004. Counterillumination in the Hawaiian bobtail squid, *Euprymna scolopes* Berry (Mollusca: Cephalopoda). Marine Biology. 144, 1151-1155

LaRoe, E.T. 1971. The culture and maintenance of the loliginid squids *Sepioteuthis sepioidea* and *Doryteuthis plei*. Marine Biology. 9, 9-25

Lavens, P. & Sorgeloos, P. (eds.). 1996. Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper No. 361. Rome, FAO. 295pp

Leporati, S.C., Pecl, G.T. & Semmens, J.M. 2007. Cephalopod hatchling growth: the effects of initial size and seasonal temperatures. Marine Biology. 151, 1375-1383

Lyle, J.M. 1983. Food and feeding habits of the lesser spotted dogfish, *Scyliorhinus canicula* (L.), in Isle of Man waters. Journal of Fish Biology. 23, 725-737

Mangold, K. 1983. Food, feeding and growth in cephalopods. Memoirs of the Natural History Museum of Victoria. 44, 81-93

Mangold, K. & Boletzky, S.v. 1973. New data on the reproductive biology and growth of *Octopus vulgaris*. Marine Biology. 19, 7-12

Mangold, K. & Boletzky, S.v. Mediterranean cephalopod fauna. 1988. In: M.R. Clarke & E.R. Trueman, (eds.). The Mollusca, Vol, 12. Paleontology and neontology of cephalopods. London, Academic Press. 315-330

Mangold, K., Froesch, D., Boucher-Rodini, R. & Rowe, V.L. 1975. Factors affecting sexual maturation in Cephalopods. Publicazzioni della Stazione Zoologica di Napoli. 39 (Suppl.), 259-266

Mangold-Wirz, K. 1963. Biologie des cephalopods benthiques et nectonique de la Mer Catalane. Vie et Milieu. 13 (suppl.), 1-285

McFall-Ngai, M.J. & Ruby, E.G. 1991. Symbiont recognition and subsequent morphogenesis as early events in an animal-bacterial mutualism. Science. 354 (5037), 1491-1494

Nabhitabhata, J., Nilaphat, P., Promboon, P. & Jaroongpattananon, C. 2005. Life cycle of cultured bobtail squid, *Euprymna hyllebergi* Nateewathana, 1997. Phuket Marine Biological Centre Research Bulletin. 66, 351-365

Naef, A. 1916. Ueber neue Sepioliden aus dem Golf von Neapel. Publicazioni della Stazione Zoologica di Napoli. 1, 1-10

Naef, A. 1928. Die Embryonalentwicklung der Sepioliden. In: Fauna Flora del Golfo di Napoli. Die Cephalopoden. Vol 35, Embryologie, pp. 235-255

Navarro, J.C. & Villanueva, R. 2000. Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. Aquaculture. 183, 161-177

Nishiguchi, M.K. 2000. Temperature affects species distribution in symbiotic populations of *Vibrio* spp. Applied and Environmental Microbiology. 66 (8), 3550-3555

Nixon, M. 1987. Cephalopod diets. In: Boyle, P.R. (eds.). Cephalopod Life Cycles, Vol II. Academic Press, New York

Okutani, T. & Sasaki, T. 2007. Eggs of *Rossia mollicella*: (Cephalopoda: Sepiolidae) deposited in a deep-sea sponge. Journal of Molluscan Studies. 73, 287-289

Patterson, K.R. 1985. The trophic ecology of whiting (*Merlangius merlangus*) in the Irish Sea and its significance to the Manx herring stock. Journal du Conseil International Pour l'Exploration de la Mer. 42, 152-161

Pearson, T.H. 1968. The feeding biology of sea-bird species breeding on the Farne Islands, Northumberland. The Journal of Animal Ecology. 37 (3), 521-552

Pepin, P. 1989. Predation and starvation of larval fish: a numerical experiment of size and growth-dependent survival. Biolological Oceanography. 6, 23-44

Rosa, R., Pereira, J., Costa, P.R. & Nunes, M.L. 2006. Distribution, biology, and biochemistry of the stout bobtail squid *Rossia macrosoma* from the Portuguese coast. Marine Biology Research. 2, 14-23

Ruby, E.G. 1999. The *Euprymna scolopes-Vibrio fischeri* symbiosis: a biomedical model for the study of bacterial colonization of animal tissue. Journal of Molecular Microbiology and Biotechnology. 1 (1), 13-21

Ruby, E.G. & Lee, K. 1998. The *Vibrio fischeri-Euprymna scolopes* light organ association: current ecological paradigms. Applied and Enviornmental Microbiology. 64, 805-812

Ruby, E.G. & McFall-Ngai, M.J. 1992. A squid that glows in the night, development of an animal-bacterial mutualism. Journal of Bacteriology. 174, 4865-4870

Silva, M.A. 1999. Diet of common dolphins, *Delphinus delphis*, off the Portuguese continental coast. Journal of the Marine Biological Association of the United Kingdom. 79, 531-540

Singley, C.T. 1983. *Euprymna scolopes* in: Cephalopod Life Cycles Vol. I Species Accounts. Acadaemic Press, London. 69-74

Steer, M.A., Pecl, G.T. & Moltschaniwskyj, N.A. 2003. Are bigger calamary *Sepioteuthis australis* hatchlings more likely to survive? A study based on statolith dimensions. Marine Ecology Progress Series. 261, 175-182

Steer, M.A., Moltschaniwskyj, N.A., Nichols, D.S. & Miller, M. 2004. The role of temperature and maternal ration in embryo survival: using the dumpling squid *Euprymna tasmanica* as a model. Journal of Experimental Marine Biology and Ecology. 307, 73-89

Summers, W.C. 1985. Ecological implications of life stage timing determined from the cultivation of *Rossia pacifica* (Mollusca: Cephalopoda). Vie et Milieu. 35 (3/4), 249- 254

Summers, W.C. & Bergström, B. 1981. Cultivation od the Sepiolid squid, *Sepietta oweniana*, and it's ecological significance. American Zoologist. 20 (4), 927

Summers, W.C. & Colvin, L.J. 1989. On the cultivation of *Rossia pacifica* (Berry, 1911). Journal of Cephalopod Biology. 1 (1), 21-31

Vidal, E.A.G., Koueta, N., Riba, J. & Boucaud-Camou, E. 2002. Influence of temperature and food availability on survival, growth and yolk utilization in hatchling squid. Bulletin of Marine Science. 7 (2), 915-932

Villanueva, R. 1994. Decapod crab zoeae as food for rearing cephalopod paralarvae. Aquaculture. 128, 143-152

Villanueva, R., Koueta, N. Riba, J. & Boucaud-Camou, E. 2002. Growth and proteolytic activity of *Octopus vulgaris* paralarvae with different food rations during first feeding, using *Artemia* nauplii and compound diets. 205, 269-286

Walsh, L.S., Turk, P.E., Forsythe, J.W. & Lee, P.G. 2002. Mariculture of the loliginid squid *Sepioteuthis lessoniana* through seven successive generations. Aquaculture. 212, 245-262

Wei, S.L. & Young, R.E. 1989. Development of symbiotic bacterial bioluminescence in a nearshore cephalopod, *Euprymna scolopes*. Marine Biology. 103, 541-546

Würtz, M., Matricardi, G. & Repetto, N. 1995. Sepiolidae (Mollusca: Cephalopoda) from the lower Tyrrhenian Sea, central Mediterranean. In S.v. Boletzky, ed. Mediterranean Sepiolidae les Sépioles de Méditerranée. Monaco, Bulletin de L' Institute Océanographique, 16 (special): 35-39

Yau, C. 1994. The ecology and ontogeny of cephalopod juveniles in Scottish waters. PhD Thesis, Aberdeen. pp 163-200

Yau, C. & Boyle, P.R. 1996. Ecology of *Sepiola atlantica* (Mollusca: Cephalopoda) in the shallow sublittoral zone. Journal of the Marine Biological Association of the United Kingdom. 76, 733-748

Young, R.E. & Harman, R.F. 1988. "Larva", "Paralarva" and "Subadult" in cephalopod terminology. Malacologia. 29 (1), 201-207

CHAPTER 4

Hatching success, survival and juvenile growth of cuttlefish *Sepia officinalis* following egg transport.

ABSTRACT

Replicate batches of fifty, wild-collected four-day old *Sepia officinalis* eggs were held in cool boxes and subjected to four different treatments during transport in the UK, from West Sussex to Menai Bridge. Eggs were transported for 8.5h using one of four treatments, either 1) Wet: in sea water, 2) Wet & aerated: eggs in sea water with continuous aeration, 3) Damp: eggs wrapped loosely in damp paper towel and 4) Dry: eggs gently blotted dry in air. Following transport, eggs from the Damp and Dry treatments appeared 'pinched' after transportation but their characteristic grape-like appearance returned after several hours in seawater. Maintained at 17°C (±1°C) in 150 L rearing tanks, hatchlings emerged from day 28 following egg transport through to day 68, with most eggs hatching between 40 and 60 days. Hatchlings from eggs transported Dry were significantly the smallest ($F= 3.59$, $p<0.015$). However no significant differences in hatching success (~85%), hatching frequency, growth (dorsal mantle length & wet weight) and survival were observed between treatments. These results indicate that it is feasible to transport the eggs of cuttlefish and to successfully rear the juveniles following transport. Using the methodologies described it should now be more cost effective to transport consignments to research laboratories and aquaria with minimal risk.

INTRODUCTION

The common cuttlefish, *Sepia officinalis* (Linnaeus 1758), is abundant in the Eastern Atlantic and Mediterranean Sea where it is found from subtidal waters (2-3m depth) down to 200m, over sandy or muddy bottoms, reaching up to 490mm in DML and 4kg in wet weight (Jereb & Roper, 2005; Guerra, 2006). The temperature limit of *S. officinalis* within its range varies from between 10 and 30°C (Guerra, 2006). In the wild, *S. officinalis* feeds on a variety of crustaceans, molluscs including other cephalopods and fish (Boletzky, 1983). *Sepia officinalis* is well suited to laboratory culture as it has; 1) large eggs, 2) high hatchling survival, 3) voracious hatchlings, 4) sedentary behaviour, 5) tolerance to crowding and manipulation, 6) acceptance of dead prey items and 7) it is easily cultured under laboratory conditions (Forsythe et al. 1994).

Sepia officinalis is often displayed in marine aquaria, whilst its organs and cells are used extensively in biomedical and environmental research (Boletzky & Hanlon, 1983) and it is the 'working model' in cephalopod research (Koueta et al. 2006). Cuttlefish that are used for these purposes are frequently caught by fishing gears and are damaged during trawling and suffer post-trawling trauma. In this condition they can often be difficult to maintain for prolonged periods in aquaria. In order to avoid reliance on wild caught animals some laboratories (i.e. the Marine Biological Association, UK; CCMar, Universidade do Algarve, Portugal; NRCC, Marine Biomedical Institute, USA) now routinely rear cuttlefish to adults from eggs collected from the field, thus ensuring a regular supply for experiments and display purposes throughout most of the year.

Laboratory-cultured cuttlefish must be fed on live prey for the first few weeks of their life, usually mysid shrimp, which produce the best growth rates, (Richard, 1975; Boletzky & Hanlon, 1983; Forsythe et al. 1994; Domingues et al. 2001a, 2002, 2004). After this time young cuttlefish will accept dead food, such as frozen shrimp, fish or crabs (De Rusha et al. 1989; Domingues et al. 2001b; Koueta & Boucaud-Camou, 1999, 2001; Koueta et al, 2002), although some researchers have cultured *S. officinalis* on live prey throughout its life cycle (Domingues et al. 2001a, 2001b, 2002). *Sepia officinalis* can be cultured at a wide range of temperatures (Domingues et al. 2006). Richard (1971) recorded growth at temperatures as low as 9.5°C, while Domingues et al. (2001b) cultured *S. officinalis* at temperatures as high as 32°C. In general, the growth of laboratory-cultured cuttlefish is lower than that of wild populations (Domingues et al. 2006).

Sepia officinalis migrates into shallow coastal waters to mate and lay eggs at depths of no more than 30 or 40m (Boletzky, 1983). In the English Channel, UK, *S. officinalis* lays batches of eggs during the main spawning season in spring and summer (Boletzky, 1983). Cuttlefish eggs consist of an outer jelly-like capsule, the chorion membrane, perivitelline fluid and the vitelline membrane which surrounds the yolk and embryo (Cronin & Seymour, 2000). The ink stained eggs (1.2-1.4 cm in diameter, 2.5-3cm in length) of *S. officinalis* are laid individually and are attached to both living and non-living objects no more than ~1cm in diameter, such as sea grasses, tube worms, tree branches, cables and netting, via a fixating ring, (a ring shaped basal structure of the envelope (Boletzky, 1983). Freshly laid eggs are both soft and gelatinous. At the late stages of embryonic development the egg envelope is dilated by the expansion of the chorion due to the osmotic pressure of the perivitelline fluid (Boletzky, 1983). According to Boletzky (1983), the oxygen concentration of

seawater surrounding the eggs must be close to saturation in order to attain optimum rates of development, while it is thought that developing embryos are provided with oxygen by diffusion through egg envelope, diffusion being most efficient at the latter stages of development when the envelope is at its thinnest (Wolf et al. 1995). Cronin & Seymour (2000) state that cuttlefish egg capsules are effectively barriers which impede oxygen diffusion, therefore limiting oxygen consumption by the developing embryos. This in turn constrains the size of the eggs such that embryos do not experience diffusion-limited V_{o2} (the rate of oxygen consumption). The duration of embryonic development varies with seawater temperature, ranging from between 40-45 days at 20°C and 80-90 days at 15°C (Boletzky, 1983). Under experimental conditions, Paulij et al. (1991) found that when maintained under a light dark cycle, hatching preferentially occurs during darkness, even during short periods of darkness, 1 to 4h in length. Hatchlings measure between 6-9mm (DML) depending on ovum size, and are similar in both morphology and their basic behaviour to adults of the species being able to feed within hours of hatching (Boletzky, 1983). In the absence of suitable prey items hatchlings can survive for several days from energy reserves obtained from an internal yolk reserve, with young animals remaining buried in sandy substrates during daylight hours (Boletzky, 1983). Once collected, cephalopods and their fertile eggs need to be transported carefully from the field to the laboratory or aquarium to ensure optimum survival (Hanlon, 1990). The best conditions for transport may vary from one species to another and therefore transport conditions need to be determined for each species (Ikeda et al. 2004). Transportation of juvenile sepioids following collection is notoriously difficult (Hanlon, 1990) although movement of the eggs is simpler since the embryo is protected inside the egg. However, despite the protection offered, encapsulation poses problems for the

embryos as they are effectively imprisoned within their egg casings throughout development (Pechenik, 1986). Fluctuations in seawater temperature, salinity and UV radiation can adversely affect their development (Przeslawski, 2004). Understanding and providing optimum conditions for survival during transport will both benefit the welfare of the embryos and maximise juvenile survival upon hatching. It will also lead to fewer eggs being required and reduce losses following transport, with the concomitant reduction in transportation costs, and promote and encourage sustainable harvesting of natural populations of eggs.

Despite the widespread culture of *S. officinalis* for experimentation from collections of wild eggs (i.e. Palmegiano & D' Apote, 1983; Forsythe et al. 1994; Koueta et al. 2000; Grigoriou & Richardson, 2004), there is a paucity of literature regarding the optimum conditions for egg and juvenile transportation and the effects of transport methods on their subsequent growth and survival. In one study by Minton et al., (2001) the transportation between Thailand and Texas (USA) of eggs of *S. pharaonis* held in plastic bags with oxygenated seawater resulted in only 1.2% of the 900 eggs producing viable hatchlings. Lee et al. (1994), Forsythe et al. (2001) and Walsh et al. (2002) successfully shipped the eggs of the oval squid, *Sepioteuthis lessoniana,* at various stages of development, for periods of between 30 & 96 h from Japan to the USA and the eggs of *S. officinalis,* successfully survived transportation (Grigoriou and Richardson, 2004). Despite the widespread transport of cephalopod species there have been no comparisons of transport methodologies and no data on the ability of the eggs to survive periods of desiccation, hypoxia, pH and temperature fluctuations associated with transport. Some limited research into the embryonic development of *S. officinalis* has been conducted which has focused on the effect of external factors e.g. seawater temperature and salinity on the incubation period and

hatching success (Richard, 1975; Palmegiano & D'Apote, 1983).

Understanding how different transportation methods affect the survival and growth of embryonic and juvenile cuttlefish will aid in improving transportation conditions and survival. This information will be valuable to institutions and individuals seeking to import the species for research and for display in aquaria, particularly in regions distant from the sea e.g. in North America where there are no native *Sepia* species (Voss, 1974) and where they are reliant on animals reared from wild collected eggs. In this chapter groups of cuttlefish *S. officinalis* eggs were transported by car from the south coast of England to North Wales (~312 miles) and the effects of four different transportation conditions on the survival and growth of embryos and their growth performance following hatching investigated. The objective of the work was to ascertain the most appropriate methodology for egg transport, which could then be applied to the transport of the eggs of the little cuttlefish, *Sepiola atlantica* to both public aquaria and research laboratories world-wide. The eggs of *Sepia officinalis* were preferentially selected for this experiment as they were readily available in large numbers from mixed parentage and share similar characteristics to the eggs of *S. atlantica* (the eggs of both species are laid individually and surrounded by a protective outer chorion) although the eggs of *S. officinalis* are ink stained and larger than those of *S. atlantica.*

METHODS

Egg Collection

Egg masses of *Sepia officinalis* were collected on 25th May 2007 from traps deployed at a depth of 8m off Bracklesham Bay, West Sussex (England, UK), where cuttlefish spawn annually. Traps were checked daily, and the eggs used in the study were known to be four days old (Stage I; see Boletzky et al. 2006). Following collection eggs were immersed in a bucket (40 L) of seawater and covered with *Fucus serratus* (L.) to reduce movement of the egg masses within the bucket and transported 35 km by boat for 3.5h to Portsmouth harbour and then held overnight for 15h in a polypropylene mesh sac (50 x 80 50 cm, 5 mm mesh diameter) suspended at a depth of ~60 cm from a pontoon located in the main channel of Portsmouth Harbour. The following morning the eggs were removed from the sac and carefully separated by hand. The eggs were then gently mixed by hand to reduce any possible effect of position in the egg mass, spawning sequence, local environmental conditions and the effect of different parentage (see Boletzky, 1983; Steer et al. 2002; Steer & Moltschaniwskyj, 2007).

Egg transport

Eggs were transported using one of four treatments with five replicates per treatment. In an earlier experimental trial (authors unpub. obs.), that had been conducted to test the feasibility of the subsequent egg transport experiment, five replicates were found to be the most appropriate number for experimentation from the point of view of space allowances within the laboratory aquaria. The treatments were 1) Wet: eggs immersed in 1.5L of Portsmouth harbour sea water, 2) Wet & aerated: eggs immersed in 1.5L of harbour sea water and continuously aerated, 3) Damp: eggs

wrapped loosely in paper towel dampened with 90ml of harbour sea water and 4) Dry: eggs gently blotted dry with paper towel and emersed in air. Each replicate (see Figure 1) consisted of a polythene bag (30 x 40 x 30 cm) containing fifty eggs (250 eggs per treatment) placed in individual polyurethane insulated cool boxes (Campingaz, dimensions 20 x 27 x 18 cm). The packing density used ensured the eggs would not clump together during transit thus reducing any effect of egg position.

Four Tinytag TGI 3080 temperature data loggers (Gemini Data Logger (UK) Ltd) placed in one replicate from each treatment, continuously monitored the sea water or air temperature inside the cool box. Additional data on the pH, salinity and dissolved oxygen content of the seawater and relative humidity (RH) of the air inside the boxes, were obtained every 2h from a sub-sample of replicates during the course of the transport. Similar data were obtained from Portsmouth harbour to evaluate whether conditions inside the cool boxes changed significantly during the road transport. Data were collected using a hand-held digital thermometer, pH meter, refractometer, dissolved oxygen meter and Tenax Thermo Hygrometer, respectively. No ammonia, nitrite or nitrate were detected during the course of the egg transport using standard laboratory kits and these compounds will not therefore be discussed further.

Cool boxes with the eggs and identical control boxes without eggs were arranged in the back of an estate car in a 5 x 5 arrangement (Latin square design), controlling for any effect of position within the car. The boxes were covered with a Gelert reflective blanket in order to reduce the effect of warming by incident sunlight. Sorting and packaging of the eggs into their transport containers took 2h during which time the eggs were stored in seawater. The egg transport from Portsmouth to Menai Bridge, North Wales took 8.5h. A further group of ~1000 eggs, transported in a

larger cool box (dimensions 42 x 27 x 33 cm) containing aerated seawater, were used to monitor the progress of development of the cuttlefish embryos inside the eggs following transport. The eggs were disturbed under experimental conditions for ~12.5 h following sorting in Portsmouth harbour, 15 hours after collection from Bracklesham Bay; the eggs were sorted and packed into the cool boxes (2 h), 8.5h in transit by car from Portsmouth to Menai Bridge (502 km) and a final 2h whilst the eggs were unpacked, sorted and placed into the experimental aquaria in Menai Bridge and accounted for the transport time. Eggs were therefore exposed to the experimental transport conditions for a minimum of 8.5 hours, and a maximum of 12.5 hours.

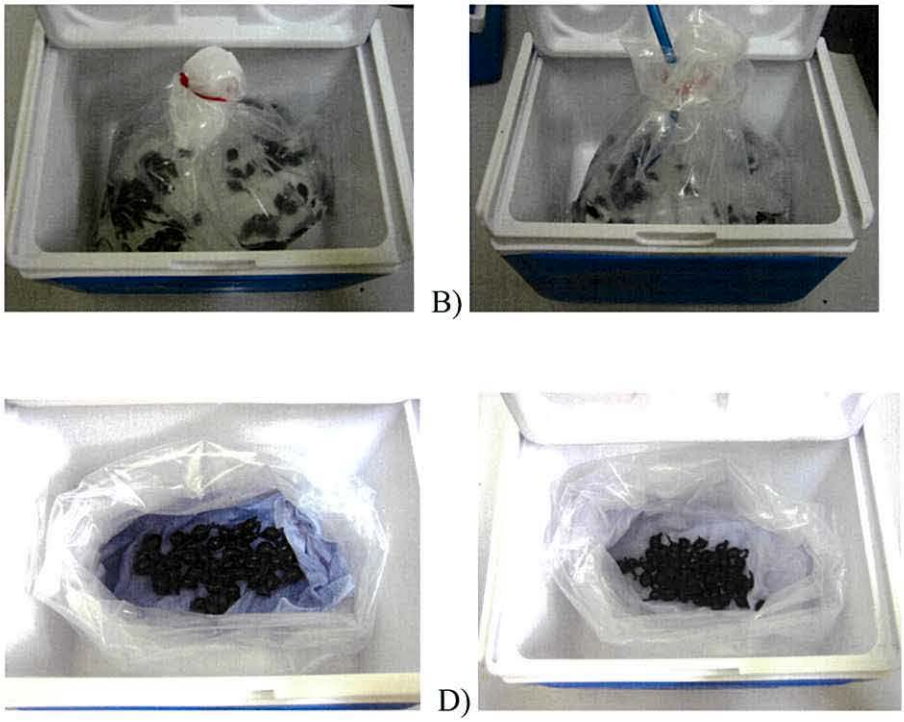


Fig. 1 The experimental setup used to transport cuttlefish eggs from Portsmouth to Menai Bridge. A) Wet, B) Wet & aerated, C) Damp and D) Dry.

Egg development & juvenile rearing

Following transport the eggs from each replicate were transferred to plastic mesh baskets (22 x 15 x 18cm, fifty eggs per basket) with lids and labelled according to their treatment and replicate number (see Figure 2). One replicate basket with eggs from each treatment was placed in one of five 150 L rearing tanks (A-E), containing sea water maintained at 17 °C (±1 °C), a temperature similar to that from which the eggs were collected (16.8 °C), and supplied with a seawater flow of 1.5L.min^{-1}, strong aeration and water circulation. The position of the baskets within the tank was changed daily to limit possible localised effects within the tanks. Due to restrictions in aquarium space eggs were maintained under subdued (~55 lux) constant illumination. The number of hatchlings in each basket was recorded daily and each individual photographed for later measurement of dorsal mantle length (DML) using analySIS® image analysis software before transfer to small plastic rearing tanks (1.8L) with perforated lids (see Figure 3). The cuttlefish hatched over a period of 40 days after which time the remaining eggs were deemed to be non-viable. The tanks were submerged within the main rearing tanks and the hatchlings fed *ad libitum* on mysid shrimp, *Neomysis integer*, for 14d after the last hatchling had emerged from all the treatments. Fourteen days was arbitrarily considered a minimum time to investigate whether development and growth following hatching had been compromised by the different transport treatments. In reality some of the early hatched cuttlefish could have been as old as 54 days. The environmental conditions within the five rearing tanks were similar so that cuttlefish in the replicates from each treatment were exposed to similar conditions amongst the rearing tanks. The DML (measured to the nearest 0.1 mm using vernier callipers) and wet body weight (Ww)

(recorded to the nearest 0.01g) of each cuttlefish from the four different replicates in a representative tank, tank C, were determined.

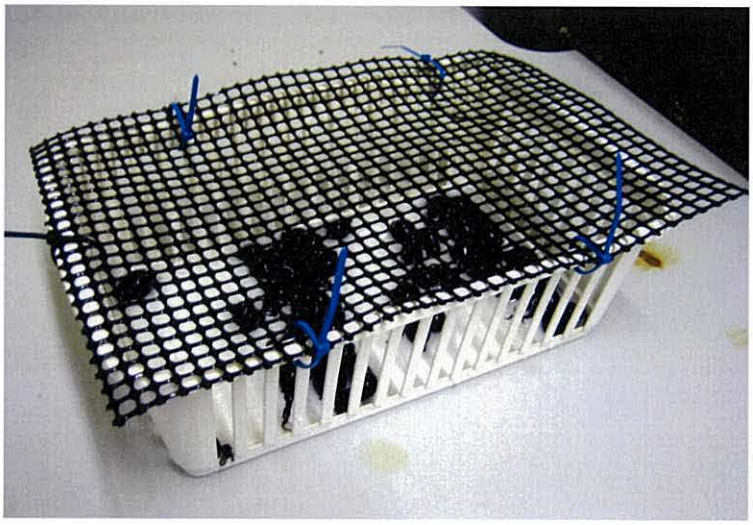


Fig. 2 Mesh baskets used to house cuttlefish eggs following transport.

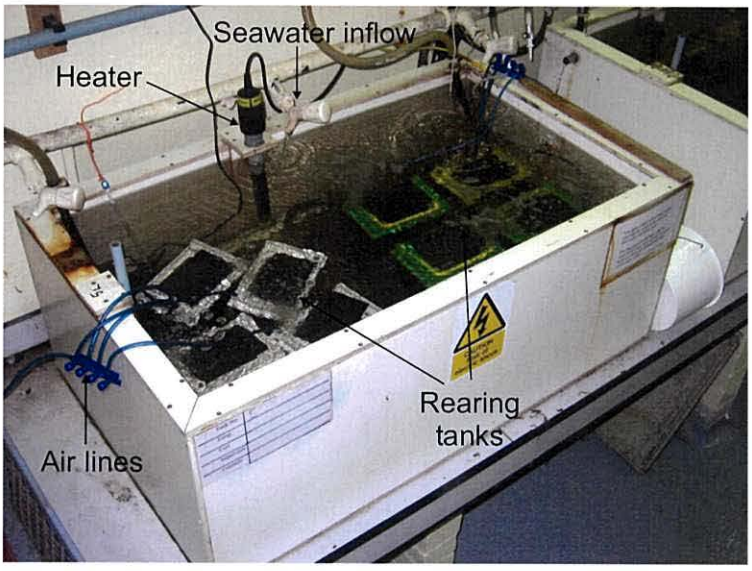


Fig. 3 Aquarium system used to rear cuttlefish hatchlings.

The group of ~1000 eggs, transported in the large cool box were transferred to baskets and held in a rearing tank (tank F) at 17 °C (±1 °C). The diameter of a random sub-sample of fifty of these eggs was measured, (see Figure 4A), using vernier callipers at the start of the laboratory rearing programme and after 40 days just prior to the cuttlefish hatching. Every 5d throughout the experiment the protective ink layer (chorion) of a sub-sample of ~10 eggs was removed in order to monitor development while the developmental stage of the cuttlefish was estimated using Boletzky's description of embryo development (Boletzky et al. 2006).

The DML and Ww data were tested for normality of distribution and homogeneity of variances and one way ANOVAs undertaken to determine whether there were significant differences in DML and Ww between the hatchlings from the different treatments in tank C at birth, and 14d after last hatching. ANOVA was used to investigate whether there were differences in hatching success, hatching frequency and hatchling survival between treatments. A Student t-test, Mann-Whitney U-test and Mood's Median tests were used to check if there were any differences in the environmental conditions amongst the treatments.

RESULTS

Egg Collection and Transport

The environmental conditions of the Portsmouth harbour water prior to the road transport and conditions within the cool boxes during transport are given in Table 1. Seawater temperature changed significantly in the wet and wet & aerated boxes ($W_{101,101}$=11231.5, p=0.033), while there was no significant difference in air temperature between the damp and dry treatments (Chi-square=0.49, p>0.05 p=0.486). However, the range of air temperatures was greater and the median air temperatures were significantly higher in the damp and dry treatments than in the wet and wet & aerated boxes (Chi-square =122.38, p<0.05). pH did not vary significantly between the wet and wet & aerated treatments ($W_{13,11}$ = 200.0, p=0.5852) (see Table 1).

There were too few measurements to allow statistical treatment of the salinity and relative humidity data; however, the mean salinity in the wet treatments was 33.40 ± 0.548 and 33.80 ± 0.447 in the wet & aerated treatments. Mean relative humidity in the damp and dry treatments was 63.2% and 61.0%, respectively. The dissolved oxygen content of the seawater in the wet and wet & aerated treatments varied significantly (t=3.30, p=0.004) and showed a high intra- and inter-treatment variability (73.06 % ± 11.41 & 89.57 % ± 4.68). No burst or physically damaged egg capsules were recorded following transport, although those transported either damp or dry looked visibly 'pinched' (Figure 4A) compared with those transported in seawater (Figure 4B). However, following re-submersion in seawater the egg capsules returned to their characteristic 'grape like' shape within 24 h of immersion.

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

Treatment	Mean Seawater Temperature (°C) ± SD	Mean Air Temperature (°C) ± SD	Air Temperature range (°C)	Mean pH ± SD	Mean Dissolved Oxygen (% saturation) ± SD	Mean Salinity ± SD	Mean Relative Humidity (%)
Harbour Water	16.8	n/a	n/a	8.54	69.8	35	n/a
Wet	18.80 ± 0.05	n/a	17.45 to 19.65	8.39 ± 0.05	73.06 ± 11.41	33.40 ± 0.548	n/a
Wet & aerated	18.80 ± 0.61	n/a	17.31 to 19.71	8.38 ± 0.06	89.57 ± 4.68	33.80 ± 0.447	n/a
Damp	n/a	19.80 ± 1.02	17.25 to 21.13	n/a	n/a	n/a	63.2
Dry	n/a	19.80 ± 1.34	16.98 to 22.05	n/a	n/a	n/a	61.0

n/a Not applicable

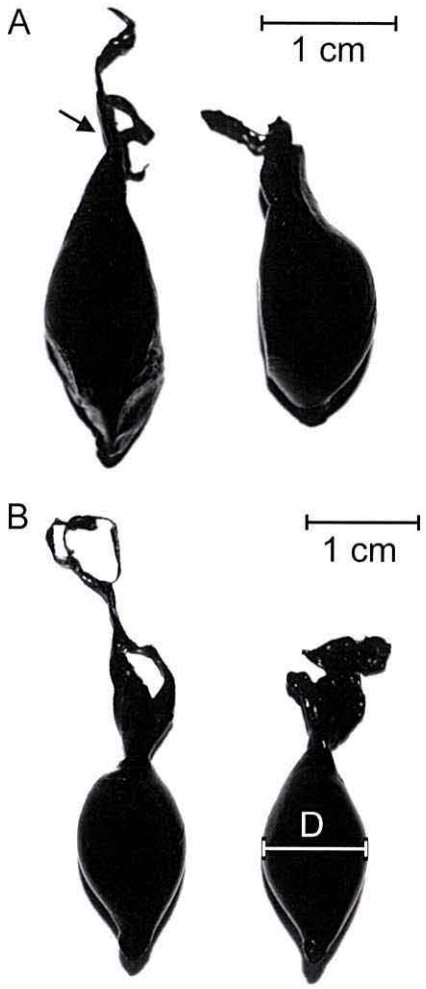


Fig. 4 Eggs of *Sepia officinalis* following 8.5h transport. A) Dry: eggs blotted dry with paper towel and emersed in air. B) Wet & aerated: eggs immersed in 2L of harbour sea water. D indicates the axis of measurement of egg diameter. The fixating rings (arrow) were removed from some of the eggs during handling. D= Egg diameter.

Egg Development

During development at 17 °C (following transport up until the first hatchling appeared), the diameter of the additional 1000 *S. officinalis* eggs increased steadily over time from an initial 9.5 ± 0.9 mm (day zero) to 17.3 ± 2.3 mm (day 38) as the embryos inside developed. The observed increase in egg diameter over time and occasional observations of the embryos inside the eggs, following chorion removal, indicated that the embryos were developing during the rearing period. The first hatchlings appeared 28 days following transport and continued to appear until day 68 with the majority of eggs hatching between 40 and 60 days after transport (see Figure 5). Thus some early hatchlings would have been 40 days old before the last hatchling appeared. After 68 days the remaining 152 of the initial 1000 eggs, which were probably infertile eggs, deteriorated and eventually ruptured. There was no significant difference in frequency of hatching ($F=0.01$, $p= 0.999$) between treatments and there was no evidence to suggest that any of the transport methods impacted on the timing of the onset of hatching (Figure 6).

The average number of hatchlings that appeared following transport was ~42 (~85% hatching success; range 68-96% amongst replicates) (Table 2). ANOVA demonstrated there was no significant difference in the number of cuttlefish hatchlings between each of the four transport treatments ($F=0.07$, $p= 0.973$). Similarly there was no significant difference in percentage hatchling survival from each replicate of each treatment following hatching ($F=1.09$, $p= 0.382$). Cuttlefish that hatched from eggs transported dry (treatment 4), (Dry = 8.6 ± 1.2 mm) were significantly smaller ($F= 3.59$, $p<0.015$) than hatchlings from the other 3 treatments (Wet = 9.3 ± 0.9, Wet & aerated = 9.2 ± 1.0 and Damp = 9.2 ± 0.9 mm). However,

following rearing of the cuttlefish for 14 days there was no significant difference (F= 1.31, p= 0.274) in hatchling size and weight (see Table 3).

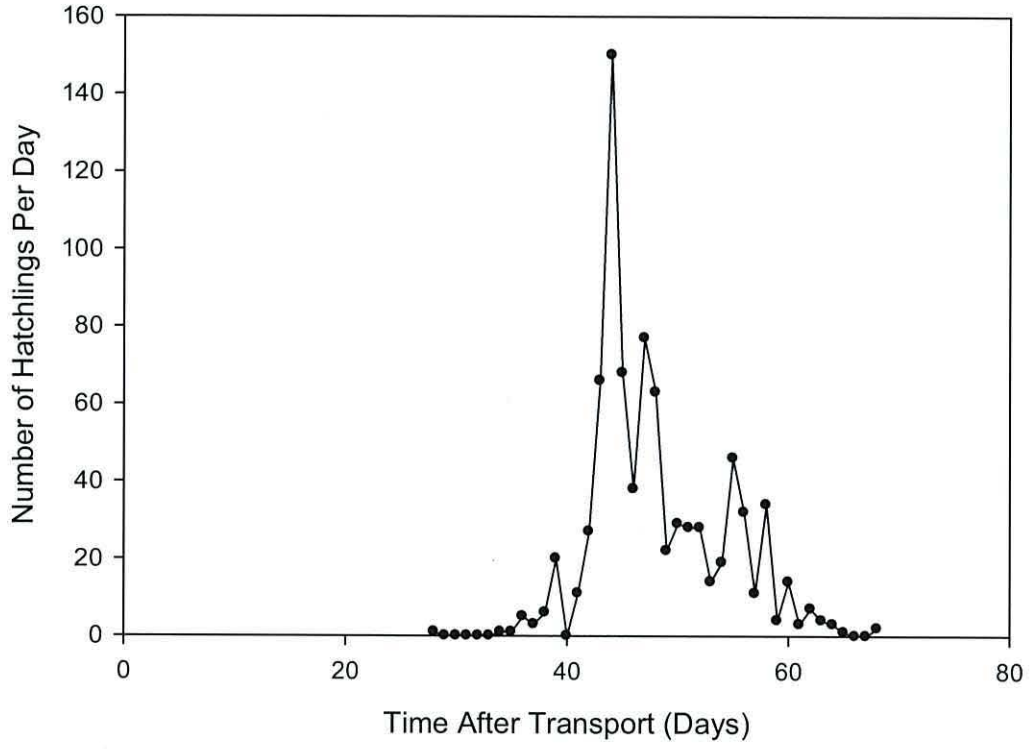


Fig. 5 The number of *Sepia officinalis* hatchlings recorded over a 68 day period following transport of a consignment of eggs from Portsmouth to Menai Bridge.

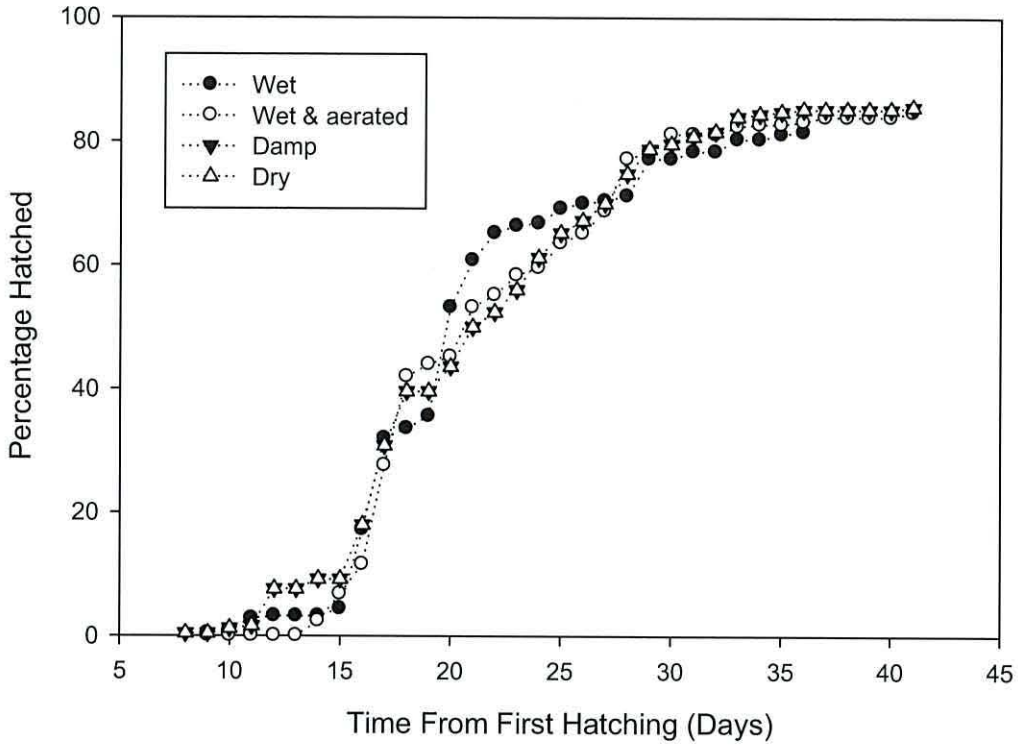


Fig. 6 The cumulative percentage of hatched *Sepia officinalis* eggs recorded over a 40 day period at 17°C.

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.

Treatment	Mean number of hatchlings ± SD	Mean hatching success (%) ± SD	Minimum and (maximum) (%)
Wet & aerated	42.2 ± 4.2	84.4 ± 8.3	74 (94)
Wet	42.0 ± 5.7	82.8 ± 10.6	68 (94)
Damp	42.8 ± 5.4	85.6 ± 10.8	68 (96)
Dry	43.2 ± 1.9	86.4 ± 3.9	82 (92)

Table 3. Dorsal mantle length and mean wet body weight of *S. officinalis* hatchlings at the time of hatching and after rearing at 17 °C for 14 days.

Treatment	Number of hatchlings at start of experiment	Mean dorsal mantle length ± SD (mm) at start of experiment	Number at end of experiment	Mean dorsal mantle length ± SD (mm) at end of experiment	Mean wet weight ± SD (g) at end of experiment
Wet & aerated	42	9.2 ± 1.0	40	14.4 ± 1.8	0.82 ± 0.24
Wet	36	9.3 ± 0.8	34	14.0 ± 2.3	0.94 ± 0.33
Damp	40	9.2 ± 0.9	32	14.8 ± 2.5	0.88 ± 0.39
Dry	34	8.6 ± 1.2	27	14.8 ± 2.0	0.94 ± 0.26

DISCUSSION

This study is the first to specifically investigate the effect of different transport methods on egg survival, juvenile hatching success and growth of *Sepia officinalis* following transport. It has been demonstrated that the developing embryos within the eggs tolerate transport over periods of 8.5h and can be transported damp, dry, in sea water with or without aeration, without any significant deleterious effects on hatching success, growth and survival following hatching. The ability to withstand transport stress may result from the protection offered by the encapsulating ink layer combined with stable environmental parameters in which the eggs were transported. Previously there have been few studies into the effects of transportation on cephalopod eggs or hatching success following transportation. This is surprising given the need to move cephalopods from coastal waters to inland laboratories. There is a basic description of a methodology used during transportation of the eggs of the oval squid, *Sepioteuthis lessoniana* (e.g. Lee et al. 1994; Forsythe et al. 2001; Walsh et al. 2002). The eggs were successfully shipped from Japan to the USA over lengthy periods between 30 and 96h in plastic bags filled with equal volumes of seawater, aeration and ice to keep the temperatures constant. Organogenesis is usually complete at larval stage 24 (stage 15 in *S. officinalis* (Boletzky, 1983)), therefore embryos are less susceptible to organ malformation but still too young to hatch prematurely during transport (Forsythe et al. 2001). When stressed in un-natural conditions, cephalopod eggs can hatch prematurely, yet under normal conditions this is prevented by a tranquilizing factor found within the perivitelline fluid (Marthy et al. 1976).

In this study, mean egg viability within each treatment ranged between 82.8 % for eggs transported wet and 86.4 % when eggs were transported dry with no significant difference in hatching success observed between the treatments. The wet

and wet & aeration treatments had the lowest hatching success rate, whilst the eggs transported damp and dry had the highest hatching success, despite the eggs being transported under conditions that potentially made the eggs more susceptible to temperature fluctuations. In previous studies, hatching success in *S. lessoniana* ranged between 37 & 49 % (Lee et al. 1994), 33 & 93 % (Forsythe et al. 2001) and 34.9 % (Walsh et al. 2002). However, in one study when the eggs of the cuttlefish *S. pharaonis*, were transported from Thailand to the USA at a density of 9.5-10.5 eggs L^{-1} only 1.2 % successfully hatched (Minton et al. 2002). However, the egg capsules of this species lack the same encapsulating ink layer as the egg capsules of *S. officinalis* (Norman, 2000). Ikeda et al. (2004) examined the appropriate transport conditions for both young and sub-adult *S. lessoniana* (22–194mm DML) under laboratory simulated (24h) and actual (22-23h) transport conditions and found survival was highest when the body weight: seawater ratio was below 30.

In the present study the eggs were the same age on collection; however, hatching took place over 40 days at ~17 °C, considerably longer than the 14 and 20 days reported by Richard (1975) in *S. officinalis* eggs reared at 20 and 15 °C respectively, yet the same as in a preliminary study under identical conditions to the present study (pers. obs). It is likely that the extended hatching period resulted from the lighting regime present in the culture room as Paulij et al. (1991) found that the time to hatching increased and was asynchronous in *S. officinalis* eggs reared under constant illumination.

The reported transport study was conducted over a relatively short period (~8 h) with few significant differences between the 4 methods of transportation found. This is perhaps not surprising since the conditions, e.g. the seawater temperature and salinity changes, experienced by the eggs were within the range of conditions the eggs

might normally experience. Longer periods under the treatment conditions may have resulted in lower hatching success. The conditions experienced by the eggs transported damp and in air would not normally be encountered and the observed slight egg shrinkage did not ultimately affect hatching success or subsequent juvenile development. When exposed to oxygen depletion, salinity fluctuations or seawater temperatures outside of the normal range for the cephalopod species the eggs can degrade or the resulting hatchlings can develop abnormalities (see Boletzky, 1998). The egg chorion of *S. officinalis* thins as the embryo develops and becomes almost transparent at the point of occlusion (Wolf et al. 1985) as a result of changes in the osmotic pressure of the perivitelline fluid (Boletzky, 1983). These observations suggest that if late stage eggs were transported then differences in survival might occur since at this stage protection from the initially thick (± 1.5 mm) chorion diminishes.

Although no ammonia, nitrite or nitrate concentrations were detected in the seawater surrounding the transported eggs of *S. officinalis*, Lee et al. (1994) found that ammonia concentrations of 0.91 and 1.55 ppm were correlated with packing densities of *S. lessoniana* eggs of 3.8 and 7.1 egg strands L^{-1}, respectively. Elevated ammonia concentrations between 0.176 and 0.333 ppm at a packing density of 2.6 egg strands. L^{-1}, were detected in seawater surrounding the eggs of *S. lessoniana* during a 30 h road transport that further rose to between 0.220 & 0.414 ppm after 96 h at a density of 2.8 egg strands. L^{-1} (Walsh et al. 2002).

Maximum hatchling size varied between 5.6mm (damp treatment) and 11.4mm DML (wet treatment) and probably reflected the staggered dates of hatching and rearing period; the earliest and last-hatched juvenile cuttlefish could have been as old as 54 days and as young as 14 days old respectively. *Sepia officinalis* hatchlings

are usually between 6 & 9mm DML, but hatching size depends on ovum size (Boletzky, 1983). Following completion of the 14d rearing trial the maximum size and weight of the hatchlings varied between 10.5mm DML (wet treatment) and 21.6mm DML (damp treatment) and 0.38g (wet & aeration treatment) and 2.02g (wet treatment), respectively. At the end of the 14d rearing period the lowest survival rate of 61 % occurred in one of the wet treatment replicates. However, each treatment had at least one replicate with 100% post-hatching survival at the end of the 14d trial. These rates are comparable with survival rates of newly born *S. officinalis* hatchlings reared for 4 weeks on the mysid *Paramysis nouvelli,* (91.1 %), fish fry *Atherina* sp. (73.3 %) and the grass shrimp *Palaemonetes varians* (100 %) (Domingues et al. 2004).

Although egg viability, hatching success and subsequent growth of hatchlings following transport are important considerations in the selection of an appropriate transport method, financial, logistic and bio-security considerations impact on the eventual method selected. A reduction in the volume of the transport water would significantly reduce shipping weight and therefore transport costs (see Bower et al. 1999). To lower freight costs, the damp and dry treatments, where water volume is reduced or eliminated and no air pumps are required, are likely to be the most economical methods. Transport of eggs without supplementary aeration is feasible as has been demonstrated in this study. From a bio-security point of view, eliminating the need to transport eggs in seawater reduces the risk of introducing non-native planktonic species from one region to another. Pests, parasites and disease can be spread from one location to another following transport and they can have serious implications to aquaculture, fisheries and the environment and they are considered to be the primary threat to global biodiversity (Bax et al. 2004). Egg masses are easier to quarantine than juvenile cephalopods and they are generally easier to transport as

has been demonstrated in this thesis. Recently laid *S. officinalis* eggs are tolerant of transport stress and were able to survive a 12.5h transport period under varying environmental conditions with no significant impact on hatching success, growth and survival between treatments. The methodology described here to transport the eggs of *S. officinalis* around the UK and Europe will enable cuttlefish to be moved with minimal fatalities to research laboratories and aquaria in regions where the species is not locally available. The methodology described in this chapter could now be used to investigate the transport of *Sepiola atlantica* eggs laid by adults (see chapter 3).

A concise version of this chapter has been published as a research note;

Jones, N.J.E., Ridgway, I.D. & Richardson, C.A. 2009. Transport of cuttlefish, *Sepia officinalis*, eggs under dry and damp conditions. Journal of Molluscan Studies. 75, 192-194

REFERENCES

Bax, N., Carlton, J.T., Mathews-Amos, A., Haedrich, R.L., Hogwarth, F.G., Purcell, J.E., Rieser, A. & Gray, A., 2001. The control of biological invasions in the world's oceans. Conservation Biology. 15, 1234-1246

Boletzky, S.v., 1983. *Sepia officinalis*. In: Boyle, P.R. (Eds.), Cephalopod Life Cycles Vol I. Academic Press, London, pp 31-53

Boletzky, S.v. & Hanlon, R.T., 1983. A review of the laboratory maintenance, rearing and culture of cephalopod molluscs. Memoirs of the National Museum of Victoria, Melbourne. 44, 147-187

Boletzky, S.v., 1998. Cephalopod eggs and egg masses. Oceanography and Marine Biology: an Annual Review. 36, 341-371

Boletzky, S. v., 2006. The *Sepia* egg: A showcase of cephalopod embryology. Vie et Milieu. 56 (2), 191-201

Cronin, E.R. & Seymour, R.S. 2000. Respiration of the eggs of the giant cuttlefish *Sepia apama*. Marine Biology. 136, 863-870

DeRusha, R.H., Forsythe, J.W. & Hanlon, R.T. 1989. Alternative diets for maintaining and rearing cephalopods in captivity. Laboratory Animal Science. 39, 306-312

Domingues, P., Sykes, A., Andrade, J.P. 2001a. The use of *Artemia* sp. or mysids as food source for hatchlings of the cuttlefish (*Sepia officinalis* L.), effects on growth and survival throughout the life cycle. Aquaculture International. 9, 319-331

Domingues, P.M., Kingston, T., Sykes, A. & Andrade, J.P. 2001b. Growth of young cuttlefish, *Sepia officinalis* (Linnaeus 1758) at the upper end of the biological distribution temperature range. Aquaculture Research. 32, 923-930

Domingues, P., Sykes, A. & Andrade, J.P. 2002. The effects of temperature in the life cycle of two consecutive generations of the cuttlefish *Sepia officinalis* (Linnaeus, 1758), cultured in the Algarve (South Portugal). Aquaculture International. 10, 207-220

Domingues, P., Sykes, A., Sommerfield, A., Almansa, E., Lorenzo, A. & Andrade, J.P., 2004. Growth and survival of cuttlefish (*Sepia officinalis*) of different ages fed crustaceans and fish. Effects of frozen and live prey. Aquaculture. 229, 239-254

Domingues, P.M., Bettencourt, V. & Guerra, A. 2006. Growth of *Sepia officinalis* in captivity and in nature. Vie et Milieu. 56 (2), 109-120.

Forsythe, J.W., De Rusha, R.H. & Hanlon, R.T. 1994. Growth, reproduction and life span of *Sepia officinalis* (Cephalopoda: Mollusca) cultured through seven consecutive generations. Journal of Zoology, London. 233, 175-192

Forsythe, J.W., Walsh, L.S., Turk, P.E., Lee, P.G., 2001. Impact of temperature on juvenile growth and age at first egg-laying of the Pacific reef squid *Sepioteuthis lessoniana* reared in captivity. Marine Biology. 138, 103-112

Grigoriou, P., Richardson, C.A., 2004. Aspects of the growth of cultured cuttlefish *Sepia officinalis* (Linnaeus 1758). Aquaculture Research. 35, 1141-1148

Guerra, A. 2006. Ecology of *Sepia officinalis*. Vie et Milieu. 56 (2), 97-107

Hanlon, R.T., 1990. Maintenance, rearing and culture of teuthoid and sepioid squids. In: Gilbert, D.L., Adelman, W.J., Arnold, J.M. (Eds.). Squid as experimental Animals. Plenum, New York, pp35-62

Ikeda, Y., Ueta, Y., Sakurazawa, I., Matsumoto, G., 2004. Transport of the oval squid *Sepioteuthis lessoniana* Ferussac, 1831 in Lesson 1830-1831 (Cephalopoda: Loliginidae) for up to 24 h and subsequent transfer to an aquarium. Fisheries Science. 70, 21-27

Jereb, P. & Roper, C.F.E. (eds.). 2005. Cephalopods of the world: An annotated and illustrated catalogue of cephalopod species known to date. Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae). FAO Species Catalogue for Fishery Purposes. No. 4, Vol. 1. Rome, 262pp

Koueta, N. & Boucaud-Camou, E. 1999. Food intake and growth in reared early juvenile cuttlefish *Sepia officinalis* L. (Mollusca: Cephalopoda). Journal of Experimental Marine Biology and Ecology. 240, 93-109

Koueta, N. & Boucaud-Camou, E. 2001. Basic growth relations in experimental rearing of early juvenile cuttlefish *Sepia officinalis* L. (Mollusca: Cephalopoda). Journal of Experimental Marine Biology and Ecology. 265, 75-87

Koueta, N., Castro, B.G. & Boucaud-Camou, E. 2000. Biochemical indices for instantaneous growth estimation in young cephalopod *Sepia officinalis* L. ICES Journal of Marine Science. 57, 1-7

Koueta, N., Boucaud-Camou, E. & Noel, B. 2002. Effect of enriched natural diet on survival and growth of juvenile cuttlefish *Sepia officinalis* L. Aquaculture. 203, 293-310

Koueta, N., Andrade, J.P., Boletzky, S.v., 2006. The cuttlefish *Sepia officinalis* a working model in cephalopod research. Vie et Milieu. 56 (2), 67

Lee, P.G., Turk, P.E., Tack Yang, W., Hanlon, R.T., 1994. Biological characteristics biomedical applications of the squid *Sepioteuthis lessoniana* cultured through multiple generations. Biological Bulletin.186, 328-341

Marthy, H.J., Hauser, R., Scholl, A., 1976. Natural tranquiliser in cephalopod eggs. Nature. 261, 496-497

Minton, J.W., Walsh, L.S., Lee, P.G., Forsythe, J.W., 2001. First multi-generation culture of the tropical cuttlefish *Sepia pharaonis* Ehrenberg, 1831. Aquaculture International. 9, 375-392

Norman, M. 2000. Cephalopods: A world guide – Octopuses, Argonauts, Cuttlefish, Squid, Nautilis. Conchbooks, Hackenheim. 320pp

Palmegiano, G.B., D'Apote, M.P., 1983. Combined effects of temperature and salinity on cuttlefish (*Sepia officinalis* L.) hatching. Aquaculture. 259-264

Paulij, W.P., Herman, P.M.J., Roozen, M.E.F. & Denucé, J.M. 1991. The influence of photoperiodivity on hatching of *Sepia officinalis*. Journal of the Marine Biological Association of the United Kingdom. 71, 665-678

Pechenik, J.A., 1986. The encapsulation of eggs and embryos by molluscs: An overview. American Malacological Bulletin. 4, 165-172

Przeslawski, R., 2004. A review of the effects of environmental stress on embryonic development within intertidal gastropod egg masses. Molluscan Research. 24 (1), 43-63

Richard, A. 1971. Contribution à l' etude expérimentale de la croissance et de la maturation sexuelle de *Sepia officinalis* L. (Mollusque: Céphalopode). Thèse no 248, Universitaire Lille. 264 pp

Richard, A., 1975. L'élevage de la seiche (*Sepia officinalis* L., Mollusque). In: 10th European Symposium on Marine Biology, Ostend, Belgium, Vol I, 155-172pp

Steer, M.A., Moltschaniwskyj, N.A., 2007. The effects of egg position, egg mass size, substrate and biofouling on embryo mortality in the squid *Sepioteuthis australis*. Reviews in Fish Biology and Fisheries. 17, 173-182

Steer, M.A., Moltschaniwskyj, N.A., Gowland, F.C., 2002. Temporal variability in embryonic development and mortality in the southern calamari *Sepioteuthis australis*: a field assessment. Marine Ecology Progress Series. 243, 143-150

Voss, G.L., 1974. *Loligo surinamensis*, a new species of loliginid squid (Cephalopoda, Myopsida) from northeastern South America. Zoologische Mededelingen Leiden.48(6),43-53

Walsh, L.S., Turk, P.E., Forsythe, J.W., Lee, P.G., 2002. Mariculture of the loliginid squid *Sepioteuthis lessoniana* through seven successive generations. Aquaculture. 212, 245-262

Wolf, G., Verheyen, E., Vlaeminck, A., Lemaire, J., Decleit, W., 1985. Respiration of *Sepia officinalis* during embryonic and early juvenile life. Marine Biology. 90, 35-39

CHAPTER 5

GENERAL DISCUSSION

According to Boyle (1991) cephalopods are primarily maintained in captivity for scientific research into aspects of their biology, physiology and biochemistry where a supply of healthy, laboratory maintained animals is essential, while Hanlon (1987) states that understanding the life cycle of a cephalopod requires observation and experimentation on live animals both in the field and the laboratory. Over sixty species of cephalopods, largely benthic octopods and sepioids have been successfully maintained under laboratory conditions (Boyle, 1991). Many public aquaria find it profitable to display cephalopods, especially large species such as the giant Pacific octopus, *Enteroctopus dofleini* (Anderson, 1987). There is also a market for small species for display in home aquaria and these include the cuttlefish *Sepia bandensis*, *S. officinalis, Metasepia pfefferi* and the Hawaiian bobtail squid, *E. scolopes* (Dunlop & King, 2009). The recent expansion of the marine aquarium trade has increased environmental concerns surrounding the sustainability of wild stocks of marine ornamental species, many of which are being increasingly threatened by environmental and anthropogenic stresses to marine ecosystems (Moe Jr, 2003). Cultured cephalopods present a desirable alternative to wild collected animals. A recent feasibility study by Nabhitabhata et al. (2005) into the small-scale culture of the bobtail squid *Euprymna hyllebergi* as an ornamental species determined that the production of animals for the aquarium market added more value to the product through higher prices and a shorter period of production compared to that of food production.

Pierce et al. (2008) discuss that the sensitivity of cephalopod stocks to environmental fluctuations is an important consideration when managing cephalopod fisheries, and also suggest that cephalopods may be used as indicators of environmental change and ecosystem conditions. Such environmental fluctuations subsequently affect the population dynamics of both higher predators and their prey. Semmens et al. (2007) suggest that in the future our understanding of the processes that impact cephalopod population variability will become increasingly important as increasing seawater temperatures, predicted as a result of global warming, may disrupt or alter cephalopod migrations. Therefore, it is necessary to fully understand the life history and movement of certain species in order for key areas such as breeding and spawning grounds to be properly managed and conserved. This would be particularly important for species such as *S. atlantica* which are consumed by a number of commercially exploited fish species whilst also being prey for a variety of seabirds and cetaceans. Therefore, changes in the distribution of *S. atlantica* may have far reaching implications further up the food chain. Data presented here in chapter 2 suggests that *S. atlantica* has a widespread seasonal distribution around the coast of Anglesey. From observations made over a three year period at Y Foryd it appears that *S. atlantica* in all states of reproductive maturity congregate at this location. Data also presented indicates that *S. atlantica* migrate to the inshore waters around the coast of Anglesey and arrive in shallow water between July and October in order to mate and spawn. Their arrival in shallow water allows them to benefit from the warm seawater temperatures in the shallow surf zone and profit form an abundance of potential prey species. Areas such as Y Foryd that are under pressure from local anglers and bait collectors and are therefore important locally to *S. atlantica* and should be conserved wherever possible.

Jackson (1994) reports that the rapid growth of squid, along with the migrations undertaken in some species, has made traditional length-frequency analysis that are traditionally used for fin-fish difficult, if not impossible to interpret when applied to cephalopod stocks. However, the discovery of incremental daily rings (Boyle & Rodhouse (2006) in the statoliths (paired calcareous stones within the equilibrium organs (Clarke, 1966) of teuthoid squid and some sepioids provides an important tool for estimating the age and understanding the growth of cephalopods. Arkhipkin (2005) describes the statoliths as 'black boxes' that contain a variety of biological and ecological information. Growth increments and other patterns within the statolith microstructure can be used to determine age, hatching date and growth of an individual and have been used to date and estimate the duration of the paralarval phase of the life cycle, the number of spawning events, while the shape of the statolith can be used in systematics and the identification of ecological life styles. Boyle & Rodhouse (2006) suggest that a better understanding of the causal mechanisms for the formation of these rings along with more validation studies through the matching of increment counts to animals of a known age should take place.

Once established, reliable and universal methods for ageing animals through statolith examination may yield invaluable information on wild cephalopod populations. According to Jackson (1994) future statolith ageing studies should include; (1) comprehensive studies on a wide variety of species including those whose life history is poorly understood; (2) an investigation of the presence of growth increments in the statoliths of other cephalopods such as sepioids; (3) a comprehensive validation of increment periodicity, encompassing long-term culture trials in which all phases of the life cycle are examined; (4) the preparation and refinement of growth models in order to establish more accurate and robust models of

squid growth; (5) an examination into what statoliths can tell us about the ecology of a species such as temporal or geographical variations in growth responses to ambient biological or physical features or how increment width may reflect changes in the availability of food or temperature; (6) age-specific information on the timing of maturation and the duration of spawning; (7) a consideration of how prominent zones or checks within the statolith microstructure may be indicative of past habitats or disturbances and (8) an investigation into how statolith length or weight may vary as a result of past growth histories. *Sepiola atlantica* is a suitable candidate for such studies as it can now be cultured throughout its life cycle in the laboratory and laboratory reared animals of known age under controlled conditions have already been cultured in the present study (chapter 3). It was my intention to extend my research into an analysis of the statoliths; however, this is another area of research and must wait for further funding. The *S. atlantica* reared in this PhD study will be available for future statolith growth increment validation studies, similar to those previously undertaken using other sepioids such as *Sepia hierredda* (Raya et al. 1994), *S. officinalis* (Bettencourt & Guerra, 2001), *Ideosepius pigmaeus* (Jackson, 1989), *Heteroteuthis dispar* (Kristensen, 1980) and *Rossia pacifica* (Arkhipkin, 1995).

The methods and results described in chapter 4 of this thesis for transporting the early developmental stage eggs of *S. officinalis* demonstrated that there were no significant deleterious effects, following movement of the eggs under a range of different conditions, on the subsequent hatching success, survival and growth of cuttlefish hatchlings. The simple and low cost transport methods described will enable cuttlefish, and potentially other cephalopods to be moved far from their country of origin to research laboratories and aquaria world-wide with minimal fatalities. At the start of my research it was intended to apply the same principles of egg transport

gained from transporting *S. officinalis* eggs (chapter 4) to the transport of *S. atlantica* eggs produced in chapter 3. However it was only towards the middle of the study that it became clear that *S. atlantica* were reluctant to mate and lay eggs in the laboratory so there were no eggs available for transport and the paralarvae were difficult to rear in laboratory aquaria. The development of the methodology for transporting the eggs will reduce the potential spread of diseases, pests and the potential introduction of non-native planktonic organisms into other areas where they may be detrimental to the environment, fisheries and aquaculture facilities, by reducing the reduction or elimination of transport water. The transport methods may also be of benefit in instances where adult mortality or damage sustained during transport is high, or where culture trials have been hampered if cues that trigger spawning in captive adults are difficult to understand, thus a cheap and simple way to transport viable eggs would be of benefit.

While my thesis has not answered all the questions regarding the laboratory culture, ecology and population biology of *S. atlantica* it has given a far greater insight into the life history of this often seen but little studied coastal water species. Future work in this area would not only increase our understanding of the life history of this species, but it would in turn benefit the conservation of other species from higher trophic levels which rely on *S. atlantica* as a food source. Further development of the culture work may enable *S. atlantica* to be cultured on demand as a model organism for biomedical studies, as an ornamental species, or for human consumption. Currently while there are no directed fisheries for *S. atlantica*, similar sized cephalopods are consumed in continental Europe. As traditional fish stocks decline as a result of over fishing it is possible that fishers may diversify and species such as *S. atlantica* may become the target of commercial fisheries. In conclusion, in order to

properly manage and conserve global cephalopod populations and to maximise the research potential of captive specimens I believe that a better understanding of the husbandry, population biology and life history of cephalopods such as *S. atlantica* is essential.

REFERENCES

Anderson, R.C. 1987. Cephalopods at the Seattle Aquarium. International Zoo Yearbook. 26, 41-48

Arkhipkin, A.I. 1995. Statolith microstructure and maximum age of the sepiolid *Rossia pacifica* (Cephalopoda, Sepioidea) in the northern part of the north Pacific. Sarsia. 80, 237-240

Arkhipkin, A.I. 2005. Statoliths as 'black boxes' (life recorders) in squid. Marine and Freshwater Research. 56, 573-583

Bettencourt, V. & Guerra, A. 2001. Age studies based on daily growth increments in statoliths and growth lamellae in cuttlebone of cultured *Sepia officinalis*. Marine Biology. 139, 327-334

Boyle, P.R. 1991. The UFAW handbook on the care and management of cephalopods in the laboratory. Universities Federation for Animal Welfare, Aberdeen. pp1-63

Boyle, P.R. & Roydhouse, P. 2005. Cephalopods- Ecology & Fisheries. Blackwell publishing, Oxford. pp 369-370

Clarke, M.R. 1978. The cephalopod statolith- An introduction to its form. Journal of the Marine Biological Association of the United Kingdom. 58, 701-712

Dunlop,C. & King, N. 2009. Cephalopods: Octopuses and Cuttlefishes for the Home Aquarium. T.F.H. Publications, Inc. Neptune City, New Jersey. 239pp

Durholtz, M.D., Lipinski, M.R. & Field, J.G. 2002. Laboratory validation of periodicity of incrementation in statoliths of the South African chokka squid *Loligo vulgaris reynaudii* (d'Orbigny, 1845): a reevaluation. Journal of Experimental Marine Biology and Ecology. 279, 41-59

Hanlon, R.T. 1987. Mariculture. In: Boyle, P.R. (eds.). Cephalopod Life Cycles, Volume II. Academic Press, London. 291-305

Jackson, G.D. 1989. The use of statolith microstructure to analyze life-history events in the small tropical cephalopod *Ideosepius pigmaeus*. Fishery Bulletin. 87, 265-272

Jackson, G.D. 1994. Application and future potential of statolith increment analysis in squids and sepioids. Canadian Journal of Fisheries and Aquatic Science. 51, 2612-2625

Kristensen, T. 1980. Periodical growth rings in cephalopod statoliths. Dana. 1, 39-51

Moe Jr, M.A. 2003. Culture of marine ornamentals: For love, for money and for science. In: Cato, J.C. & Brown, C.L. (eds). Marine Ornamental Species: Collection, culture and conservation. Iowas State Press, Ames. 11-28

Nabhitabhata, J., Nilaphat, P., Promboon, P. & Jaroongpattananon, C. 2005. Life cycle of cultured bobtail squid, *Euprymna hyllebergi* Nateewathana, 1997. Phuket Marine Biological Centre Research Bulletin. 66, 351-365

Pierce, G.J., Valavanis, V.D., Guerra, A., Jereb, P., Orsi-Relini, L., Bellido, J.M., Katara, I., Piatkowski, U., Pereira, J., Balguerias, E., Sobrino, I., Lefkaditou, E., Wang, J., Santurum, M., Boyle, P.R., Hastie, L.C., MacLeod, C.D., Smith, J.M., Viana, M., González, A.F. & Zuur, A.F. 2008. A review of cephalopod-environment interactions in European Seas. Hydrobiologia. 612, 49-70

Raya, C.P., Fernandez-Ninez, M., Balguerias, E. & Hernandez-Gonzalez, C.L. 1994. Progress towards ageing cuttlefish *Sepia hieredda* from the northwestern African coast using statoliths. Marine Ecology Progress Series. 114, 139-147

Semmens, J.M., Pecl, G.T., Gillanders, B.M., Waluda, C.M., Shea, E.K., Jouffre, D., Ichii, T., Zumholz, K., Katugin, O.N., Leporatti, S.C. & Shaw, P.W. 2007. Approaches to resolving cephalopod movement and migration patterns. Reviews in Fish Biology and Fisheries. 17, 401-423