

2013

# The Ecology, Distribution and Spawning Behaviour of the Commercially Important Common Cuttlefish (*Sepia officinalis*) in the Inshore Waters of the English Channel

Bloor, Isobel

<http://hdl.handle.net/10026.1/1494>

---

<http://dx.doi.org/10.24382/3827>

University of Plymouth

---

*All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.*

2013

# The Ecology, Distribution and Spawning Behaviour of the Commercially Important Common Cuttlefish (*Sepia officinalis*) in the Inshore Waters of the English Channel

Bloor, Isobel

<http://hdl.handle.net/10026.1/1494>

---

University of Plymouth

---

*All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.*

This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognize that its copyright rests with its author and that no quotation from the thesis and no information derived from it may be published without the author's prior consent.



**The ecology, distribution and spawning behaviour of  
the commercially important common cuttlefish  
(*Sepia officinalis*) in the inshore waters of the  
English Channel**



Isobel Bloor

The Marine Institute & The Marine Biological Association of the  
United Kingdom

Plymouth University

A thesis submitted to Plymouth University in partial  
fulfillment of the requirements for the degree of:

*PhD Marine Ecology*

16th October 2012



# Abstract

## The ecology, distribution and spawning behaviour of the commercially important common cuttlefish (*Sepia officinalis*) in the inshore waters of the English Channel

Isobel Bloor

Over the last 50 years there has been a rapid increase in global landings of cephalopods (octopus, squid and cuttlefish). In European waters, cuttlefish are among the most important commercial cephalopod resources and within the North-East Atlantic, the English Channel supports the largest cuttlefish fishery, with the common cuttlefish, *Sepia officinalis* (Linnaeus, 1758), dominating landings. *S. officinalis* has a short (2 year) life cycle in the English Channel that is punctuated by seasonal migrations inshore and offshore. Using a combination of different métiers including beam trawling, otter trawling and coastal trapping, this shared fisheries resource is targeted at nearly every phase of the life cycle. Despite this continuing increase there remain only minimal management measures in place, with no quotas, no total allowable catches, no closed areas, no minimal landing size and no routine assessment of stocks. In order to provide sustainable fisheries management advice for *S. officinalis* populations it is essential that a thorough understanding of the ecology and life history of this species, in particular the factors affecting spawning and recruitment variability, is attained.

In this thesis, I examine critical gaps in our understanding of the distribution, movements, habitat use and behaviours of spawning and sub-adult *S. officinalis*. This research provides baseline data for this species within the inshore waters of the English Channel and uses a combination of novel field-based electronic tracking techniques, *in situ* subtidal observations of spawning patterns within natural environments and presence-only species distribution modelling. A maximum entropy (MaxEnt) modelling approach was used to predict the distribution of benthic egg clusters using presence-only data. The model showed very good performance in terms of predictive power and accuracy (test area under the receiver operating characteristics curve [AUC] = 0.909) and among the explanatory variables used to build the model, depth (gain = 1.17), chlorophyll-*a* concentration (used here as a proxy for turbidity; gain = 1.06) and distance from coastline (gain = 1.02) were shown to be the greatest determining factors for the distribution of *S. officinalis* spawning. As part of the model output, maps (logistic and binary) of the predicted spawning distribution of *S. officinalis* within the English Channel were produced.

Subtidal observation were undertaken at spawning grounds on both the North and South coast

of the English Channel to investigate spawning habitat and structure use. A total of 15 types of natural spawning structures were identified. The range of spawning structures used varied among sites with *Zostera marina* identified as the dominant spawning structure at two of the UK sites (Torbay and Poole Bay), potentially indicating a ‘preference’ for this structure within localities. Fractal dimension analysis of the seagrass beds at Torbay revealed that the spatial dynamics of seagrass beds within this site varied significantly between 2011 and 2012 (Mann-Whitney U:  $Z = 4.92$ ,  $P < 0.0001$ ) as a result of both anthropogenic and natural disturbance. Interannual changes in the spatial dynamics of these beds could affect the annual pattern and intensity of spawning at a site. The use of structures with small diameters was found to occur, with cuttlefish adapting the device to their requirements by utilising multiple leaves or thalli in order to achieve a suitable diameter for egg attachment, this was evident in their use of both *Chorda filum* and *Z. marina*.

This research also provided the first data on the fine-scale movements and behaviours of adult and sub-adult individuals, tracked within their natural environments, using electronic tagging methodologies. That expected patterns of short-term spawning site fidelity at a local level were observed in only two individuals, whilst larger scale movements (up to 35 km) along the coast-line were observed in three individuals, indicated that a range of behaviours and movement patterns could occur among spawning adults. Similarly varied patterns of site fidelity were also observed in tagged sub-adults, tracked over an extended period (up to 73 days), using a static acoustic array. These results highlight the complex range of patterns and plasticity in behaviour that exist within natural populations.

In summary, a series of different approaches was used within this thesis in an effort to improve our understanding of the fine-scale movement, behaviours and habitat use of *S. officinalis* (in both spawning adults and non spawning sub-adults), as well as their potential spawning distribution within the inshore waters of the English Channel. Observing the movements and behaviours of small marine animals like *S. officinalis* in their natural environments has traditionally been difficult. Recent developments in technologies and techniques however, including those used within this thesis (e.g. electronic tagging), have highlighted the potential capacity of novel tools to monitor the *in situ* movements and behaviour of cuttlefish. By providing important insights into the ecology of this species these new tools can aid conservation and management advice for this important commercial fishery species, both within the English Channel and further afield.



# Contents

<b>Abstract</b>	<b>v</b>
<b>Acknowledgements</b>	<b>xxvii</b>
<b>Author's declaration</b>	<b>xxix</b>
<b>1 General introduction and exploratory case study of the English Channel cuttlefish fishery</b>	<b>1</b>
1.1 Introduction . . . . .	1
1.1.1 English Channel cuttlefish fishery . . . . .	1
1.1.1.1 Species . . . . .	2
1.1.1.2 Fishery . . . . .	3
1.1.1.3 Landings . . . . .	4
1.1.1.4 Management measures . . . . .	5
1.2 Aims and objectives . . . . .	6
1.3 Methods . . . . .	7
1.3.1 Landings . . . . .	7
1.3.2 Market sampling . . . . .	7
1.3.3 Sampling of landings from cuttlefish traps . . . . .	7
1.3.4 Eggs laid on cuttlefish traps . . . . .	8
1.3.5 Potential mitigations for eggs laid on cuttlefish traps . . . . .	8
1.4 Results . . . . .	9
1.4.1 Landings . . . . .	9
1.4.2 Market sampling . . . . .	10
1.4.3 Inshore cuttlefish trap fishery at Torbay (Brixham) . . . . .	13
1.4.4 Sampling of landings from cuttlefish traps . . . . .	14

1.4.5	Eggs laid on cuttlefish traps . . . . .	15
1.4.6	Potential mitigations for eggs laid on cuttlefish traps . . . . .	16
1.5	Discussion . . . . .	16
1.5.1	Eggs laid on cuttlefish traps . . . . .	17
1.5.2	Potential mitigations for eggs laid on cuttlefish traps . . . . .	18
1.5.3	Conclusions. . . . .	19
1.6	Overall study aims and objectives . . . . .	19
<b>2</b>	<b>A review of the factors influencing spawning, early life stage survival and recruitment variability in the common cuttlefish (<i>Sepia officinalis</i>) within the inshore waters of the English Channel</b>	<b>23</b>
2.1	Introduction . . . . .	23
2.1.1	Life history and life cycle . . . . .	23
2.1.2	Stock definition and assessment . . . . .	24
2.1.3	Recruitment variability . . . . .	25
2.1.4	Review aims . . . . .	26
2.2	Maternal effects and their potential mechanisms . . . . .	27
2.2.1	Mate choice . . . . .	27
2.2.1.1	Precopulatory female mate choice . . . . .	28
2.2.1.2	Postcopulatory female mate choice . . . . .	29
2.2.2	Offspring release (Oviposition timing) . . . . .	29
2.2.2.1	Spawning season . . . . .	30
2.2.2.2	Reproductive strategies . . . . .	30
2.2.2.3	Migration cues . . . . .	31
2.2.2.3.1	Temperature . . . . .	32
2.2.2.3.2	Sexual Maturation . . . . .	32
2.2.2.3.2.1	<i>Photoperiod:</i> . . . . .	32
2.2.2.3.2.2	<i>Temperature:</i> . . . . .	33
2.2.3	Offspring release (Oviposition Site selection) . . . . .	34

2.2.3.1	Migration patterns . . . . .	35
2.2.3.1.1	Water circulation and prevailing current . . . . .	36
2.2.3.1.2	Temperature . . . . .	37
2.2.3.2	Navigation and orientation . . . . .	38
2.2.3.3	Site selection . . . . .	39
2.2.3.3.1	Multiple sites . . . . .	39
2.2.3.3.2	Habitat Selection . . . . .	39
2.2.4	Offspring provisioning . . . . .	41
2.3	Effect of early life stage environments on growth and survival . . . . .	42
2.3.1	Local environment pre-hatching . . . . .	43
2.3.1.1	Temperature . . . . .	43
2.3.1.2	Nutrition . . . . .	46
2.3.1.2.1	Food imprinting . . . . .	47
2.3.1.3	Salinity. . . . .	48
2.3.1.4	Oxygen Saturation. . . . .	49
2.3.1.5	Light-Dark cycles . . . . .	51
2.3.1.6	Water quality. . . . .	52
2.3.1.7	Predation . . . . .	54
2.3.1.8	Storm Events . . . . .	56
2.3.1.9	Eggs on pots . . . . .	56
2.3.2	Local environment post-hatching . . . . .	57
2.3.2.1	Temperature . . . . .	58
2.3.2.2	Food availability . . . . .	59
2.3.2.3	Size-at-hatching . . . . .	61
2.3.2.4	Survival . . . . .	63
2.4	Recruitment to the fishery . . . . .	65
2.4.1	Recruitment variability . . . . .	65
2.4.2	Fecundity: . . . . .	66

2.4.3	Persistence of maternal effects . . . . .	67
2.4.4	Age-at-recruitment. . . . .	67
2.4.5	Timing of Recruitment (onshore/offshore spawning). . . . .	69
2.4.6	Juvenile and nursery habitats. . . . .	70
2.4.7	Connectivity . . . . .	71
2.4.8	Climate change . . . . .	71
2.5	Summary . . . . .	73
<b>3</b>	<b>Species distribution modelling of potential spawning habitat</b>	<b>77</b>
3.1	Introduction . . . . .	77
3.1.1	Review of models . . . . .	80
3.1.2	Presence-only model selection . . . . .	84
3.1.3	MaxEnt . . . . .	86
3.1.4	The presence-only problem . . . . .	89
3.1.4.1	Lack of absence data . . . . .	89
3.1.4.2	Spatial sampling bias . . . . .	90
3.2	Objectives . . . . .	92
3.3	Materials and methods . . . . .	92
3.3.1	Location (Step i.) . . . . .	93
3.3.2	Species occurrence data (Step ii.) . . . . .	94
3.3.3	Environmental predictor variables (Step i. and ii.) . . . . .	95
3.3.3.1	Collinearity among predictor variables . . . . .	96
3.3.3.2	Preparation of environmental layers . . . . .	98
3.3.3.2.1	Attenuation coefficient, K490 (March - September)	100
3.3.3.2.2	Chlorophyll- <i>a</i> concentration (March - September)	100
3.3.3.2.3	Sea surface temperature, SST (March - September)	100
3.3.3.2.4	Depth . . . . .	100
3.3.3.2.5	Bed shear stress . . . . .	101
3.3.3.2.6	Distance from the nearest coastline . . . . .	101

3.3.3.2.7	Sea surface salinity . . . . .	101
3.3.3.2.8	Sediment . . . . .	101
3.3.4	Non-spatial analysis . . . . .	102
3.3.5	MaxEnt pre-run settings (Step iii.) . . . . .	102
3.3.5.1	Background points . . . . .	102
3.3.5.2	Random test percentage . . . . .	103
3.3.5.3	Other settings . . . . .	104
3.3.6	MaxEnt model output and evaluation (Steps iv. and v.) . . . . .	104
3.3.6.1	Predictive habitat suitability maps . . . . .	104
3.3.6.2	Analysis of Variable contributions . . . . .	105
3.3.6.3	Jackknife . . . . .	106
3.3.6.4	Marginal response curves . . . . .	107
3.4	Results . . . . .	107
3.4.1	Non-spatial analysis . . . . .	107
3.4.2	MaxEnt model . . . . .	108
3.4.2.1	Collinearity among predictor variables . . . . .	108
3.4.2.2	Presence maps . . . . .	111
3.4.2.3	Model evaluation . . . . .	113
3.4.2.3.1	Area under the receiver operating characteristics curve (AUC) . . . . .	113
3.4.2.3.2	Predictor variable importance . . . . .	114
3.4.2.3.3	Jackknife . . . . .	115
3.4.2.3.4	Marginal response curves . . . . .	118
3.5	Discussion . . . . .	120
3.5.1	Spawning distribution of <i>S. officinalis</i> within the English Channel . . . . .	120
3.5.1.1	Western and Eastern English Channel . . . . .	120
3.5.2	Species-habitat relationship . . . . .	122
3.5.2.1	Depth . . . . .	123
3.5.2.2	Chlorophyll- <i>a</i> . . . . .	124

3.5.2.3	Distance from the coastline . . . . .	125
3.5.2.4	Bed shear stress . . . . .	126
3.5.2.5	Salinity . . . . .	127
3.5.2.6	SST . . . . .	128
3.5.3	Data limitations . . . . .	131
3.5.3.1	Long-term averages . . . . .	131
3.5.3.2	Spatial resolution and data availability . . . . .	131
3.5.3.3	Sample selection bias . . . . .	132
3.5.3.4	Data errors: . . . . .	133
3.5.4	Conclusions . . . . .	134
<b>4</b>	<b>Observations of natural spawning substrates on the UK and French coastlines of the English Channel</b>	<b>135</b>
4.1	Introduction . . . . .	135
4.1.1	Aims and objectives . . . . .	138
4.2	Methods and Materials . . . . .	139
4.2.1	Study sites . . . . .	139
4.2.1.1	Torbay . . . . .	139
4.2.1.2	Selsey . . . . .	139
4.2.1.3	Poole Bay . . . . .	140
4.2.1.4	Agon-Coutainville . . . . .	140
4.2.1.5	Langrune-sur-Mer . . . . .	141
4.2.2	Survey methods . . . . .	141
4.2.2.1	Circular transect (radius of 4 m, area of 50.3 m <sup>2</sup> ) . . . . .	142
4.2.2.2	Line belt transect (area of 100 m <sup>2</sup> ) . . . . .	142
4.2.2.3	GPS-tracked drift transect (15 minutes; variable length) . . . . .	144
4.2.3	Survey design . . . . .	144
4.2.3.1	Qualitative assessment of natural spawning structures . . . . .	144
4.2.3.2	Comparison of spawning strata (depth) . . . . .	144

4.2.3.3	Comparison of spawning strata (structures) . . . . .	145
4.2.3.4	Temporal analysis of spawning in seagrass beds . . . . .	147
4.2.4	Data collection . . . . .	147
4.2.5	Data analysis . . . . .	147
4.2.5.1	Kruskal-Wallis test . . . . .	148
4.2.5.2	Mann-Whitney U test . . . . .	148
4.2.5.3	Qualitative assessment of natural spawning structures . . . .	148
4.2.5.3.1	Characteristics of natural spawning structures . . . .	148
4.2.5.3.2	Variability in egg cluster size . . . . .	148
4.2.5.4	Comparison of spawning strata (Depth) . . . . .	149
4.2.5.5	Comparison of spawning strata (Structures) . . . . .	149
4.2.5.6	Temporal analysis of spawning within seagrass beds (Torbay 2010-2012) . . . . .	149
4.2.5.6.1	Egg density . . . . .	149
4.2.5.6.2	Variability in the number and size of egg clusters .	150
4.2.5.6.3	Fractal dimension . . . . .	150
4.2.5.6.4	Seagrass analysis . . . . .	150
4.3	Results . . . . .	151
4.3.1	Qualitative assessment of natural spawning structures . . . . .	151
4.3.1.1	Characteristics of natural egg laying structures . . . . .	151
4.3.1.2	Structure use among sites . . . . .	155
4.3.1.3	Variation in cluster size among natural structures . . . . .	156
4.3.2	Comparison of spawning strata (depth) . . . . .	159
4.3.3	Comparison of spawning strata (structures) . . . . .	159
4.3.4	Temporal analysis of spawning within seagrass beds (Torbay 2010-2012)	161
4.3.4.1	Egg density . . . . .	161
4.3.4.2	Variability in the number and size of egg clusters . . . . .	163
4.3.4.3	Fractal dimension . . . . .	165
4.3.4.4	Seagrass analysis . . . . .	165

4.4	Discussion . . . . .	167
4.4.1	Qualitative assessment of natural spawning structures . . . . .	167
4.4.1.1	Spawning structures among sites . . . . .	168
4.4.2	Comparison of spawning strata (depth) . . . . .	168
4.4.3	Comparison of spawning strata (structures) . . . . .	169
4.4.4	Temporal analysis of spawning within seagrass beds (Torbay 2010-2012) . . . . .	170
4.4.5	Selection Strategies . . . . .	172
4.4.6	Data limitations . . . . .	174
4.4.6.1	Methodological variation . . . . .	174
4.4.7	Conclusions . . . . .	176
<b>5</b>	<b>Acoustic tagging for the study of adult and sub-adult <i>S. officinalis</i> in inshore waters</b>	<b>177</b>
5.1	Introduction . . . . .	177
5.2	Objectives . . . . .	180
5.3	Methods . . . . .	181
5.3.1	Study location . . . . .	181
5.3.1.1	Torbay, Devon, U.K. . . . .	181
5.3.1.2	Whitsand Bay, Cornwall, U.K. . . . .	181
5.3.2	Study animals . . . . .	184
5.3.3	Tagging methodology . . . . .	184
5.3.4	Sedation procedures . . . . .	185
5.3.5	Tagging procedure . . . . .	187
5.3.6	Vemco Radio Acoustic Positioning Array . . . . .	188
5.3.6.1	Moorings . . . . .	189
5.3.6.2	Base station . . . . .	192
5.3.6.3	Passive monitoring . . . . .	192
5.3.6.3.1	Positional accuracy . . . . .	193
5.3.6.4	Active monitoring . . . . .	194
5.3.7	Static acoustic array . . . . .	195



5.3.8	Tag recovery . . . . .	196
5.3.9	Data processing . . . . .	196
5.3.9.1	VRAP array . . . . .	196
5.3.9.1.1	Track cleaning . . . . .	196
5.3.9.2	Static acoustic array . . . . .	198
5.3.10	Data analysis . . . . .	198
5.3.10.1	Presence/absence . . . . .	198
5.3.10.2	Maximum net displacement . . . . .	198
5.3.10.3	Habitat and area use . . . . .	199
5.3.10.3.1	Diel activity patterns . . . . .	200
5.3.10.3.2	Swimming speeds . . . . .	201
5.4	Results . . . . .	201
5.4.1	Sedation . . . . .	201
5.4.2	Tag retention and recovery . . . . .	202
5.4.3	Presence/absence . . . . .	204
5.4.3.1	Active monitoring . . . . .	209
5.4.4	Maximum net displacement . . . . .	209
5.4.5	Habitat and area use . . . . .	211
5.4.6	Diel activity patterns . . . . .	216
5.4.7	Speed and distance analysis . . . . .	220
5.5	Discussion . . . . .	220
5.5.1	Evaluation of tagging methodology for field use . . . . .	221
5.5.1.1	Tag retention . . . . .	221
5.5.1.2	Tag recovery . . . . .	222
5.5.1.3	Tag presence . . . . .	222
5.5.2	Presence/absence . . . . .	223
5.5.3	Maximum net displacement . . . . .	224
5.5.4	Habitat and area use . . . . .	225

5.5.4.1	Site fidelity . . . . .	226
5.5.4.2	Reproductive strategies . . . . .	228
5.5.5	Diel activity patterns . . . . .	229
5.5.6	Swimming speeds . . . . .	230
5.5.7	Conclusions . . . . .	230
<b>6</b>	<b>Long-term tagging of <i>S. officinalis</i> using data storage tags</b>	<b>233</b>
6.1	Introduction . . . . .	233
6.2	Objectives . . . . .	235
6.3	Methods . . . . .	236
6.3.1	Study animals . . . . .	236
6.3.2	Tagging methodology . . . . .	237
6.3.3	Sedation procedures . . . . .	239
6.3.4	Tagging procedure . . . . .	242
6.3.5	Tag recovery . . . . .	242
6.3.6	Data analysis . . . . .	243
6.4	Results . . . . .	244
6.4.1	Sedation . . . . .	244
6.4.2	Tag retention and recovery . . . . .	244
6.4.3	Data analysis . . . . .	246
6.5	Discussion . . . . .	248
6.5.1	Sedative procedures . . . . .	248
6.5.2	Tag recovery . . . . .	251
6.5.3	Data analysis . . . . .	253
6.5.4	Predation rates of juvenile cuttlefish . . . . .	254
6.5.5	Conclusions . . . . .	257
<b>7</b>	<b>General discussion</b>	<b>259</b>
7.1	Summary of new contributions of this thesis . . . . .	259

7.2	Discussion . . . . .	261
7.2.1	Spawning location and distribution . . . . .	261
7.2.2	Characteristics of spawning habitat . . . . .	262
7.2.3	Spawning behaviour . . . . .	264
7.3	Conclusions . . . . .	266
7.4	Future work . . . . .	266
<b>A</b>	<b>Chapter 4: Raw data tables</b>	<b>269</b>
A.1	Results . . . . .	269
A.1.1	Comparison of spawning strata (structures) . . . . .	269
A.1.2	Comparison of spawning strata (depth) . . . . .	273
A.1.3	Fractal dimension . . . . .	275
<b>B</b>	<b>Laboratory Observations 2011.</b>	<b>277</b>
B.1	Introduction. . . . .	277
B.1.1	Aims and objectives . . . . .	278
B.2	Methodology . . . . .	279
B.2.1	Study animals . . . . .	279
B.2.2	Prey . . . . .	280
B.2.3	Predators . . . . .	280
B.2.4	Water quality . . . . .	280
B.2.5	Experimental design . . . . .	280
B.2.6	Data collection . . . . .	282
	B.2.6.0.1 Computer-aided video monitoring . . . . .	282
B.2.7	Data analysis . . . . .	282
	B.2.7.1 Predation . . . . .	282
	B.2.7.2 Defence . . . . .	284
	B.2.7.3 Movement . . . . .	284
B.3	Results . . . . .	284

B.3.1	Study animals . . . . .	284
B.4	Discussion. . . . .	285
<b>C</b>	<b>Supplementary information</b>	<b>287</b>
C.1	Species occurrence data [DAASH] . . . . .	287
C.2	‘Cuttlewatch’ sightings scheme . . . . .	287
C.3	Poster display on cuttlefish acoustic tagging at Living Coasts . . . . .	289
C.4	DST recovery . . . . .	290
	<b>List of references.</b>	<b>292</b>

# List of Figures

1.1	Global production landings (t) of cuttlefish, squid and octopus from 1950-2010 (FAO 2012) . . . . .	2
1.2	Cuttlefish species known to occur within the English Channel (illustrations from Reid and Jereb (2005); Reid et al. (2005)) . . . . .	3
1.3	Eggs laid on cuttlefish traps (Eastbourne 2011) . . . . .	6
1.4	Egg removal and redeployment trial (Torbay 2011) . . . . .	9
1.5	Cuttlefish annual landings (solid black line) and price per kg (dotted red line) at Brixham fish market (1988 to 2011) . . . . .	10
1.6	Brixham market sampling combined overall results presented for the years 2011 and 2012 indicating length (DML) frequency . . . . .	11
1.7	Brixham market sampling results presented by month for 2011 . . . . .	12
1.8	Relationship between dorsal mantle length and body weight calculated using market sampling data from Brixham fish market (2011 and 2012) . . . . .	13
1.9	Sampling of landings from commercial cuttlefish trap fisheries in May 2010 (Torbay, Selsey, Eastbourne) and in June 2011 (Eastbourne) indicating length (DML) frequency . . . . .	14
1.10	Visual comparison of length (DML) frequency between landing samples from Brixham fish market (all métiers, 2011 and 2012) and from cuttlefish trap landings (2010 and 2011). . . . .	15
2.1	A diagram showing the biannual life cycle of <i>S. officinalis</i> within the English Channel. . . . .	24
2.2	A diagram illustrating the main migrational patterns and movements for <i>S. officinalis</i> within the English Channel. . . . .	36
2.3	A diagram illustrating the major water currents within the English Channel. . . . .	37
2.4	A graph illustrating temperature dependent embryogenesis. . . . .	44
3.1	Trends in the number of marine applications of SDMs . . . . .	81
3.2	Steps in making a SDM in MaxEnt . . . . .	93

3.3	A map showing the locations of the 217 sample points for <i>S. officinalis</i> egg occurrence within the English Channel . . . . .	95
3.4	Maps showing the data for each predictor variable . . . . .	97
3.5	Correlation between Chlorophyll- <i>a</i> concentration and the attenuation coefficient (K490) within the English Channel . . . . .	109
3.6	Predicted Habitat Suitability Map for <i>S. officinalis</i> spawning distribution within the English Channel (Logistic and Binary) . . . . .	112
3.7	Receiver operating characteristic (ROC) curve averaged over 15 replicate runs for both training and test data . . . . .	114
3.8	Jackknife of training gain, test gain and AUC . . . . .	117
3.9	The marginal response curves for each predictor variable . . . . .	119
3.10	Maps showing the data for SST as a long-term median over seven months and long-term medians for each month . . . . .	130
4.1	English Channel Study Sites (Torbay, Selsey, Poole Bay, Agon-Coutainville and Langrune-sur-Mer) . . . . .	141
4.2	Survey methods (circular belt transect, line belt transect and GPS-tracked drift transect) . . . . .	143
4.3	Study sites for comparison of natural spawning structures with subtidal survey sites marked. . . . .	146
4.4	Examples of egg clusters laid on spawning structures intertidally . . . . .	154
4.5	Examples of egg clusters laid on subtidal spawning structures . . . . .	154
4.6	Examples of egg clusters laid on spawning structures both subtidally and intertidally . . . . .	155
4.7	<i>C. filum</i> with cuttlefish eggs attached . . . . .	155
4.8	A box plot showing the variation in egg cluster size (numbers of eggs per cluster) among natural spawning structures . . . . .	157
4.9	Diagrams illustrating the natural structures to which egg clusters have been found attached . . . . .	158
4.10	A graph showing the proportion of egg clusters recorded within each depth stratum by spawning structure type and is independent of the area surveyed (Agon 2011) . . . . .	159

4.11	Variability in egg clusters size for 2011 and 2012 at Millstones Bay and Torre Abbey Sands . . . . .	164
4.12	A box plot showing the variation in seagrass length (cm) in May, June and July in Torbay (2011) . . . . .	166
4.13	Photographs showing eggs attached at the base of a <i>Z. marina</i> plant and eggs attached higher up <i>Z. marina</i> plants plant . . . . .	167
4.14	A wind rose showing the mean wind speed and direction for April 2011 and 2012	172
5.1	Map of south-west England showing the location of the study site at Millstones Bay . . . . .	182
5.2	A photograph depicting the layout of the study site at Millstone bay . . . . .	182
5.3	The location and spatial arrangement of the static acoustic array (6 VR3-UWM receivers) at Whitsand Bay, Cornwall, U.K. . . . .	183
5.4	Attachment of acoustic transmitters (V9, VEMCO) to adult cuttlefish in Torbay	188
5.5	Schematic diagram illustrating the set up of the radio acoustic positioning system (VRAP) . . . . .	189
5.6	A diagram of the moorings used for the VRAP array . . . . .	190
5.7	Simulation plot of the accuracy of positions determined within the detection range of the VRAP array with a receiver timing error of 0.5 ms and a transmitter depth of 10 m. The default speed of sound (1,500 ms) was also used . . . . .	194
5.8	A diagram illustrating the seabed landers at Whitsand Bay . . . . .	196
5.9	A habitat map for the Millstones Bay study area, produced using drop down video surveys and interpolated from point data to a raster layer using ArcGIS. .	199
5.10	Types of movement pattern . . . . .	201
5.11	Somatic growth at surgical tag attachment site for recovered Cuttlefish 5 . . . .	203
5.12	Telemetry data points and tracks (Cuttlefish 3, 5, 6 and 8) . . . . .	207
5.13	Telemetry data points and tracks (Cuttlefish 1, 2, 4 and 7) . . . . .	208
5.14	Data from tagged sub-adults within the Whitsand Bay static acoustic array . . .	209
5.15	Maximum displacement for three tagged cuttlefish . . . . .	210
5.16	Dissection and macroscopic maturation analysis of Cuttlefish 5 . . . . .	211
5.17	Habitat use patterns for Cuttlefish 7 . . . . .	212

5.18	Example movement patterns exhibited by Cuttlefish 7 . . . . .	213
5.19	A minimum convex polygon to estimate area use (or home range) for Cuttlefish 7 over 9 days of tracking . . . . .	215
5.20	Daily minimum convex polygons (day and night) for Cuttlefish 7 . . . . .	217
5.21	Directed movement patterns for Cuttlefish 7 indicating the percentage of hours for directed movement during each phase of the day . . . . .	218
5.22	Graph of net distance moved by hour of the day for Cuttlefish 7 indicating dominant habitat type . . . . .	219
6.1	Map of south-west England showing the location of the Whitsand Bay study site	237
6.2	DST wired into harness prior to attachment . . . . .	242
6.3	Photographs showing crabs eating the flesh off the dead cuttlefish . . . . .	245
6.4	Cuttlebone with DST attached from recovered Cuttlefish D10 . . . . .	246
6.5	The retrieved data storage tag track from Cuttlefish D10. . . . .	247
6.6	The average depth over time calculated for the retrieved data storage tag track from Cuttlefish D10. . . . .	248
B.1	Tank setup showing experimental arena and video monitoring system . . . . .	283
C.1	Poster for the cuttlefish sightings scheme ‘Cuttlewatch’ to enable members of the general public to report cuttlefish sightings . . . . .	288
C.2	Poster display (1 of 2) for Living Coats on the general ecology of the common cuttlefish . . . . .	289
C.3	Poster display (2 of 2) for Living Coats on the VRAP acoustic tagging study conducted in Torbay . . . . .	290
C.4	Reward poster for DSTs aimed at Fishermen . . . . .	290
C.5	Reward poster for DSTs aimed at recreational beach goers . . . . .	291



# List of Tables

1.1	General characteristics of three cuttlefish species known to occur within the English Channel . . . . .	3
1.2	Numbers of eggs recorded laid on traps in July 2010 (Babbacombe Bay), July 2011 (Torbay) and June 2011 (Eastbourne) . . . . .	16
2.1	A table describing the known structures used by <i>S. officinalis</i> for egg laying . .	40
3.1	Main species distribution modelling methods available . . . . .	83
3.2	Area under the curve values for use in assessment of MaxEnt model . . . . .	88
3.3	A list of predictor variable datasets, their description and source . . . . .	99
3.4	Environmental limits for spawning in the common cuttlefish <i>S. officinalis</i> in the English Channel based on the outputs of the non-spatial model (NSM) and spatial model (SM) . . . . .	108
3.5	Pearson correlation coefficient analysis among pairs of predictor variables . . .	110
3.6	Relative contributions of the predictor variables to the MaxEnt model . . . . .	115
3.7	General characteristics of the Western and Eastern basins of the English Channel	122
4.1	A list of natural spawning supports for <i>Sepia officinalis</i> found by Blanc (1998) in a survey of sites in the west and east of Morbihan Bay situated in the northern part of the Bay of Biscay. . . . .	136
4.2	A table indicating the characteristics of natural spawning structures recorded during qualitative subtidal and intertidal surveys (2010-2012) . . . . .	153
4.3	A table indicating the natural structures, with <i>S. officinalis</i> egg clusters attached, recorded (by study site) during qualitative subtidal and intertidal surveys . . . .	156
4.4	Results of the Mann-Whitney U test for differences between seagrass and mixed strata (Poole and Torbay , June 2011). . . . .	161
4.5	Temporal variation in mean egg density at Millstones Bay seagrass site (2010-2012) . . . . .	162

4.6	Temporal variation in mean egg density at Torre Abbey Sands seagrass site (2010-2012) . . . . .	162
4.7	Results of Mann-Whitney U tests for differences between egg cluster size at Millstones Bay and Torre Abbey Sands between 2011 and 2012. . . . .	165
4.8	Results of Kruskal-Wallis and Mann-Whitney U tests for differences between seagrass height among the months of May, June and July at Torbay (2011). . .	166
5.1	Steps of visible sedation in cuttlefish using $MgCl_2$ . . . . .	186
5.2	Locations and details for the moorings for the VRAP array . . . . .	191
5.3	Summary details of sub-adult cuttlefish (CF-SA) receiving V9-2L coded tags .	204
5.4	Summary details of adult cuttlefish receiving Vemco V9 continuous tags . . . .	205
5.5	The percentage (%) of time each of the nine sub-adult cuttlefish (CF-SA) were tracked by each Receiver . . . . .	216
5.6	Maximum swimming speeds of tagged cuttlefish within Millstones Bay . . . .	220
6.1	Summary details of cuttlefish receiving Cefas G5 (20bar 2MB) DST tags . . . .	238
6.2	Summary details of cuttlefish receiving anaesthetic ( $MgCl_2 \cdot 6H_2O$ ) . . . . .	241
6.3	Review of anaesthesia and analgesia in <i>S. officinalis</i> . . . . .	250
6.4	Review of predators of <i>S. officinalis</i> . . . . .	256
A.1	Torbay 2010 subtidal survey results for May (seagrass and mixed strata) . . . .	269
A.2	Torbay 2010 subtidal survey results for July (seagrass and mixed strata) . . . .	270
A.3	Torbay 2011 subtidal survey results for June (seagrass and mixed strata) . . . .	271
A.4	Poole Bay 2011 subtidal survey results for June (seagrass and mixed strata) . .	272
A.5	Summary details of subtidal survey transect data for Agon-Coutainville in June 2011 (by depth strata) . . . . .	273
A.6	Summary details of subtidal survey transect data for Langrune in June 2011 (by depth strata) . . . . .	274
A.8	Fractal dimension slopes for seagrass transects at Torre Abbey Sands 2011 and 2012 . . . . .	275
A.7	Fractal dimension slopes for seagrass transects at Millstones Bay 2011 and 2012	275

B.1	A description of the eight different laboratory trials . . . . .	282
-----	--	-----



# Acknowledgements

It is a pleasure to thank the many wonderful and varied people who made this research possible:

I would like to acknowledge my PhD supervisors Emma Jackson, Martin Attrill and David Sims, for their guidance, advice and assistance during this research. I would particularly like to thank Emma, for giving me the opportunity to undertake this research and for providing great support and supervision since the beginning of my PhD and particularly during my thesis-writing period, providing timely feedback and sound advice.

I would like to thank the EU Interreg IV for funding this PhD studentship as part of the Cephalopod Recruitment from English Channel Spawning Habitats (CRESH) project. I am grateful to all the partners and participants of the CRESH project for their support and advice. In particular, Sarah Clarke from Devon and Severn IFCA who got up very early once a month to accompany me to the fish market, even during the cold and wet winter months. To Michael and Georges, PhD students on the CRESH project, who have been great fun and provided support and entertainment at the many CRESH meetings and conferences we have attended over the three years. An extra special mention also goes to Jean-Paul Robin who has led the CRESH project with such enthusiasm and dedication.

The fieldwork that was undertaken as part of this research involved the assistance of a number of people including Viki Wearmouth and Matthe McHugh. I would also like thank Viki for her expertise and advice with experimental design for acoustic tagging experiments. I am also grateful to all the undergraduate diving volunteers from Plymouth University and the dive team in France who contributed their time to the fieldwork involved in this research. The cuttle fishers who provided their time, boats, knowledge and help and to Rick Parker, whose help, local knowledge and expert dive boat skippering made our diving research in Torbay such a smooth operation.

At the MBA I would like to thank Charli Marshall for guiding me in my first foray into the world of modelling and for all her subsequent advice and Charly Griffiths for GIS advice. John Rundle for his expertise, help and advice in the maintenance and rearing of cuttlefish. Peter Rendle and Kevin Atwood for all their technical expertise and help in building and constructing laboratory set ups and equipment, their ability to find and make anything and everything even at a moments notice is amazing and they never faltered to deliver no matter how strange and bizzare my request and a thank you to the MBA boat crew who provided cuttlefish for us to tag. From the MarLIN office I would like to everyone for making me so welcome, especially Fiona for her role as recreational dive buddy and for accompanying me to the yard arm on occasion for 'just a quick pint' and Jack who answered my many questions about photoshop and who

constantly kept me on my toes with his many varied plastic and home made spider creations!. In addition I would like to thank Martin Coath for introducing me to the ways of  $\text{\LaTeX}$  and providing a wonderful and simple to use template for this thesis.

I would also like to thank my friends, particularly Jessicar, Vickytoria and Jay whose friendship, support and trips to the pub (...and of course further afield- to pubs in Panama) have helped keep me sane. In addition they have provided me with beds, cups of tea and lovely meals on my many visits to London, whether for pleasure or ahead of early morning flights to conferences.

Finally I would like to thank my family (you are all amazing), but in particular my wonderful and loving parents, who are an inspiration to me, both in my work and in my daily life. Who have shown me their support in all ways and who have demonstrated to me the true meaning of strength and determination, proving that it is possible to overcome any challenge, and teaching me more than they will ever know. I am so glad that you are both here to have shared this experience with me.

## Author's declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

Relevant scientific seminars and conferences were regularly attended at which work was often presented.

**Signed:** \_\_\_\_\_

**Date:** \_\_\_\_\_

### Chapter acknowledgements :

Chapter 3: Thanks to all the volunteer divers that assisted with diving surveys in the UK and to Jean-Paul Robin for his assistance in organising the French diving surveys and for providing the data from these.

Chapter 4: Thanks to the Behavioural Ecology group at the MBA for their expert advice in planning the tagging field studies and in deployment of the acoustic tagging array. All surgical tagging procedures were undertaken by a team of scientists which included Prof. D.W. Sims (MBA) who provided surgical techniques, Isobel Bloor, Dr. V.J. Wearmouth and M. McHugh.

Chapter 5: Thanks to the Behavioural Ecology group at the MBA for their expert advice in tagging field studies. All surgical tagging procedures were undertaken by a team of scientists which included Prof. D.W. Sims (MBA) who provided surgical techniques, Isobel Bloor, Dr. V.J. Wearmouth and M. McHugh.

### Publications :

Wearmouth, V.J., Durkin, O.C., **Bloor, I.**, McHugh, M., Rundle, J. & Sims, D.W. *A method for long-term electronic tagging and tracking of juvenile and adult common cuttlefish Sepia officinalis*. Journal of Experimental Marine Biology and Ecology **In press**.

**Bloor, I.**, Robin, J.P., Attrill, M. & Jackson, E. *A review of the factors influencing spawning, early life stage survival and recruitment variability in the common cuttlefish Sepia*

*officinalis*. Advances in Marine Biology, Vol. 65 **Submitted**.

**Bloor, I.**, Wearmouth, V.J., McHugh, M., Humpries, N., Cotterell, S., Jackson, E., Attrill, M. & Sims, D.W. *Investigating the movement and behaviour of the common cuttlefish Sepia officinalis in the inshore waters of the English Channel using acoustic telemetry*. Journal of Experimental Marine Biology and Ecology **Submitted**.

Revill, A., **Bloor, I.**, & Jackson, E. *Survival of discarded juvenile Sepia officinalis in the English Channel* Fisheries Research **Submitted**.

#### **Posters and conference presentations :**

##### **2010:**

International Council for Exploration of the Seas (ICES) Working Group on Cephalopod Fisheries and life history (WGCEPH), 9<sup>th</sup> to 11<sup>th</sup> March 2010 *Bilbao, Spain* Oral presentation: **CRESH: Cephalopod Recruitment from English Channel Spawning Habitats**.

Marine Biological Association of the United Kingdom (MBA), 22<sup>nd</sup> April 2010 *Plymouth, UK* Poster presentation: **An evaluation of the relative importance of benthic habitats in the recruitment of the common cuttlefish Sepia officinalis within the English Channel**.

GCOE INET, International Summer School "Cascading interactions among ecosystems", 19<sup>th</sup> June - 1<sup>th</sup> July 2010 *Hokkaido, Japan* Poster presentation: **An ecosystem based approach to determining the real CRESH nurseries for the common cuttlefish (Sepia officinalis) within the English Channel**.

ICES, Annual Science Conference (ASC), 20<sup>th</sup> to 24<sup>th</sup> September 2010 *Nantes, France* Poster presentation: **Inshore spawning habitats for English Channel cuttlefish (Sepia officinalis): preliminary observations of substratum choice for egg attachment**.

Cephalopod Recruitment from English Channel Spawning Habitats (CRESH) Annual Meeting, 24<sup>th</sup> September 2010 *Nantes, France* Oral presentation: **Compilation of pre-existing knowledge about egg and juvenile observations**.

CRESH Annual Meeting, 24<sup>th</sup> September 2010 *Nantes, France* Oral presentation: **Observations of natural spawning substrates**.

Marine Biology and Ecology Research Centre, Seminar Series, 1<sup>st</sup> December 2010 *Plymouth, UK* Oral presentation: **(What's the story) Spawning Glory? Habitat preferences in spawning and juvenile cuttlefish**.

##### **2011:**



ICES WGCEPH, 28<sup>th</sup> February to 3<sup>rd</sup> March 2011 *Lisbon, Portugal* Oral presentation: **Habitat utilisation and preferences in spawning cuttlefish (*Sepia officinalis*) in the English Channel.**

University of Caen, 16<sup>th</sup> March 2011 *Caen, France* Oral presentation: **Habitat utilisation and preferences in spawning and juvenile cuttlefish in the English Channel**

Marine Conservation Society, 24<sup>th</sup> March 2011 *Plymouth, UK* Oral presentation: **Habitat preferences in spawning and juvenile cuttlefish in the English Channel.**

EUROCEPH, 7<sup>th</sup> to 10<sup>th</sup> April 2011 *Vico Equense, Naples, Italy* Poster presentation: **A preliminary investigation into habitat modelling of the common cuttlefish (*Sepia officinalis*) spawning grounds in the English Channel, using presence only data.**

Marine Biological Association, 19<sup>th</sup> April 2011 *Plymouth, UK* Poster presentation: **An evaluation of the contribution of benthic habitats to spawning in the common cuttlefish (*Sepia officinalis*) within the English Channel.**

ICES ASC, 19<sup>th</sup> to 24<sup>th</sup> September 2011 *Gdansk, Poland* Oral presentation: **Environmental and habitat effects on early life stages and subsequent recruitment variability of the common cuttlefish (*Sepia officinalis*) within the English Channel.**

ICES ASC, 19<sup>th</sup> to 24<sup>th</sup> September 2011 *Gdansk, Poland* Oral presentation: ***Sepia officinalis*: Spawning habitat modelling in the English Channel and its potential for fisheries management.**

Blue Horizon, 14<sup>th</sup> December 2011 *Plymouth, UK* Oral presentation: **Environmental and habitat effects on early life stages and recruitment variability of the common cuttlefish (*Sepia officinalis*) within the English Channel.**

## **2012:**

CRESH Stakeholders dissemination meeting, 12<sup>th</sup> March 2012 *Brixham, Devon* Oral presentation: **Adaptation of cuttlefish traps to minimise wastage of eggs: A study to ascertain the best working practices for cuttlefish trap fisheries.**

Marine Biological Association, 19<sup>th</sup> April 2011 *Plymouth, UK* Poster presentation: **An acoustic tracking study to investigate the spawning behaviour of the common cuttlefish (*Sepia officinalis*) within the English Channel.**

Malacological Society of London, 20<sup>th</sup> to 21<sup>st</sup> April 2012 *Portsmouth, UK* Oral presentation: **(What's the story) Spawning Glory? The life history and spawning behaviour of the common cuttlefish (*Sepia officinalis*) within the English Channel.**

Blue Sound diving group, 15<sup>th</sup> May 2012 *Plymouth, UK* Oral presentation: **The common cuttlefish (*Sepia officinalis*).**

The English Channel and its marine resources -dynamics of research for the benefits of society, 14<sup>th</sup> June 2012 *Boulogne-sur-mer, France* Oral presentation: **Presence-only modelling to investigate large-scale distribution of *Sepia officinalis* habitat within the English Channel.**

The English Channel and its marine resources -dynamics of research for the benefits of society, 14<sup>th</sup> June 2012 *Boulogne-sur-mer, France* Oral presentation: **Subtidal surveys to investigate fine-scale distribution of *Sepia officinalis* spawning habitat within the English Channel.**

The English Channel and its marine resources -dynamics of research for the benefits of society, 14<sup>th</sup> June 2012 *Boulogne-sur-mer, France* Oral presentation: **Electronic tagging: New tools for examining *Sepia officinalis* movement and behaviour.**

CRESH Stakeholders dissemination meeting, 17<sup>th</sup> to 18<sup>th</sup> July 2012 *Hastings and Selsey, UK* Oral presentation: **Adaptation of cuttlefish traps to minimise wastage of eggs: A study to ascertain the best working practices for cuttlefish trap fisheries.**

Marine Biological Association of the United Kingdom, internal seminar series, 14<sup>th</sup> August 2012 *Plymouth, UK* Oral presentation: **Spawning in the commercially important common cuttlefish (*Sepia officinalis*), within the English Channel.**

Oceans of Potential Conference, 11<sup>th</sup> and 12<sup>th</sup> September 2012 *Plymouth, UK* Poster presentation: **Subtidal surveys to investigate fine-scale distribution of *Sepia officinalis* spawning habitat within the English Channel.**

Oceans of Potential Conference, 11<sup>th</sup> to 12<sup>th</sup> September 2012 *Plymouth, UK* Poster presentation: **Presence-only modelling to investigate large-scale distribution of *Sepia officinalis* spawning grounds within the English Channel.**

Cephalopod International Advisory Council Symposium, 27<sup>th</sup> October to 2<sup>nd</sup> November 2012 *Florianopolis, Brazil* Oral presentation: **Investigating the movement, behaviour and habitat use of *Sepia officinalis* on inshore juvenile and spawning grounds in the English Channel.**

Cephalopod International Advisory Council Symposium, 27<sup>th</sup> October to 2<sup>nd</sup> November 2012 *Florianopolis, Brazil* Poster presentation: **Subtidal surveys to investigate fine-scale distribution of *Sepia officinalis* spawning habitat within the English Channel.**

Cephalopod International Advisory Council Symposium, 27<sup>th</sup> October to 2<sup>nd</sup> November 2012 *Florianopolis, Brazil* Poster presentation: **Presence-only modelling to investigate large-scale distribution of *Sepia officinalis* spawning grounds within the English Channel.**

**Word count for the main body of this thesis: 79,982**



# **Chapter 1**

## **General introduction and exploratory case study of the English Channel cuttlefish fishery**

### **1.1 Introduction**

As a general introduction to this thesis a short description is provided on the commercial cuttlefish fishery within the English Channel. An exploratory case study of this fishery is also presented using results collected during three years of research (2009-2012) highlighting data from Brixham (Devon) which is one of the most important ports for cuttlefish landings in the UK. This Chapter finishes with a general overview of the aims and objectives of this thesis.

#### **1.1.1 English Channel cuttlefish fishery**

Global landings of cephalopods (cuttlefish, squid and octopus) have increased dramatically over the last 50 years. Rising from approximately 0.5 million tonnes (t) in 1958 (FAO 1964) to over 4 million t in 2008 (FAO 2010), cephalopod landings now constitute almost 5% of the total world's fisheries production (FAO 2010). At a time when landings of many traditional fin-fish stocks are continuing to experience a global decline as a result of over-exploitation; it is expected that fishing pressure on cephalopod stocks will continue rising as the fishing industry switch their focus onto these non-quota species. However, long term trends may indicate that global cephalopod landings have now begun to plateau or even show a slight decline and a better understanding of these commercial cephalopod species is required in order to sustainably manage these stocks (Figure 1.1).

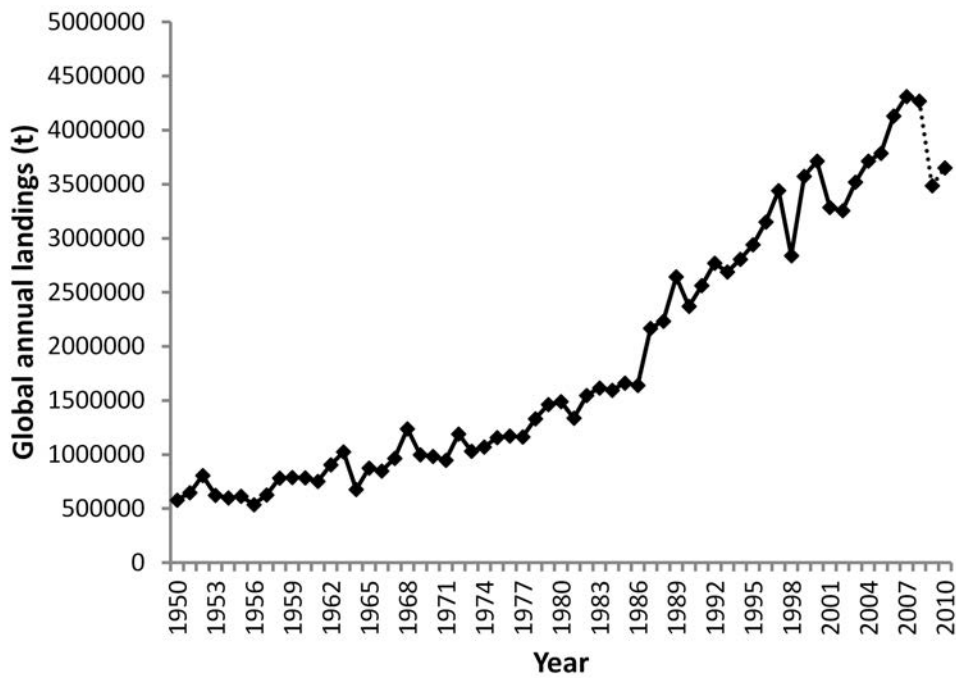


Figure 1.1: Global production landings (t) of cuttlefish, squid and octopus from 1950-2010 (FAO 2012)

In European waters, cuttlefish are among the most important commercial cephalopod resource (Perez-Losada et al. 1999; Denis and Robin 2001; Pierce et al. 2010) and the highest yielding cephalopod group harvested in the north-east Atlantic (Royer et al. 2006).

#### 1.1.1.1 Species

Three species of cuttlefish occur in the English Channel viz., *Sepia officinalis* (Linnaeus, 1758), *Sepia elegans* (Blainville, 1827) and *Sepia orbignyana* (Férussac, 1826) (Figure 1.2) (Reid and Jereb 2005; Reid et al. 2005). Of these, *S. officinalis* is considered the only species to be abundant (Dunn 1999). These species can be distinguished by differences in external colouration, morphology, size or the presence and shape of the cuttlebone (Table 1.1).

---

**Figure 1.2 has been removed from this version of the thesis due to Copyright restrictions.**

---

*Table 1.1:* General characteristics of three cuttlefish species known to occur within the English Channel (information from Reid and Jereb (2005); Reid et al. (2005)). DML = Dorsal mantle length.

Species	Max DML	Max depth	Cuttlebone	Colouration
<i>Sepia officinalis</i>	490 mm	200 m	rounded posterior	brownish
<i>Sepia elegans</i>	89 mm	500 m	kite shaped	reddish-brown
<i>Sepia orbignyana</i>	120 mm	570 m	long spine	reddish-brown

#### 1.1.1.2 Fishery

Although several species of cuttlefish are present in the area, it is landings of the common cuttlefish *S. officinalis* which dominate (Denis and Robin 2001), with the English Channel (International Council for Exploration of the Seas (ICES) division VIIId and VIIe) supporting the main fishery for this species (Dunn 1999; Royer et al. 2006).

The English Channel cuttlefish fishery is a shared resource dominated by French and U.K. fishing fleets and has become an increasingly important component of many of these fishermen's

incomes. Using a combination of different métiers including beam trawling, otter trawling and coastal trapping (e.g. Denis and Robin 2001; Royer et al. 2006; Pierce et al. 2010). On both sides of the Channel, inshore landings are highest between March and June (ICES 2003) coinciding with the peak in breeding season. Offshore landings meanwhile are concentrated in the centre of the Channel with a peak between November and March (ICES 2003), coinciding with the known migration pattern of this population (Boucaud-Camou and Boismery 1991). Whilst originally considered as a pest species in the UK due to its low value and copious ink production (Dunn 1999), landings of cuttlefish by UK vessels have seen a period of rapid increase over the last three decades, rising from approximately 26 t (£12,000) in 1980 (Dunn 1999) to almost 4,000 t (approximately £5,500,000) in 2007 (MMO 2010).

In contrast to the U.K., the French fishery is longer established and better developed, both in terms of volume and value, with landings remaining fairly consistent at around 10,000 t a year between 2002 and 2007 (ICES 2010). An analysis of the 1996 landings indicate that the largest portion of cuttlefish landings was taken by trawlers (Denis et al. 2002). Furthermore, a spatial analysis of catch indicate that the French fleet target cuttlefish at nearly all stages of their life-cycle, exploiting both their offshore wintering areas and inshore spawning and nursery grounds (Denis et al. 2002). Whilst offshore landings were almost exclusively derived from trawling, in coastal areas a variety of métiers co-exist, with coastal traps consistently contributing only a minor proportion of these landings (Denis et al. 2002).

### 1.1.1.3 Landings

As reported by (Dunn 1999), the shift from by-catch to directed fishery was the result of several key factors including an increase in the market value, which rose from only £0.45 per kilogram (kg) in 1980 (Dunn 1999), to £1.45 per kg in 1996 (Dunn 1999), £1.97 per kg in 2007 (MMO 2010) and up to £3.00 per kg in 2011 (Pers comm R. Smith (Brixham Trawler Agents)). Currently, landings of cuttlefish within the English Channel are not separated by species and in the UK are only sorted into two size classes. Whilst *S. officinalis* is known to be the dominant constituent of the catch, the exact composition of species within the landings remains unknown. In addition, as a non-quota species, there has been no mandatory reportings of landings and so the



available statistics may under represent the true landing values for this fishery (ICES 2003). On the UK coast of the English Channel inshore cuttlefish landings are known to be greatest at the ports of Brixham, Shoreham, Portsmouth, Hastings and Eastbourne (ICES 2003). Data for UK cuttlefish landings (1992 - 2002) presented in the ICES 2002 report from the Working Group on Cephalopod Fisheries and Life History (WGCEPH) (ICES 2003) showed that annual landings by offshore trawlers (beam and otter) comprised 90 % of total UK landings for cuttlefish within the English Channel, whilst for the smaller inshore trap fishery landings comprised only 4.3% (ICES 2003).

### 1.1.1.4 Management measures

To date no specific management measures have been introduced in the UK to maintain and manage the English Channel cuttlefish stock (e.g. no total allowable catch, no minimum landing size and no fisheries closures), despite the significant increase in exploitation levels (e.g. Dunn 1999; Challier et al. 2005a). The short-lived, fast-growing life cycle of *S. officinalis* means that within the English Channel each year's stock is composed entirely of only two overlapping generations (e.g. Royer et al. 2006), with half the standing crop of biomass replaced on an annual basis (Boyle and Boletzky 1996). This lack of 'demographic buffer' leaves these populations vulnerable to the effects of unsuccessful annual recruitment (Moltschaniwskyj et al. 2003). As a result large and unpredictable, interannual fluctuations in the stock (Koueta et al. 2000) and landings (Piatkowski et al. 2001) are known to occur and the lack of regular stock assessment data for this species severely limits the ability of fisheries managers to assess the resulting risk of overfishing or stock collapse for this fishery.

The use of traps has been encouraged as they specifically target spawning cuttlefish, which are at the end of their life cycle (e.g. Dunn 1999). A potential issue surrounding their use is that their efficiency in attracting spawning adults is considered, at least in part, due to the use of these devices by female cuttlefish as spawning structures (Figure 1.3) and raises concerns regarding the potential long-term sustainability of the inshore trap fishery. The issue arises when the eggs laid on traps are subsequently lost from the system, which occurs when the traps are removed at the end of the spawning season and cleaned off using pressure hoses to remove

any eggs or biofouling, before being placed in storage over the autumn and winter (e.g. Blanc and Daguzan 1998). The potential impact of egg mortality from cuttlefish traps has yet to be quantified within the English Channel, but is a potential area of concern for fishermen, scientists and fisheries managers.



Figure 1.3: Eggs laid on cuttlefish traps (Eastbourne 2011)

## 1.2 Aims and objectives

The overall aim of this chapter was to assess and update our current understanding of the English Channel fishery *S. officinalis* and the issues that it currently faces. This chapter will focus on the smaller inshore fishery due to the interesting interactions with spawning cuttlefish in this inshore area. The objectives of this Chapter were to:

- Summarise known information on the English Channel cuttlefish fishery
- Using Brixham port as a case study discuss up to date data on landings
- Present new data on the inshore fishery and landings collected as part of this thesis, including:

Monthly market sampling scheme (2010-2012)

Samplings of landings from cuttlefish traps

- Trial three new methodologies in order to find a suitable technique for rapid and accurate assessment of eggs counts that can be used either by scientific observers on board fishing vessels or by trained divers during subtidal surveys.

## 1.3 Methods

### 1.3.1 Landings

Data on cuttlefish landings were obtained for the port of Brixham from 1989 to 2011 from Brixham Trawler Agents (BTA) (Pers comm R. Smith). The data includes both quantity (tonnes (t)) and value (£) per kg. This data is compiled from actual sales figures at Brixham market and is not divided by gear or boat size.

### 1.3.2 Market sampling

As part of this research, monthly market sampling was undertaken between January 2011 and July 2012 with the assistance of Devon and Severn Inshore Fisheries Conservation Authority (DSIFCA). For each monthly survey a total of 200 individuals (when available) were weighed and measured by hand. Length measurements were recorded using dorsal mantle length and reported in centimetres (cm), whilst weight measurements were recorded using electronic scales and reported in grams (g). In some cases the sex of individuals was also recorded, but this information was difficult to collect year round due to the variation in visible sexual maturity stages and condition. In general cuttlefish collected from inshore traps were free of ink and sand and in better visible condition, this made it easier for the sex to be identified. In these cases, sex was determined by examining the interior of the mantle cavity to check for visual signs of spermatophores (males) or nidamental glands and oocytes (female), identification was therefore only possible for individuals that had reached a visible macroscopic state of sexual maturity.

### 1.3.3 Sampling of landings from cuttlefish traps

To examine the landings of the trap fishery in more detail, 400 cuttlefish, caught by trap fishermen, were sampled between 2010 and 2011, with the dorsal mantle length (cm) and weight (g) recorded for each individual.

#### 1.3.4 Eggs laid on cuttlefish traps

During the course of this research three methodologies were trialled for counting eggs on a small number of traps:

- At Babbacombe Bay in July 2010 as part of an MSc project that was undertaken to monitor spawning on artificial structures, the eggs from three traps were counted in July (Brigden 2010). An assessment of the numbers of eggs laid on traps was obtained *in situ* by two pairs of divers, the results from each pair were then cross-validated and compared with reference to photographic and video footage (Brigden 2010).
- At Torbay in July 2011 at the end of the cuttlefish trap season, the eggs from three cuttlefish traps were removed by hand and individually counted as part of a study looking at the feasibility of removing eggs from traps to redeploy into purpose built egg receptors within sheltered areas.
- At Eastbourne in June 2011, eggs were recorded from a further three traps. In this instance, eggs quantities were estimated from a series of photographs and video footage obtained on board a commercial inshore cuttlefish trap fishing vessel during routine hauling of pots. The data from these images was subsequently analysed on return to the laboratory.

#### 1.3.5 Potential mitigations for eggs laid on cuttlefish traps

The issue of egg mortality on cuttlefish traps has been cited in the literature as a potential area of concern (e.g. Bouchaud 1991b; Blanc and Daguzan 1998). These issues were discussed directly with the fishermen during a series of presentations and discussions that were organised between March and June 2012 at three separate locations (Torbay, Selsey and Hastings) where active inshore cuttlefish trap fisheries operate. A series of practical mitigation measures were investigated and discussed to gauge the feasibility of these options. As a non-quota species with no direct management in place, there is no legal requirement for fishermen to undertake any such mitigation actions and so self-regulation of such techniques would be required in order to develop a best working practice for the minimisation of egg mortality from cuttlefish traps.

## 1.4. RESULTS

---

In addition, a small project was undertaken with the help of DSIFCA to test the feasibility of one mitigation techniques. In 2011 a method was trialled to remove the eggs from cuttlefish traps by hand and replace them in a pre-made egg receptor that would subsequently be redeployed into a sheltered area of Torbay to allow the eggs to develop (Figure 1.4). For this purpose the eggs from three traps were removed, counted and placed into the receptor. The device was then redeployed at a sheltered area of Hope's Cove (Torbay, Devon). Due to limited resource availability the egg receptor was monitored *in situ* by a dive pair on one occasion, to check that the device was in place and that significant bio-fouling had not occurred, but a quantitative assessment of hatching rates was not made.

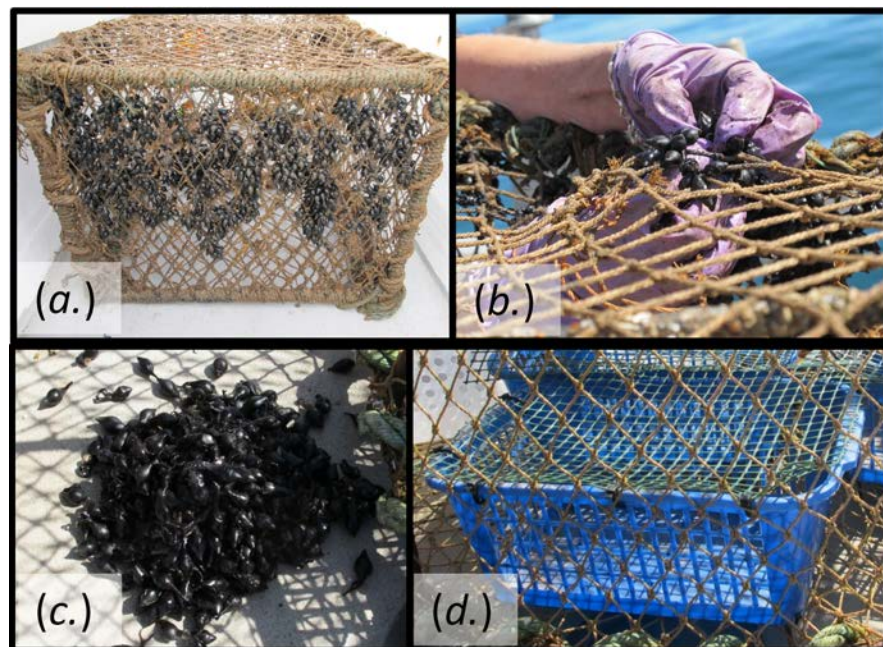


Figure 1.4: Eggs removal and redeployment trial (Torbay 2011). (a.) One of the three traps randomly selected for egg removal, (b.) eggs being removed by hand from the traps, (c.) eggs counted prior to redeployment, (d.) trial egg receptors for redeployment at a sheltered location at Hope's Cove (Torbay)

## 1.4 Results

### 1.4.1 Landings

On the UK coast of the English Channel, Brixham is one of the largest ports for cuttlefish landings. Data from Brixham Trawler Agents (Pers comm. R. Smith) managers of the market at

#### 1.4. RESULTS

this location are presented in Figure 1.5. The landings data showed a substantial increase within this region from approximately 200 tonnes (t) in 1989 to a peak of almost 3,500 t in 2004. Over the last ten years (2001 to 2011), annual landings of cuttlefish at this port had averaged 2,096 t (£2,800,000). In the UK from 2000 to 2002, the average percentage of landings from inshore cuttlefish traps was 4.3 % (ICES 2003). This estimate was used to approximate the total average annual quantity and value of landings by cuttlefish traps at Brixham over the last ten years (2001 to 2011) and equated to approximately 90 t (£120,400) per year.

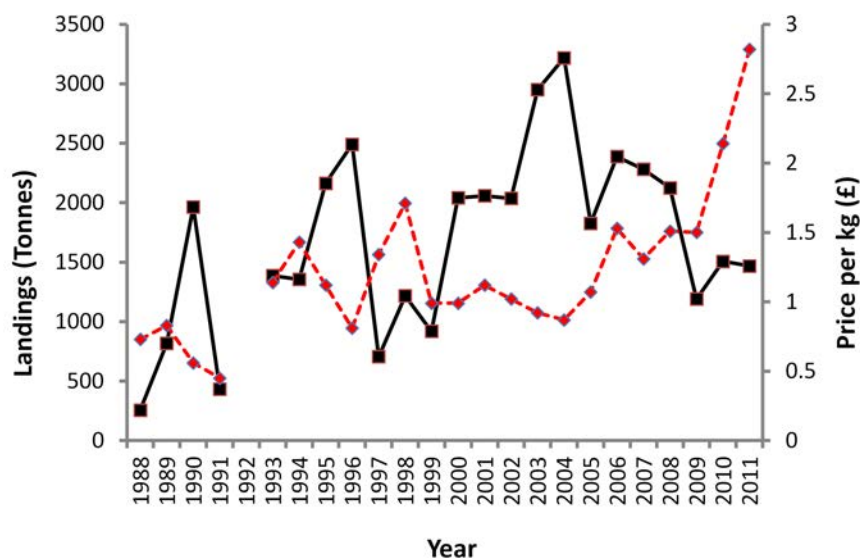


Figure 1.5: Cuttlefish annual landings (solid black line) and price per kg (dotted red line) at Brixham fish market (1988 to 2011) (Pers comm Brixham Trawler Agents).

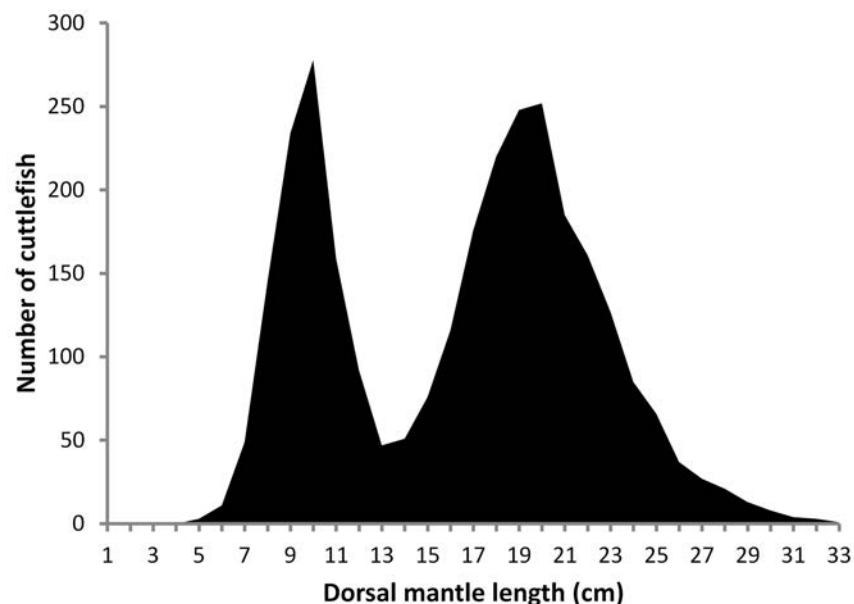
##### 1.4.2 Market sampling

The results of the market sampling from 2011 and 2012 combined are presented by month in Figure 1.6. The results indicate that two peaks are evident throughout several months of the year, representing ‘Year 1’ individuals and ‘Year 2’ individuals. The data collected from 2011 are presented by month in Figure 1.7 to visualise changes in the length frequency distribution within landings over the year. In January, February, March and April both ‘Year 1’ and ‘Year 2’ peaks are separate and evident. In May an intermittent peak is evident and by June the landings are represented only by small individuals. In July and August, there were none or

#### 1.4. RESULTS

---

minimal landings available to sample as the inshore trap fishery had finished for the season and the offshore trawling fishery had yet to commence. In September when landings recommenced, a single cohort was observed following mass mortality of spawning adults at the end of the season, whilst the new cohort of cuttlefish were still too small to enter the fishery (Figure 1.7). By October, the new cohort had begun entering (or recruiting to) the fishery as evidenced by the introduction of smaller size frequency classes and by November and December the two ‘Year’ peaks were again evidenced. It is of interest to note, that although not prevalent in terms of frequency, that individuals as small as 5 cm dorsal mantle length (DML) and 30 g in weight are captured within the fishery (Figure 1.6). The results of the 18 months sampling are presented in combined format in Figure 1.6 and reflect the two overlapping generations that occur within the population. In addition, the relationship between length and weight data is also presented in Figure 1.8 and show the rapid growth rate that occurs within this species. The data are not separated by sex and it is possible that the increased variability in weight and length seen in individuals over 15 cm DML could be attributed to sex related differences in growth and investment in reproductive resources.



*Figure 1.6:* Brixham market sampling combined overall results presented for the years 2011 and 2012 indicating length (DML) frequency.



#### 1.4. RESULTS

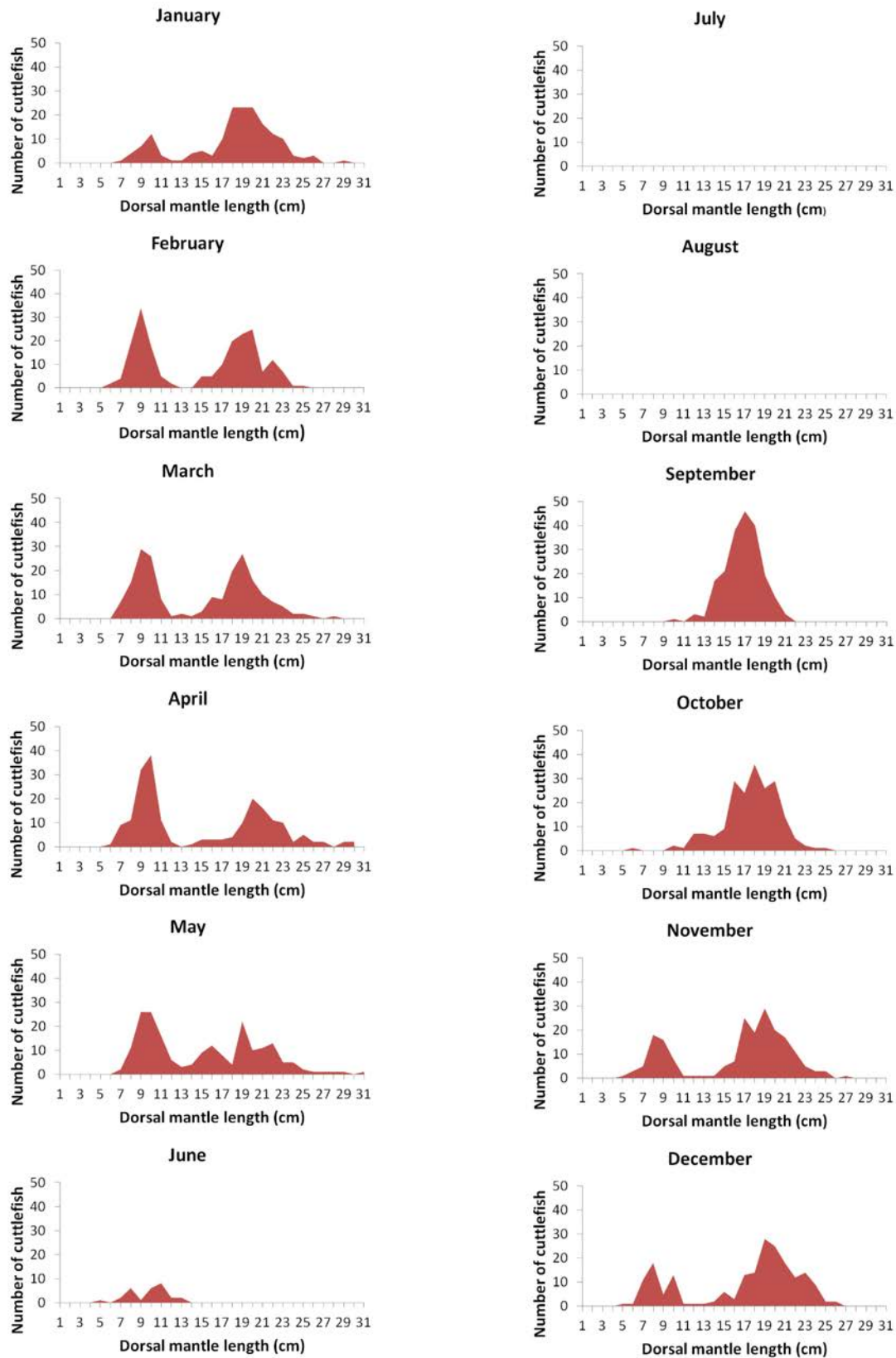
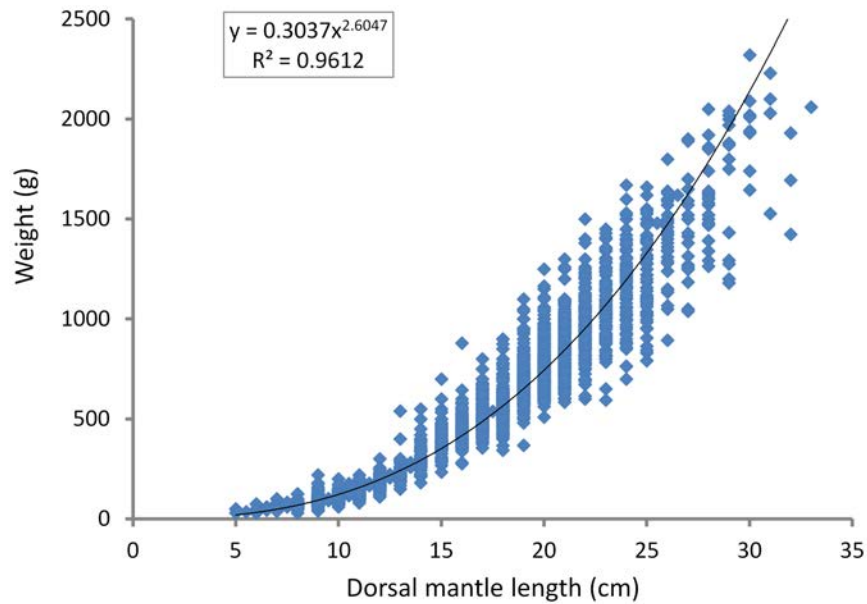


Figure 1.7: Brixham market sampling results presented by month for 2011





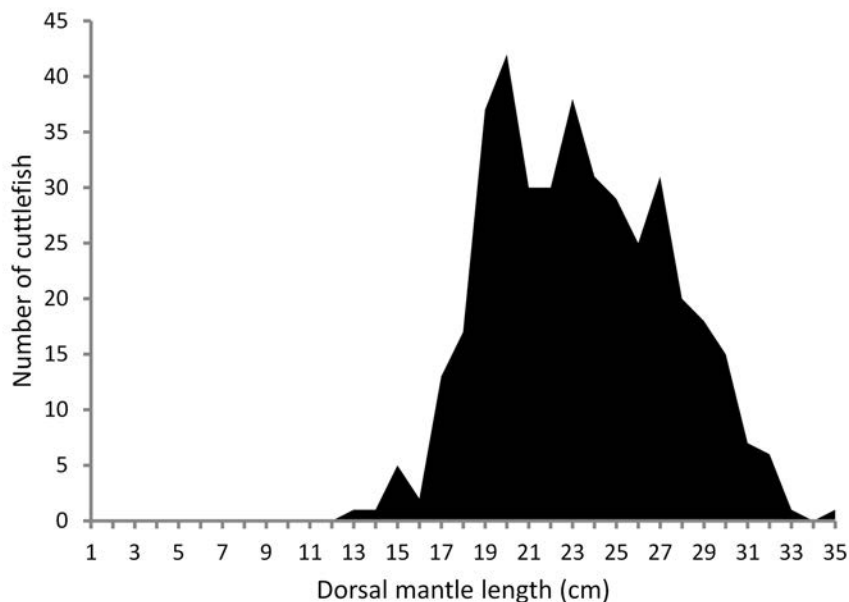
*Figure 1.8:* Relationship between dorsal mantle length and body weight calculated using market sampling data from Brixham fish market (2011 and 2012). A power trend line (black line) was also fitted to the data.

#### 1.4.3 Inshore cuttlefish trap fishery at Torbay (Brixham)

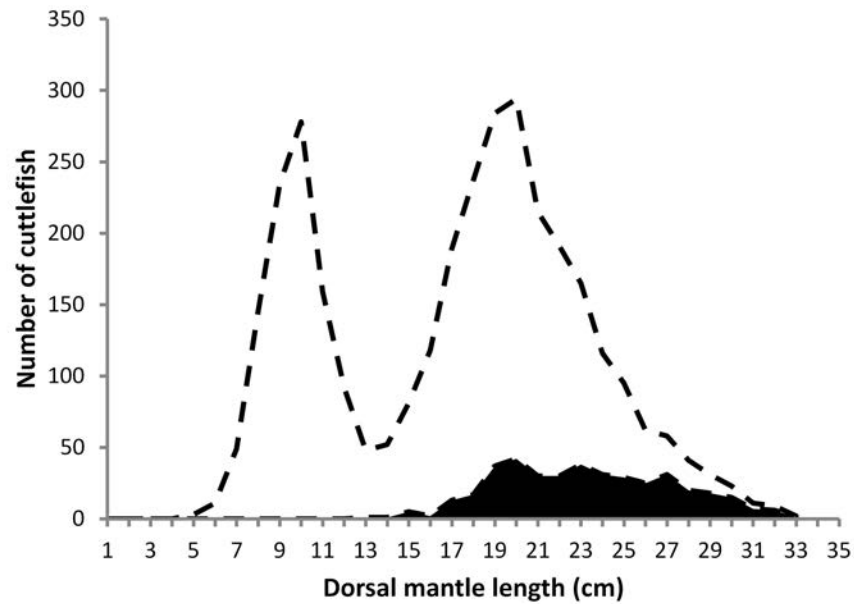
The inshore cuttlefish trap fishery in operation at Torbay (Brixham) is discussed here in more detail as an example of the fisheries that occur at the study sites discussed within this thesis. The inshore trap fishery operates during the spring and summer, it is a relatively short fishing season and lasts for approximately four months beginning in late February/early March and concluding late June/early July, although the start and end of the fishing season can vary interannually by around three weeks and is dependent on environmental conditions. The trap fishery in Brixham is well established and the most up to date information suggests that in 2010 approximately eight boats operated commercial cuttlefish traps in the area, with around 466 cuttlefish traps in total (Pers comm. DSIFCA). However, because cuttlefish are a non-quota species and licences are not required to fish for this species, this data was recorded by voluntary submission only and may be an underestimation of the total fishing effort for this métier within the region.

#### 1.4.4 Sampling of landings from cuttlefish traps

The assertion that inshore cuttlefish trap fisheries specifically target spawning adults was tested by analysing the length frequency distribution of a random sample of catch from three fishing boats in May 2010 and one in June 2011. The average DML of cuttlefish landed by these boats was found to be 23.3 cm (range = 13 - 35 cm, mean = 23.3 cm, SD =  $\pm 4$ ,  $n = 400$ ) with the smallest cuttlefish landed 13 cm, which would suggest that landings are composed mainly of spawning adults (Figure 1.9). This is in contrast to the data reported from Brixham fish market (data from all métiers) for which the average DML of cuttlefish landed is 16.4 cm (range = 5 - 33 cm, mean = 16.4 cm, SD =  $\pm 5$ ,  $n = 2894$ ), with the smallest cuttlefish landed 5 cm. A visual comparison of this data can be seen in Figure 1.10 illustrating the distribution of landings within the ‘Year 2’ peak only.



*Figure 1.9:* Sampling of landings from commercial cuttlefish trap fisheries in May 2010 (Tor-bay, Selsey, Eastbourne) and in June 2011 (Eastbourne) indicating length (DML) frequency



*Figure 1.10:* Visual comparison of length (DML) frequency between landing samples from Brixham fish market (all métiers; 2011 and 2012; indicated by black dotted line) and from cuttlefish trap landings (2010 and 2011; indicated by black fill).

#### 1.4.5 Eggs laid on cuttlefish traps

At Babbacombe Bay (2010) the eggs from three cuttlefish traps were counted by pairs of divers the average number of eggs laid on each trap was approximately 1050 (Table 1.2). In Torbay (July 2011) the eggs from three cuttlefish traps were removed by hand and counted the average numbers of eggs for these three traps was approximately 3,000 (Figure 1.4). At Eastbourne on 2<sup>nd</sup> June 2012, the average over these three traps was approximately 1,500 eggs (Table 1.2).

*Table 1.2:* Numbers of eggs recorded laid on traps in July 2010 (Babbacombe Bay), July 2011 (Torbay) and June 2011 (Eastbourne)

Site	Date	Trap	Number of eggs
Babbacombe Bay	July 2010	1	1210
Babbacombe Bay	July 2010	2	1770
Babbacombe Bay	July 2010	3	150
Torbay	July 2011	1	3400
Torbay	July 2011	2	3700
Torbay	July 2011	3	2100
Eastbourne	June 2011	1	1005
Eastbourne	June 2011	2	1470
Eastbourne	June 2011	3	725

#### 1.4.6 Potential mitigations for eggs laid on cuttlefish traps

One of the potential mitigation measures proposed was the removal of eggs from cuttlefish traps at the end of the season by hand. These eggs could subsequently be redeployed in egg receptors placed into sheltered areas during the summer until hatching. As a trial study, the eggs from three pots were removed by hand at the end of the cuttlefish trap season in July 2011. In total around 9,000 eggs were removed from the three traps, a process that took three people, three hours to complete. It was considered that even if the hatching rates using such methods was high, such a method could not be implemented practically by fishermen (some of whom work single handed and have up to 100 traps) as part of their routine work.

## 1.5 Discussion

The status of the English Channel cuttlefish fishery as a non-quota stock, combined with the increase value of landings for *S. officinalis* (currently around £3.00 per kg) has caused a dramatic increase in UK landings of cuttlefish over the past thirty year with a value of approximately 4,000 t recorded in 2007 (MMO 2010). However, despite this increase in fishing pressure and value with the English Channel cuttlefish fishery, there remain minimal management measures

in place and only a limited understanding of the interactions between fishing pressure, environmental conditions and recruitment variability. In particular, although one of the smallest métiers in operation, the interaction of coastal trap fisheries which occur on inshore spawning grounds during the spring and summer and which target spawning adults (who may or may not have spawned prior to capture) and that utilise these traps as spawning structures, is an area where more research is required in order to gain a full understanding of the impacts and interactions within this natural population.

### 1.5.1 Eggs laid on cuttlefish traps

Small-scale pilot studies investigating quantification of eggs laid on cuttlefish traps were undertaken. The method of counting eggs individually, by hand, produced the highest recorded values for the numbers of eggs laid on traps (average 3,000) and is the most accurate method of quantification. However, the time taken to count eggs in this manner is prohibitive for large-scale, standardised use, with egg counts for three traps taking three people three hours, for fishermen with 100's of traps, such a method is prohibitively time consuming. Additional methods of video and photographic measurement and *in situ* measurement by divers were also trialled. The use of divers for regular monitoring of eggs laid on traps would not be practical for traps in commercial operation due to the dangers associated with this task (the traps surveyed within this research were part of an experimental trial and as such the traps were not hauled during the study). The most promising method for fast and efficient collection of data is the use of photographic and/or video records that could be collected by an onboard scientific observer during regular fishing activities of commercial trap fishermen. Photographs were simply recorded as the pots were hauled and the data subsequently analysed on return to the laboratory. However, two major limitations are currently evident in this technique. Despite the addition of a scale to the photographs, the field of view within the photographs is likely to vary unless each is taken at a set distance, for this purpose a frame, similar to that used during subtidal video transects could be of use. The three dimensional nature of egg clusters also provides a limit to this method (and may result in underestimation of egg counts) as only those eggs visible can be counted and in many instances the three dimensional aspects of the egg clusters are not well represented by

two-dimensional photographs. Analysis of photographic records collected in this manner are still time consuming and a way of automating this process in an image counting software such as Image J would be required before this technique could be utilised at a larger scale to quantify the numbers of eggs laid on traps within the English Channel during a spawning season. A potential estimate of predation rates for eggs laid on traps together with an assessment of the proportion of eggs that hatch from these traps prior to cleaning would be required for a study of this kind.

### 1.5.2 Potential mitigations for eggs laid on cuttlefish traps

In some areas of the UK English Channel coastline cuttlefish traps are simply left underwater until the cuttlefish eggs have hatched. For example, the Southern Inshore Fishery and Conservation Authority (SIFCA) promote a voluntary code of conduct that requests all cuttlefish trap fishermen to leave their cuttlefish traps in the water until September to give the eggs time to develop and hatch. This solution is by far the simplest and has been demonstrated in experimental trials, undertaken in France, to give high rates of hatching (e.g. up to 95 % Gouyen (2001)).

Unfortunately, in many areas, the fishermen consider the practice of leaving cuttlefish traps, unworked, in the water following the end of the cuttlefish season to be of high risk to their gear. Disturbance and damage from bottom trawlers, storms or just increasing wear and tear are all issues that have been raised as concerns regarding this practice. For this purpose research and discussions with local fishermen were undertaken to assess alternative options that would be a feasible method for mitigating against egg mortality from cuttlefish traps. One such method, using the removal of eggs from cuttlefish traps at the end of the spawning season and redeploying them in egg receptors within sheltered areas was trialled in July 2011. The trial study undertaken in Torbay showed that the method was labour intensive and even at a small scale (e.g. three traps) would not be sustainable, taking three people three hours to complete the process. For many of the fishermen who work in Torbay operation of the boats is undertaken single handedly, working up to 100 traps, in this context such a method is not practical. Alternative methods proposed by the fishermen could be to only remove the eggs that are laid on the fingers of the trap entrances, as these eggs can simply be slipped off the plastic fingers and redeployed

very quickly. Another method that was proposed to fishermen was an idea first proposed by a French cuttlefish fishermen and involved the adaptation of cuttlefish traps at the end of the season to include an opening in the top of the trap, allowing them to continue to be worked in the months following the end of the cuttlefish trap season to fish for spider crabs (Malgrange 2009). Whilst this idea was considered to be feasible in practice, two major issues would need to be overcome in the UK before such a method could be trialled at any scale. Firstly, in the UK shellfish licensing requirements would need to be met and the majority of the <10 m boats that operate cuttlefish traps in Torbay do not have the required licence. Secondly, in the UK whilst there is certainly the capacity to fish for spider crabs, at present there is little to no infrastructure in place to market this species and so there is no financial incentive to fish for this species. The results of this work indicate that communication and discussion with fishermen will be vital to the development of any mitigation measures proposed to reduce the mortality of eggs from cuttlefish fishing traps. This is essential as the feasibility and practicality of any method needs to be assessed and due to the non-quota nature of this species, for which there is currently minimal management in place, the implementation of any such methods would require voluntary agreement by the fishermen.

### 1.5.3 Conclusions.

To conclude, there are still many aspects of the inshore trap fishery and its interactions with natural spawning grounds that remain unknown. An assessment of the quantity of eggs laid on traps within these inshore areas is the basic starting point for investigating the potential impacts of egg mortality from this métier. Whilst landings of this non-quota species continue to increase, steps towards developing targeted stock assessment methods and the knowledge on natural movements, behaviours and spawning patterns of these individuals will be required in order to provide the necessary advice for future management of this species.

## 1.6 Overall study aims and objectives

The main aim of this thesis was to investigate the free-ranging movements, behaviours and distribution of spawning *S. officinalis* within the inshore waters of the English Channel and the potential impacts of spawning habitat ‘selection’ on early life stage (ELS) growth rates

and survival, in order to begin to address critical gaps in our knowledge for this commercially important species and provide baseline data that can be used for the future management and conservation of this species. The main objectives of this thesis were to:

1. Compile pre-existing information on the distribution of *S. officinalis* spawning locations within the English Channel using records of egg cluster presence; these data were then used in (Chapter 3).
2. Undertake a spatial analysis of potential spawning areas in order to examine the environmental conditions of known cuttlefish spawning locations and to build a predictive model describing areas within the English Channel where the environmental conditions are within the preferred range for spawning (Chapter 3). The maps of predicted potential spawning habitat/locations produced will provide an informed knowledge base for *in situ* observations (Chapter 4).
3. Collect *in situ* observations of natural substratum where spawning females attach their eggs, in both UK and French coastal waters and assess patterns of spawning within study locations.
4. Describe the movement and activity patterns of free-ranging adult and sub-adult cuttlefish in the inshore waters of the English Channel using acoustic telemetry (Chapter 5).
5. Undertake a long-term archival tagging study using sub-adult cuttlefish to provide a better understanding of the movements and migration patterns of *S. officinalis* within the English Channel (Chapter 6).
6. Investigate the impact of heterogeneous conditions within early life stage (ELS) habitats (as a result of variations in spawning habitat selection) on growth and survival rates of ELS, with a particular focus on the physical complexity of these different habitats (Appendix B).

In Chapter 1, a general introduction to this thesis is made and combined with a short study introducing the commercial cuttlefish fishery within the English Channel. Several key aspects



of this commercial fishery are highlighted and results collected during the three years of this research are presented. This research focuses on the fishery that operates from the port of Brixham (Torbay), one of the most important ports for cuttlefish landings in the UK.

The detailed review provided in Chapter 2 identified and highlighted the factors considered to affect spawning, early life stage survival and recruitment variability in the common cuttlefish (*S. officinalis*) within the English Channel. Despite the great body of literature that already exists for this species and the advances in many areas of study that have been made there clearly still exists the need for further study that addresses the main knowledge gaps highlighted within this review. Specifically the need for information on the free-ranging behaviours and movements of *S. officinalis* and their habitat use in inshore waters is key. Such information is vital for non-quota commercial species like *S. officinalis* for which fishing pressure continues to increase, whilst minimal management exists.

Despite the growing importance of *S. officinalis* as a commercial fishery species, there has to date, been no direct efforts to predict the distribution of spawning habitat within the English Channel. Chapter 3 highlighted the potential spawning habitat/locations of *S. officinalis* within the English Channel and produced maps of this data, contributing to the available baseline data for spawning habitats and location for *Sepia officinalis* around the coast of the English Channel.

Within the English Channel, a clear definition of spawning habitat for *S. officinalis* was lacking. In Chapter 4 a series of surveys was undertaken to record *in situ* observations of spawning on natural structures within both the UK and French inshore waters of the English Channel. Using the outputs produced from Chapter 3, to provide an informed knowledge base to guide the study locations used for this research, surveys at five study sites were undertaken over a three year period with the aim of identifying the structures and habitats used for spawning and to make an assessment of the spawning patterns among different depth strata, habitat strata and between different years.

Previously the movement patterns of free-living *S. officinalis* have been little studied, but advances in electronic tagging technologies and new methods for tag attachment have provided the opportunity for these novel tools to be used in the study of *S. officinalis* in the field for

the first time. Chapters 5 and 6 provide details of the first use of electronic tags (acoustic and archival) for the study of the free-ranging movements and behaviours of this species within their natural environment. The aim was to provide information to address the major knowledge gaps that currently exist and to help shed light on habitat use and selection of spawning adults. This information is essential for understanding the spatial distribution patterns of this species, which in turn will help provide better information and advice for sustainable management and conservation of this species.

Laboratory studies were designed and partially implemented in order to address Objective 6. An example of these laboratory studies, which aimed to investigate the effects of different levels of habitat complexity on ELS behaviour, survival and growth rates, is described in summary in Appendix B. However, the presence of a *Vibrio* sp. bacterial infection in the sea water reservoir system at the MBA laboratory in both 2011 and 2012 affected the completion of this work which had to be terminated in both years as the bacterial infection was fatal for cuttlefish hatchlings. As such, whilst the methodology and laboratory set up are described within the appendices, the results collected were too limited in scope to provide any robust analysis or discussion. However, it is hoped that the methods described will provide the basis for additional future studies.

Finally in Chapter 7 a summary of the key findings and contributions of this thesis to the current literature are highlighted and a brief discussion and integration of this work and its conclusions are provided.

## Chapter 2

**A review of the factors influencing spawning, early life stage survival and recruitment variability in the common cuttlefish (*Sepia officinalis*) within the inshore waters of the English Channel**

---

**Chapter 2 has been published in Advances in Marine Biology Volume 65.**

**Chapter 2 (pages 23 – 82) of this thesis has been removed due to Copyright restrictions.**

---

## Chapter 3

# Species distribution modelling of potential spawning habitat

### 3.1 Introduction

Describing the spatial patterns of species distribution and understanding the driving factors behind them, is a key objective in ecology dating back over 100 years (e.g. Grinnell 1904). It is widely acknowledged that the geographic distribution of a species is shaped by a wide range of factors including dispersal capacity, climatic conditions and biotic interactions (Soberon 2005). Of these factors, climatic conditions (e.g. environmental and physical variables) are often considered the most important, especially in short lived species like cephalopods, where large variations in annual abundance are often related to variations in environmental conditions (Pierce et al. 2008). As survey data are rarely available for every location within the extent of a species' distribution range, species distribution models (SDMs) are often used to interpolate (or extrapolate) the limited data that are available for species distribution by relating species presence (and absence) to a set of environmental variables (Pearce and Boyce 2005). The relationship between environmental and physical variables and the distribution of the reproductive behaviour of a species is a key research focus (Sanchez et al. 2008), especially for commercially important species like *S. officinalis* for which this information provides an essential base from which to propose sustainable fisheries management.

Spawning grounds are essential for the maintenance of recruitment, particularly in cephalopods which are heavily dependent on successful annual recruitment to sustain population levels. By understanding which areas within the heterogeneous marine environment have a potentially

high capacity for the biological production of a specific species, the prioritisation of conservation or management of these areas can be undertaken in order to sustain the long-term viability of a population and its associated fishery and landings (Valavanis et al. 2008). As such, the identification of potential spawning locations for commercial fisheries species is an essential basis for sustainable management of a fishery resource. In *S. officinalis*, spawning females attach clusters of eggs to erect flora or fauna that radiate from the seabed, which are left to develop without parental care. The benthic and static nature of these eggs makes them an ideal measure of true spawning, in contrast to the presence of spawning adults which indicates, but does not confirm potential spawning events at a location. In addition, the use of static, benthic egg clusters as opposed to mobile adults, provides a life-stage that is easier to model as during the predefined modelling period (March to September) movement does not need to be taken into account for these individuals. The use of egg cluster sample points rather than those of spawning adults is a more certain way to ensure that the distribution of spawning locations are accurately modelled. Within the English Channel, the spawning distribution of *S. officinalis* is already known in part, at a large scale, from the presence of inshore cuttlefish trap fisheries, which target spawning adults. However, despite the commercial importance of this species, the location and description of fine-scale spawning grounds and habitats are not yet well defined, especially in areas where coastal trap fisheries do not operate.

The literature highlights a range of variables that may be important in determining suitable spawning habitat for *S. officinalis*. Cuttlefish are highly visual animals (e.g. Hanlon and Messenger 1996) which may rely on visual cues for navigation to spawning locations, finding suitable mates as well as assessing the suitability of fine-scale spawning habitats, the success of which have the potential to be affected by variation in the water clarity.  $K_{490}$  is the attenuation coefficient at 490 nm and is one of the indicators used to represent water column turbidity. In addition, the spring bloom, which in the English Channel can cause an increase in Chlorophyll-*a* concentrations from June through to August (Smyth et al. 2010) and may also have an effect on the turbidity of the water in coastal regions causing additional impacts on spawning in this species in the same manner. Chlorophyll-*a* concentration also reflects the concentration of phytoplankton, which forms the basis of the marine food chain and is considered a good indicator

of primary productivity (Pierce et al. 2008), which is thought to be an important determinant of cephalopod distribution and abundance (Pierce et al. 2008). This is particularly the case for paralarval and juvenile cephalopods (Vidal et al. 2010) with primary productivity potentially indicating areas of favourable feeding habitat which is essential for newly emerged hatchlings. SST has been shown by numerous authors to affect cephalopod abundance and distribution (e.g. Waluda and Pierce 1998; Wang et al. 2003). In particular, for *S. officinalis*, SST was found to correlate with the annual migration patterns of *S. officinalis* in the English Channel (Wang et al. 2003). That temperature is a key regulating factor in recruitment of cephalopod populations, as a result of its effects on ELS (e.g. rate of embryogenesis, yolk utilisation, size and weight at hatching) is an idea supported by numerous authors (e.g. Boyle and Boletzky 1996; Forsythe et al. 2001; Waluda et al. 1999; Challier et al. 2005a; Hatfield et al. 2001). *S. officinalis* is relatively tolerant to variations in salinity however, a salinity of 28 or greater is considered optimal for spawning (Paulij et al. 1990a; Mangold-Wirz 1963) and so maybe an important determining factor in spawning distribution, within the English Channel salinities of between 32 and 35 have been recorded at spawning sites on the French coast (Boucaud-Camou and Boismery 1991). Within the English Channel, salinity can vary significantly both spatially and temporally (within and between years). For example, areas such as the Baie de Seine are strongly influenced by river input, the rate of flow from which is variable (Garnaud et al. 2002) and can cause fluctuations in the salinity of the area; whilst the salinity in areas of the English Channel which are not influenced by river input may remain at a higher and more stable level. Reduced salinity may influence both the abundance of prey in the area and the success of hatching rates in *S. officinalis*, with salinity found to have a statistically significant affect between the range of 28 to 33, with a hatching rate of only 50 % at a salinity of 28 (e.g. Palmegiano and d'Apote 1983). As such, salinity may have an effect on the use of sites for spawning within the English Channel.

*S. officinalis* is a nekto-benthic species which occurs, from the coastline (2-3 m) to approximately 200 m depth (Guerra 2006), beyond which, the shell is vulnerable to implosion (Ward and Boletzky 1984). Depth has also been shown to play a part in the spawning distribution of this species with eggs generally thought to occur at depths of less than 40 m (Guerra and

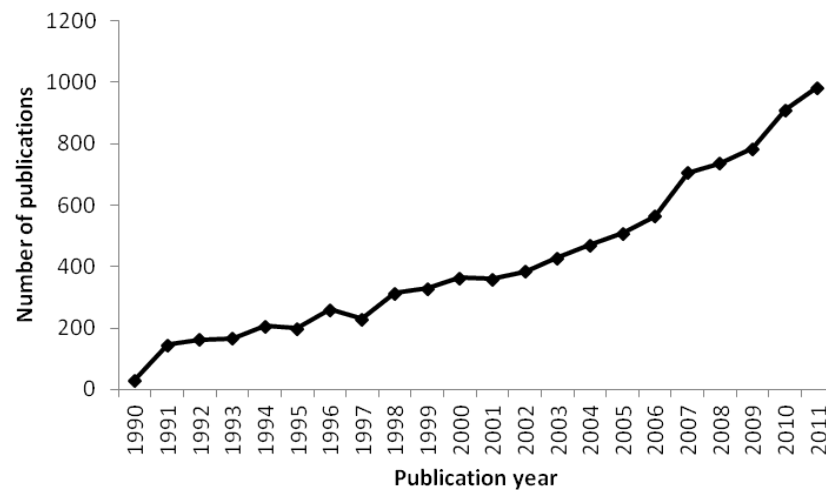
González 2011) or 50 m (Valavanis et al. 2002). *S. officinalis* undertake seasonal migrations from the deeper, offshore waters of the Channel to the shallower, inshore waters. In spring adults migrate to inshore waters to spawn and so distance from the nearest coastline has been included here as a predictor variable. *S. officinalis* is a benthic spawner, with spawning thought to occur predominantly on muddy and sandy sediments (Guerra 2006). As such sediment type is likely to be an important predictor variable for spawning distribution in this species. Bed shear stress (Newtons per m<sup>2</sup>) is a measure of the friction exerted on the seabed by the mean tidal current, and is often associated with the type of sediment predominant at a location. In general, bed shear stress is considered to be an important determinant in the distribution patterns of many species (Freeman and Rogers 2003), and is likely to be important to some degree for benthic spawners such as *S. officinalis*.

This current study aims to use presence-only sample points of *S. officinalis* egg clusters to provide the first SDM for spawning of this species within the English Channel. Presence-only datasets have no information about areas where the species is absent and have often been collected without a specific sampling method and include ad-hoc or opportunistic records. Despite the increase in landings of cuttlefish within the English Channel by UK fishing vessels (26 t in 1980 (Dunn 1999) to 4,000 t in 2007 (MMO 2010)), there remains little or no directed management in place for this fishery, with no total allowable catch, no minimum landing size and no closures (either spatial or temporal). The increased popularity of this species as a commercial fisheries resource, with fisheries targeting nearly every stage of their life cycle (e.g. offshore wintering grounds, migration routes and coastal spawning grounds) and across a wide range of habitats, combined with large fluctuations in recruitment (reflected in inter-annual variability of landings), identifies a need for basic information of the distribution of spawning locations and habitats to be determined (both at the broad and finer-scale) within the English Channel.

#### 3.1.1 Review of models

Within the marine environment it is often only possible to obtain fragmentary information regarding species and habitats. SDMs provide important tools with which the distribution of a species can be predicted, based on the partial information that is available (Guisan and Zim-

mermann 2000; Guisan and Thuiller 2005). Whilst the application of SDMs within the marine environment has been a relatively recent phenomenon (Valavanis et al. 2008; Robinson et al. 2011), their increasing use (Figure 3.1) has allowed a wide range of practical and theoretical issues to be addressed including: the relationships between species occurrence and environmental conditions (e.g. Lefkaditou et al. 2008), planning of marine protected areas and conservation networks (e.g. Leathwick et al. 2008), identification of essential fish habitat (e.g. Valavanis et al. 2004b) and forecasting how species distribution is affected by climate change or environmental disturbance (Cheung et al. 2009).



*Figure 3.1:* Trends in the number of marine applications of SDMs. The data in this graph were derived from an ISI Web of Science search using the search criteria specified by Robinson et al. (2011): Search topic = ‘species distribution’ OR ‘ecological niche’ OR ‘habitat preference’ OR ‘environmental preference’ OR ‘bioclimate envelope’ OR ‘bioclimate’ OR ‘environmental niche’ OR ‘habitat suitability’ AND ‘model\*’. In order to obtain only marine records, a subsearch within the original results was also undertaken using the search topic = ‘marine’ to obtain only the marine records

Rapid developments in statistical techniques and geographical information systems combined with a greater availability and access of remotely sensed environmental datasets have enabled a rapid development in the number and types of SDM techniques that are now available (Guisan and Zimmermann 2000). This vast array of SDM techniques can be loosely divided either by their basic approach (e.g. correlative, coupled correlative or mechanistic) or by the type of data that they require (e.g. presence-absence, presence-pseudo absence, presence-only or



abundance). The common methods used for modelling presence-absence data include: generalised linear models (GLMs) (e.g. McCullagh and Nelder 1983), a mathematical extension of linear models that fits parametric terms and utilises a link function to assume a relationship between the mean of the response variable and the linear combination of the explanatory variables (Guisan et al. 2002). Generalised additive models (GAMs) (Hastie and Tibshirani 1986), provide a semi-parametric extension to GLMs that offer a more flexible approach so as to deal with highly non-linear relationships between response and explanatory variables (Guisan et al. 2002). A link function provides a relationship between the mean of the response variable and a ‘smoothed’ function of the explanatory variables (Guisan et al. 2002). The main underlying assumption of this technique is that the functions are additive and the components are smooth (Guisan et al. 2002); multiple adaptive regression splines (MARS) (Friedman 1991), a flexible, non-parametric, regression based approach that fits non-linear responses using piecewise linear fits rather than smoothing functions (Elith and Leathwick 2007); boosted regression trees (BRTs) (e.g. Elith et al. 2008; De’Ath 2007), an ensemble approach that combines two algorithms (regression trees and boosting) based on an additive regression model in which individual terms are simple trees, fitted in a forward, stagewise fashion (Elith et al. 2008) and finally, artificial neural networks (ANNs) (e.g. Lek and Guegan 1999), a highly flexible, non-linear mapping structure based on the function of the human brain (Lek and Guegan 1999). It has been termed a ‘black box’ approach in which all the characters describing the unknown situation are presented to the trained ANN and a prediction is provided (Lek and Guegan 1999).

The common methods used for presence-only data include: bioclimatic envelopes (BIOCLIM) (Busby 1991), an environmental envelope algorithm which identifies locations that have environmental values that fall within the range measured from the presence-only dataset provided for the target species (Busby 1991); environmental niche factor analysis (ENFA) (Hirzel et al. 2002), a multivariate modelling approach that performs a factor analysis within the multidimensional space of ecological variables, to assess the distribution of the localities that a target species was observed within, against a reference set that describes the entire modelling extent (Hirzel et al. 2002); genetic algorithm for rule-set production (GARP) (Stockwell 1999), an artificial intelligence-based approach that employs the rules from four distinct modelling methods

(atomic, logistic regression, bioclimatic envelope and negated bioclimatic envelope) to derive several different rules which are then used to iteratively search for non-random correlations between the presence and background absence observations and the environmental predictors (Stockwell 1999; Hernandez et al. 2006) and finally, maximum entropy modelling (MaxEnt) (Phillips et al. 2006), part of the machine learning community of models, MaxEnt estimates the species' distribution of maximum entropy (or that which is closest to uniform) across the study area, given the constraint that the expected value of each environmental predictor variable under this estimated distribution matches its average value for the set occurrence data (Phillips et al. 2006). Summary details for each of these methods can be found in Table 3.1. Despite the intrinsic differences between these available techniques (e.g. presence-absence, presence-only, presence-pseudoabsence etc.) they are all numerical tools which are generally used within ecology for one of three main purposes as described by Elith and Graham (2009):

Table 3.1: Main species distribution modelling methods available

Method	Type	Data requirement	Reference
Generalised linear model (GLMs)	Statistical (regression based)	Presence/Absence	McCullagh and Nelder (1983)
Generalised additive model (GAMs)	Statistical (regression based)	Presence/Absence	Hastie and Tibshirani (1986)
Multivariate adaptive regression splines (MARS)	Statistical (regression based)	Presence/Absence	Friedman (1991)
Boosted regression trees (BRTs)	Machine learning	Presence/Absence	Elith et al. (2008)
Artificial Neural Networks (ANNs)	Machine learning	Presence/ Absence	Lek and Guegan (1999)
Maximum Entropy (MaxEnt)	Machine learning	Presence only	Phillips et al. (2006)
Genetic Algorithm for Rule-set production (GARP)	Machine learning	Presence only	Stockwell (1999)
Bioclimatic envelopes (e.g. BIOCLIM)	Profile technique	Presence only	Busby (1991)
Ecological Niche Factor Analysis (ENFA)	Profile technique	Presence only	Hirzel et al. (2002)

1. To describe and understand the relationships between species occurrence and environmental conditions (e.g. define its ecological niche)
2. To predict and map those geographic areas within a study region that are more or less suitable for use by a target species (e.g. to extend the prediction of species distribution to unsampled locations within the study region)
3. To extrapolate to environmental conditions or spatial locations outside of the sample space (e.g. to predict changes in species distribution as a result of climate change)

#### 3.1.2 Presence-only model selection

The type of environment that *S. officinalis* uses for spawning in the English Channel can be investigated by modelling the probability that at least one cuttlefish egg cluster is present at a particular location within the modelling extent, given a particular set of environmental variables. Ideally, this model would be produced using a rigorously defined sampling scheme that covers the entire extent of the English Channel (with an equal representation of sites both environmentally and spatially) with surveys undertaken at each of the sampling locations to determine whether egg clusters of this species are present or absent. However, one of the greatest challenges within the marine environment remains the collection of high quality datasets containing both presence and absence records, as collection is often difficult, due to the intrinsic nature of the marine environment and or the mobility of the target species, and because such sampling is costly (and often prohibitive) in terms of both time and expense (Tsoar et al. 2007). As a result there has been an increase in interest in methods that allow the utilisation of pre-existing and readily available presence-only data sets. Sources of species presence records include archival datasets, incidental observation databases, museums and bibliographic records.

The range of methods available for using presence-only data to create SDMs has rapidly expanded over the last twenty years (Phillips et al. 2009), with Pearson (2010) describing three main approaches:

1. The first approach centred around ‘true’ presence-only models that are specifically designed to use only presence records, without reference to the set of environmental condi-

tions available to the species within the region (Elith et al. 2006). These use envelope or distance based methods and include BIOCLIM and DOMAIN (e.g. Busby 1991; Carpenter et al. 1993).

2. A subsequent approach adapted existing presence-absence modelling methods to use with presence-only datasets. By taking into account the available environment ('pseudo-absence' data; selected randomly or according to a set of weighting criteria) as well as observed presence-only species records these methods allowed better discrimination and included methods such as GLMs, GAMs and MARS which are all multiple regression based techniques which differ in the fitting procedures and methods used to model complex responses (Guisan et al. 2007).
3. The most recent approach utilises novel methods specifically designed to work with 'noisy' presence-only datasets whilst additionally taking account of the environmental conditions (Elith et al. 2006). Methods for this approach include MaxEnt and BRT which focus on how the environment where the species has been observed to occur relates to the environment across the rest of the study area (the 'background'). An important distinction between the use of background and pseudo-absence data is that observed occurrence localities are included as part of the background datasets, whilst pseudo-absence datasets are not (Pearson 2010).

Whilst at present there is no clear advice within the SDM community as to what is the best choice among presence-only modelling techniques for a given application, several thorough reviews have now been undertaken to compare the ability of such modelling techniques to accurately model species distribution within specific situations (e.g. Segurado and Araujo 2004; Elith et al. 2006; Tsoar et al. 2007; Elith and Graham 2009; Franklin and Miller 2009; Hernandez et al. 2006; MacLeod et al. 2008). The most extensive of these reviews was undertaken by Elith et al. (2006) and used 16 different presence-only modelling approaches to model the distribution of 226 species from across the globe. Whilst many of the tested methods produced differences in predictions within different situations, others consistently outperformed the rest (Elith et al. 2006). Two of the best performing models were novel presence-only mod-

elling methods from the machine-learning community, MaxEnt and a naive boosted trees model, which both consistently outperformed established envelope and regression methods both within this study (Elith et al. 2006) and in other comparative studies (e.g. Guisan et al. 2007; Hernandez et al. 2006). Of these two methods, MaxEnt was selected as the focus of this study as it operates using presence-only data combined with a background dataset, whilst BRTs require presence/absence or presence/pseudo-absence data to operate. MaxEnt operates without absence or pseudo-absence data and is flexible in using both categorical and continuous data types for environmental variables (Wilson et al. 2009). It also performs well with small (as few as four records) datasets and its comparative analysis is one of the best and most consistent tools for presence only modelling (e.g. Elith et al. 2006; Guisan et al. 2007; Hernandez et al. 2006); the ability for the user to account, at least in part, for sampling bias within the pre-run settings and that the mathematics implemented in the software has been subjected to rigorous analysis (Dudík et al. 2004; Dudík et al. 2005, 2007).

#### 3.1.3 MaxEnt

Maximum entropy is a general purpose technique for estimating a probability distribution from partial information (Jaynes 1957). In the context of a probability distribution, the term (or concept) of *entropy* has been used interchangeably with that of *uncertainty*, such that entropy could be defined ‘as a measure of our degree of ignorance as to the state of a system’ (Jaynes 1957). Jaynes (1957) was the first author to propose the maximum entropy approach to probability distribution. The principle is that estimates of a probability distribution are generally based on only partial information, and any inference about the distribution should be based on maximum entropy (or maximum uncertainty), subject to whatever information is known. This distribution is calculated given the constraint that the expected value of each environmental predictor variable under this estimated distribution matches its average value for the set occurrence data (Phillips et al. 2006). In other words, any estimate of probability distribution that is based on only partial information should be maximally non-committal in order to ensure that no arbitrary assumptions are introduced (Jaynes 1957). Maximising entropy is a desirable aim in species distribution modelling as to do otherwise would be to impose additional (unfounded) constraints on

the predicted species distribution (Phillips et al. 2009). The modern day approach to maximum entropy species distribution modelling was developed within the machine learning community by Phillips et al. (2004, 2006) and is delivered through a free-ware software platform known as MaxEnt, which is available for download from: <http://www.cs.princeton.edu/~schapire/maxent>.

For the purpose of this study the following key terms and concepts that will be used in reference to MaxEnt throughout the rest of this chapter will be introduced and defined. A *sample point* is defined as an occurrence (or presence) record of the target species for which the probability distribution is being predicted, a *feature* refers to a predictor variable (environmental or physical) or a function thereof. In order to encompass the range of possible responses of a species to these features, MaxEnt currently has six possible feature classes that can be used : Linear, product, quadratic, hinge, threshold and categorical (Phillips and Dudík 2008; Elith et al. 2011). Finally, the term *location* refers to the geographic extent which outlines the geographic study area of the model (Dudík et al. 2004; Phillips et al. 2004) which is pre-ordained by the user. These parameters are then provided by the user as the input data for MaxEnt, such that a set of sample points (occurrence/presence locations) for the target species and a set of features (predictor variables) that are relevant to the distribution of the target species are uploaded to the software interface within the user defined location (geographic extent). Whilst a number of different distributions exist that will satisfy the constraints of the partial information available, MaxEnt uses the supplied information to produce the probability distribution of maximum entropy (i.e. the distribution closest to uniform) for the target species (Phillips et al. 2004), subject to the constraints of the supplied features, with the expectation that each feature should match its empirical average (Phillips et al. 2004). MaxEnt has a range of built in evaluation techniques which allow the user to compute a test statistic known as the area under the receiver operating characteristic curve (AUC), which enables models performance to be evaluated. The computation of a receiver operating characteristic (ROC) curve enables a threshold independent analysis of the model's performance (i.e. overall model fit) to be made. The ROC curve is created by plotting sensitivity values (the true-positive fraction) against 1-specificity (the false positive fraction) for all available probability thresholds (Fielding and Bell, 1997; Manel et al., 2001). An ROC curve for which the sensitivity is maximised at low values of the false-positive

fraction is considered to be a good model and is quantified by calculating the area under the receiver operating characteristic curve (AUC). The AUC has been extensively used within SDM research and is a useful measure of how well a model is able to discriminate between areas where a species is present and those where it is absent (Hanley and McNeil 1982). The AUC is calculated automatically by MaxEnt and with values ranging from 0 to 1. Where 1 represents perfect discrimination and 0.5 representing a model that is no better than random. An interpretation of intermediary AUC values is presented in Table 3.2. ROC curves are not restricted to use with traditional presence/absence data and can also be generated with presence and background data (Phillips et al. 2006). When using presence and background data to generate the ROC plot the AUC value can be interpreted as a measure of the models ability to discriminate between a suitable environmental condition (occurrence point) and a random background pixel (background point), as opposed to the traditional interpretation used with presence/absence data of discrimination between suitable (presence) and unsuitable (absence) conditions (Phillips et al. 2006). As a result the AUC calculated by MaxEnt is based on the *fractional predicted area*, defined as the fraction of the total study area predicted present, rather than the traditional *fraction of absences predicted present* (Phillips 2010). Using the data from the entire replicate set it is possible to calculate average AUC values for both training and test data as well as the standard deviation to assess the degree of variability within the replicate set. It is also possible to run a jackknife test to determine which predictor variable contributes most to the model prediction (Pearson 2010).

*Table 3.2:* Area under the curve values for assessment of the performance and predictive ability of the MaxEnt model (Phillips et al. 2009; Hosmer and Stanley 2000; Swets 1988)

AUC value	Prediction assessment
1	perfect prediction
$\geq 0.9$	excellent prediction
0.7 - 0.9	good prediction
0.5-0.7	poor prediction
$\leq 0.5$	prediction no better than random



Since its introduction in 2004, MaxEnt has been used to model a wide range of species distributions within both the terrestrial (e.g. Lizards: Gadsden et al. (2012), wild dogs: Jenks et al. (2012) and bird species: Brambilla and Ficetola (2012)) and marine environments (e.g. harbour porpoises: Edrén et al. (2010), cold water corals: Yesson et al. (2012) and humpback whales: Smith et al. (2012)). In terms of cephalopods, whilst a variety of studies have been undertaken to assess the relationship between cephalopod distribution and environmental conditions using solely GIS or GIS combined with SDM techniques (e.g. Valavanis et al. 2002; Sanchez et al. 2008; Moreno et al. 2009; Waluda and Pierce 1998) to date only one published study has used MaxEnt to model the distribution of a cephalopod species e.g. *Octopus vulgaris* in the Mediterranean and Eastern Atlantic waters (Hermosilla et al. 2011). This study used 213 presence records of octopus collected from surveys and bibliographic records to model the distribution of this species (Hermosilla et al. 2011). The authors found that MaxEnt was able to successfully predict octopus distribution in the Mediterranean whilst additionally evaluating which variables used within the model were the most important in predicting this distribution (Hermosilla et al. 2011).

Whilst *S. officinalis* has yet to be modelled using presence-only methods, the EU Interreg IV funded Channel Integrated Approach for Marine Resource Management (CHARM II) project modelled the distribution of *S. officinalis* in the eastern English Channel (for July and October) using GLM and GAM methods and a series of structured fisheries survey data from the ground fish survey (October) and bottom trawl survey (July) that enabled presence-absence modelling methods to be utilised. These models use data from adult and juvenile life stages and do not focus on the spawning locations and conditions of this species.

#### **3.1.4 The presence-only problem**

##### **3.1.4.1 Lack of absence data**

Before using presence-only techniques to build SDMs, an understanding of the inherent issues associated with these presence-only datasets is required. Two main problems exist regarding their use to model species distribution. Firstly, whilst these datasets inform us of the locations in which cuttlefish spawning is observed, they often contain no information regarding where

a species is absent (Elith et al. 2006). When using these presence-only datasets to assess the distribution of cuttlefish spawning it is not possible to know for sure where cuttlefish spawning does not occur.

Ward (2007) summarises this presence-only problem stating that the aim of presence-only modelling is to predict the probability of true presence ( $y = 1$ ) or absence ( $y = 0$ ) of a species, given the environmental covariates  $\mathbf{x}$ . The presence-only datasets that are utilised however provide information only on observed presences ( $z = 1$ ). Depending on the modelling method used, a set of background or pseudo-absence data ( $z = 0$ ) is then also created. Whilst within the presence-only dataset an observed presence ( $z = 1$ ) implies a true presence ( $y = 1$ ), for the background data or pseudo-absence dataset it is unknown whether ( $z = 0$ ) equates to a true presence ( $y = 1$ ) or a true absence ( $y = 0$ ). In presence modelling, the sampled locations (i.e. all locations which are represented in the presence-only dataset or the background or pseudo-absence datasets) can be defined using the notation  $s = 1$ . Any location which is within the study location but that is not part of the dataset is subsequently denoted as  $s = 0$ . Whilst the aim of presence-only modelling is to use the observed  $z$  and  $\mathbf{x}$  to estimate the model of interest,  $P(y=1|\mathbf{x})$ , as a result of the presence-only problem the data are actually generated instead by the probability  $P(z=1|\mathbf{x}, s=1)$  (Ward 2007).

#### 3.1.4.2 Spatial sampling bias

Secondly, these datasets often have no defined sampling scheme (e.g. the aims and methods of collection for data within these datasets are generally unknown). The lack of known sampling scheme can mean that these datasets exhibit a level of spatial bias in survey effort (e.g. Reddy and Dávalos 2003), reflecting the haphazard manner in which these datasets may have been collated. For example, some sites (e.g. those that are easily accessible) within the modelled area could potentially have been surveyed more than others, this is known as ‘sample selection bias’. Sample selection bias within these presence-only datasets is generally unquantified but can theoretically impact the quality of the SDM produced (Phillips et al. 2009). Whilst presence-only datasets can provide an important data source for SDMs, a critical assessment of the limitations and biases associated with these models is required in order to realise the full potential of these

methods (Elith et al. 2006).

A number of issues can affect the accuracy of presence-only modelling techniques such as MaxEnt, including geographic bias in the sample point dataset, which is a greater problem for presence-only models than for presence-absence models, since the bias afflicts presence data but not background data (Phillips 2008). Presence-absence models are not however without issues of bias or error in terms of the absence data. For example, absence records may be misleading as the species may not be easily detected or because they can include strong imprints of biotic interactions, disturbances or dispersal constraints that preclude modelling of potential distributions (Elith and Leathwick 2009; Elith et al. 2011). The presence-only datasets that are utilised for presence-only modelling are collated from a range of different sources and often records will have no known sampling scheme. A degree of spatial sampling bias is likely, however, the precise structure or extent of this bias is unknown (Ward 2007). These datasets are vulnerable to bias from a range of sources including:

- Sample points may be correlated to ease of access to a site (Phillips et al. 2006), such that areas that are hard to access are underrepresented within the dataset.
- Datasets may exhibit spatial-autocorrelation if the sample points are collected from a limited number of nearby sites within a restricted portion of the total area available for the study location (Phillips et al. 2006).
- Sampling intensity and/or methodology may vary significantly across the study area (Phillips et al. 2006).

If unaccounted for, spatial and temporal sampling bias within a dataset can affect the quality of the model produced, regardless of the modelling method used (e.g. Dudík et al. 2005; Phillips et al. 2009). When using only random background sampling with no assessment of the spatial bias, the majority of modelling methods will subsequently make predictions biased towards intensively sampled areas (Phillips et al. 2009). MaxEnt was developed to allow the user to account for such sampling bias through two pathways which help guide the training of the model in the presence of sampling bias (Wilson et al. 2009). The first allows a ‘bias density

map’ to be included with the input data, providing MaxEnt with a grid of cells that are weighted according to the degree of sampling bias that is expected to occur within that cell (Dudík et al. 2005). Model quality can be affected by the geographical scope of the region from which the set of random background points that are included during the creation of the model can be attained. If the geographical scope from which environments can be sampled is too broad, then the model may be over-fitted, and if it is too narrow, then the predicted distribution may be uninterpretable (Wilson et al. 2009). Providing a bias density map helps to define the optimal geographic scope from which the set of random background points can be selected, creating a similar spatial sample bias within the background data as is prevalent in the original presence-only dataset. Guidelines for the appropriate selection of background points are still a focus of active research for both MaxEnt and other modelling tools (e.g. Phillips et al. 2009). The second pathway allows the user to create a ‘target group’ (a defined group of species that are ecologically or behaviourally similar to the target species and for which locations show similar sampling bias) which is used to select the set of background points for use in the model creation (Phillips et al. 2009). This second pathway of bias adjustment is however, restricted for use with species or situations, where reasoned and justified arguments can be presented for the identification of species within the target group (Wilson et al. 2009).

### 3.2 Objectives

1. Collate information on the occurrence of *S. officinalis*, at all life stages, throughout the English Channel and store this information in a specialised geo-database.
2. Identify the environmental and physical drivers (predictor variables) of *S. officinalis* spawning distribution in the English Channel.
3. Create a model to produce predictive maps based on pre-selected predictor variables and known occurrence records of *S. officinalis* egg clusters, extracted from the geo-database.
4. Evaluate and assess the predictive capabilities of the model.
5. Identify how the information from the output of the model can be used for future conservation or fisheries management of this species.

## 3.3 Materials and methods

The creation of a SDM follows a number of steps that are undertaken in order to construct the model as outlined in Figure 3.2. As illustrated in Figure 3.2a, these steps include (i.) conceptualisation, (ii.) data preparation, (iii.) model fitting, (iv) model evaluation, (v.) spatial prediction, and (vi.) assessment of model applicability (Guisan and Zimmermann 2000; Guisan and Thuiller 2005). Where relevant these steps will be highlighted throughout this section and further details of the subphases involved are illustrated in Figure 3.2b. During the conceptual phase (Step i.), the concept behind the model is proposed, this includes defining the objectives of the model (Section 3.2), assessing which environmental predictors may be relevant for modelling the target species (for which a review of the literature was conducted) as well as an appreciation of the scale (both temporal and spatial) for which the model should be created (e.g. Section 3.3.1). In addition during the conceptual phase it is also necessary to identify the most appropriate method for modelling the response variable (Section 3.1.2).

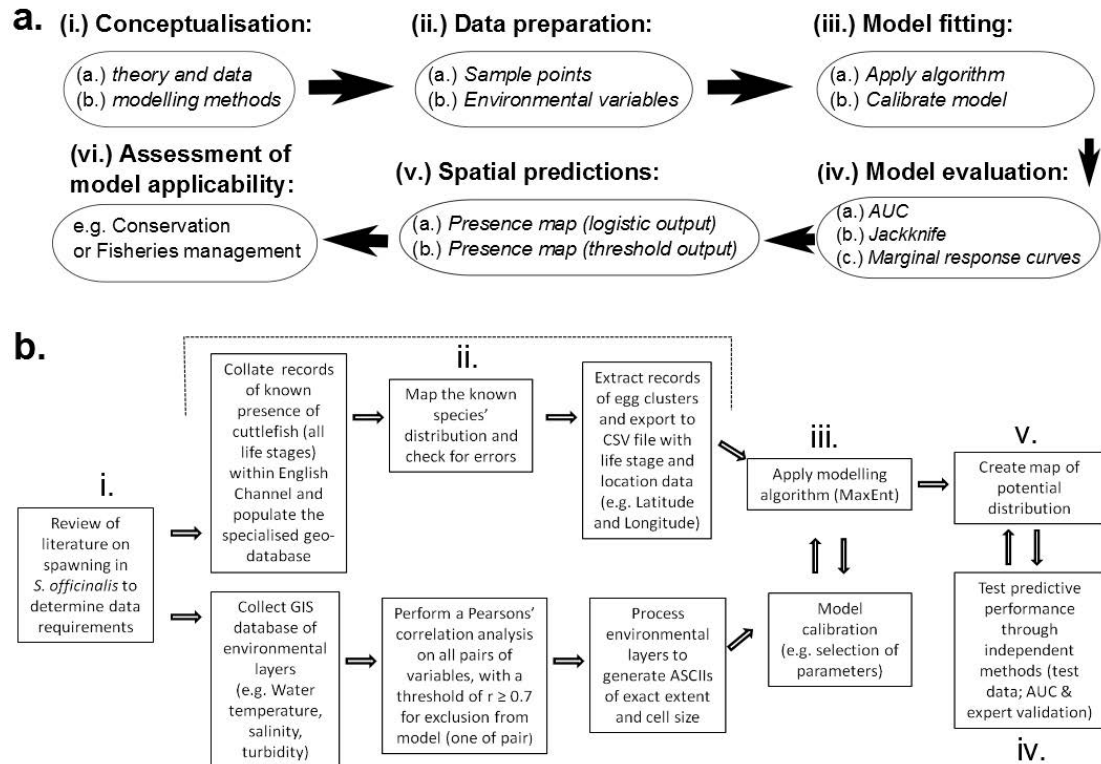


Figure 3.2: Steps in making a SDM in MaxEnt (adapted from (Pearson 2010; Guisan and Zimmermann 2000; Guisan and Thuiller 2005). These steps include (i.) Conceptualisation, (ii.) data preparation, (iii.) model fitting, (iv.) model evaluation, (v.) spatial prediction and (vi.) assessment of model applicability

### 3.3.1 Location (Step i.)

This study focused on the discrete spawning population that is known to occur within the English Channel. As such the location (or extent) of this study will extend across the entire area of the English Channel (approximately 51° 3'N, 1° 50' E and 48° 25'N, 5° 37'W).

The English Channel is a shallow epicontinental shelf system that extends over an area of 77,000 km<sup>2</sup> and is bordered by both the U.K. and France (Dauvin 2012). The Channel extends approximately 750 km from the Dover strait (east) to the Celtic Sea (west) with the deepest areas in the west, reaching 174 m in the deep central trench and the shallowest areas in the east, diminishing to 40 m at the Dover strait (Dauvin 2012). The sediment varies within the Channel with a predominance of pebble based substrates in the strong tidal currents that prevail offshore and fine or muddy sand in the weaker tidal currents of the inshore estuaries and bays (Dauvin 2012). The

Channel is considered a transitional area between the warm temperate waters of the Atlantic ocean system and the colder waters of the North Sea (Dauvin 2012). The distinction between the biological and physical features of the western and eastern Channel has led to it's consideration for many purposes as two separate basins, divided along a vertical line between Start Point on the U.K. coast and the Cotentin Peninsula on the French coast (Dauvin 2012). One of the reasons for the disparity in conditions between the two basins is the dominant influences on the hydrologic and oceanographic features of the areas, with the Eastern Channel affected predominately by the Seine Estuary and the Western Channel by Atlantic waters (Dauvin 2012).

#### 3.3.2 Species occurrence data (Step ii.)

A total of 217 sample points of presence records for *S. officinalis* egg clusters was collated and stored within an ArcGIS geo-database (Esri, Version 10) (Figure 3.3). These records were obtained from five main sources which included:

- Fisheries surveys data (e.g. Centre for Environment, Fisheries and Aquaculture Science [CEFAS] and French Research Institute for Exploration of the Sea [IFREMER]), where cuttlefish are not the target species, but information has still been collected on their presence and abundance
- Bibliographic records (e.g. current and historical published literature)
- Current research (e.g. Cephalopod Recruitment from English Channel Spawning Habitats [CRESH]. subtidal surveys)
- Historical data archives (e.g Data Archive for Seabed Species and Habitats [DASSH]; see Appendix C.1 for details)
- Current sightings scheme (e.g. Cuttle-Watch) (see Appendix C.2 for further details)

The data set was extracted from the geodatabase and saved as a comma-delimited (CSV) file with the life stage and associated latitude and longitude coordinates extracted for each record, ready for incorporation into the MaxEnt software. Records of eggs, rather than records of

### 3.3. MATERIALS AND METHODS

spawning adults, were used as a basis for the model as eggs represent a true measure of spawning. Presence data included a total of 217 records of *S. officinalis* eggs within the English Channel area from 1995-2012, and between March and September. The data was further subdivided by MaxEnt into two randomly allocated data sets, a larger training data set (163 records) and a smaller test data set (54 records).

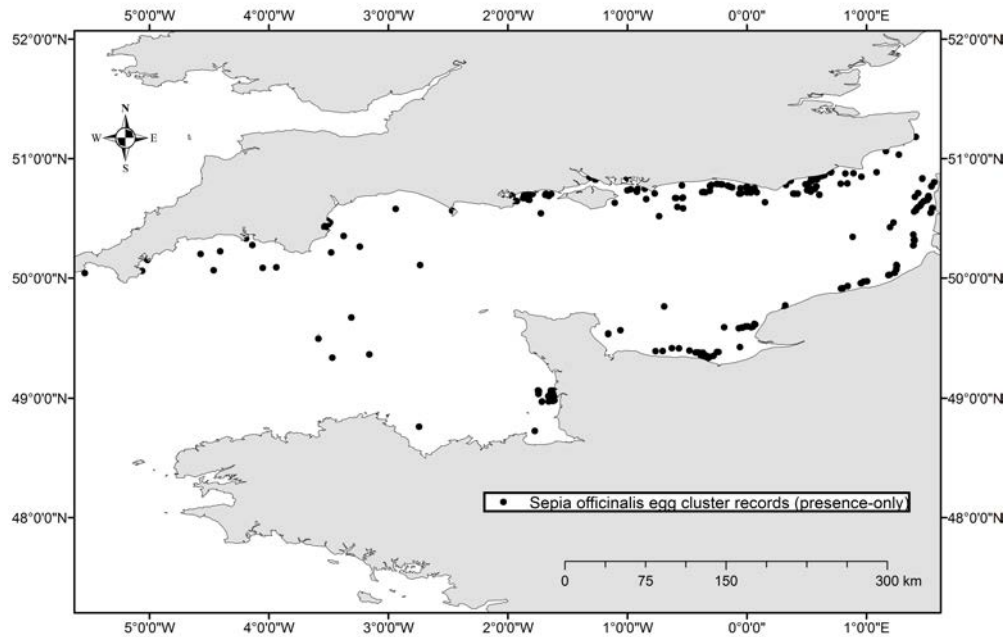


Figure 3.3: A map showing the locations of the 217 sample points for *S. officinalis* egg occurrence within the English Channel (March to September, 1995-2012)

#### 3.3.3 Environmental predictor variables (Step i. and ii.)

A review of the literature concerning spawning in *S. officinalis* was undertaken to ascertain which environmental and physical variables were ecologically relevant for use as predictor variables within the model (see Chapter 2). The list of variables highlighted included both categorical and continuous forms of data such as: sediment type, depth, distance from coastline, sea surface temperature, sea bottom temperature, sea surface salinity, productivity, thermal fronts, turbidity, current velocity and habitat type.

Of the variables highlighted in the literature a set of eight were selected to incorporate this information into the model, based on knowledge of the species life cycle in the English Channel,



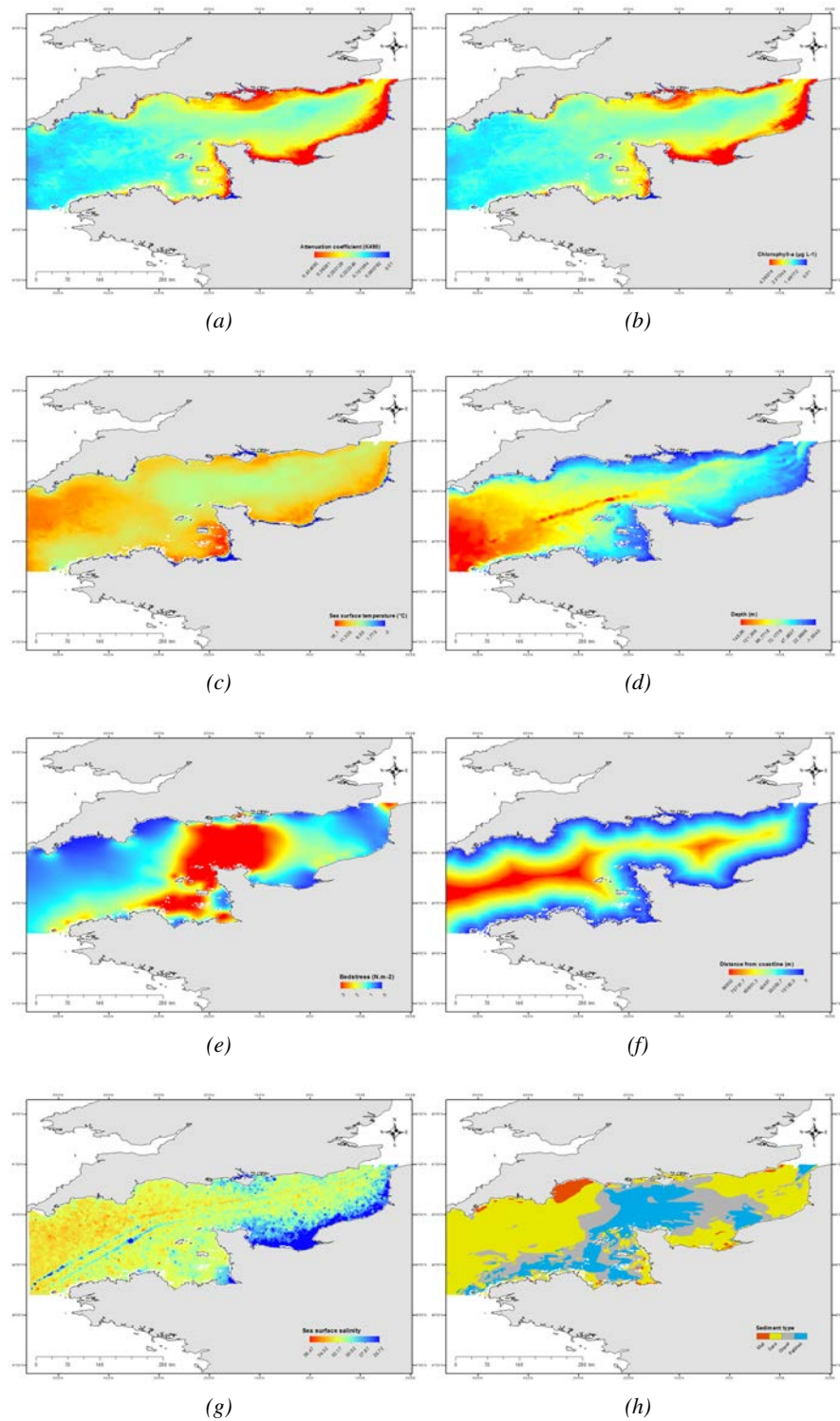
and/or accessibility of data layers at resolution and quality high enough for useful inclusion (Figure 3.4):

- Attenuation coefficient K<sub>490</sub> (representing turbidity).
- Chlorophyll -a (representing turbidity and primary productivity).
- Sea surface temperature (SST) [known to be correlated with sea bottom temperature (Wang et al. 2003)].
- Depth (bathymetry plus mean sea level).
- Bed shear stress (representing current flow and sediment type).
- Distance from coastline.
- Sea surface salinity.
- Sediment type

#### 3.3.3.1 Collinearity among predictor variables

Prior to running the model with all eight pre-selected predictor variables, it was first necessary to assess the degree of collinearity among them. It is not recommended to use highly correlated variables within the MaxEnt modelling process and so in this study, Pearson's correlation was used to assess collinearity among the variables. To avoid issues of colinearity, only variables with a Pearson correlation between -0.7 and 0.7 were included (Ommen Kloeke et al. 2012). If variables had a Pearson correlation greater than these values one of the pair of variables was excluded from the model construction phase.

In order to assess whether collinearity among variables did exist, the data were extracted from the raster layers using ArcGIS. An *environmental grid* consisting of 132492 points was created which covers the whole extent of the study area. The environmental grid was used to extract point data for each of the predictor variables in order to perform correlation analysis. Extraction was performed using the ArcGIS tool 'Extract multi values to points' (ArcGIS Toolbox -> Spatial Analyst Tools -> Extraction -> Extract Multi Values To Points). Following extraction



*Figure 3.4:* Maps showing the data for each of the eight predictor variables used in the MaxEnt model, (a) Attenuation coefficient K490, (b) Chlorophyll-*a*, (c) Sea surface temperature, (d) Depth, (e) Bed shear stress, (f) Distance from coastline, (g) Salinity, (h) Sediment

of the data from each variable to the environmental grid, the data within the grid was saved and exported to a Microsoft Excel file (Microsoft 2007) where the Pearson's correlation function was used to calculate the degree of correlation between each pair of variables.

#### **3.3.3.2 Preparation of environmental layers**

The sourcing and pre-processing of data layers is summarised in Table 3.3. For all variables with a temporal element (SST, Chlorophyll-*a* and K490) long-term averages (median) were assembled over the period 2000-2010 and incorporating the months when eggs were recorded as being present in the English Channel (March to September). This was done as MaxEnt does not have the ability to include time as a variable in this way. Long-term averages were created from the weekly composite data which were combined to create monthly and seasonal long term averages (median) in ArcGIS using the 'Cell statistic tool' (Spatial analyst -> Local -> Cell statistics), seasonal averages were then combined for each year in order to produce a single layer.

To execute the model, data layers (saved in ASCII format) for all predictor variables were converted to the same resolution (cell size), projection system and clipped to the same pixel extent to ensure data availability for every cell and to provide MaxEnt with information on the modelling extent. Data layers were processed using spatial analyst tools.

Table 3.3: A list of predictor variable datasets obtained for use in the MaxEnt model together with a description and source

Parameter	Sensor/Model	Units	Resolutions	Years	Source
Sea surface Chlorophyll-a	MERIS	mg mm <sup>-3</sup>	1 km	2002 to 2010	NEODAAS
Sea surface Temperature	AVHRR	°C	1 km	2000 to 2010	NEODAAS
Attenuation Coefficient (K490)	MERIS	m <sup>-1</sup>	1 km	2002 to 2011	NEODAAS
Depth	SHOM and MARS	m	1 km	-	CHARM III Sextant (Carpentier et al. 2009)
Substrate type	-	-	0.009	-	(Larsonneur et al. 1979)
Sea surface salinity	-	-	Point data	1981 to 2012	ICES Oceanographic Data Centre
Bed shear stress	POL	N.m <sup>-2</sup>	1 km	-	CHARM III Sextant (Carpentier et al. 2009)
Distance from coastline	-	m	0.009	-	ArcGIS (ESRI V.10)

**3.3.3.2.1 Attenuation coefficient, K490 (March - September)** These data were obtained from NEODAAS (National Environment Research Council Earth Observation Data Acquisition and Analysis Service; [www.neodaas.ac.uk](http://www.neodaas.ac.uk)) and were generated from MERIS (MEdium Resolution Imaging Spectrometer) satellite/sensor with a spatial resolution of 1 km. The data set consists of 1359 weekly composite images (layers) for a period of 10 years between 2002 and 2011. The data were received in Geo-Tiff format having already been processed for cloud cover and in digital number format which required extraction to real-world values (range 0.01 to 7.08  $\text{m}^{-1}$ ) prior to use in the model. Data extraction (SST, Chlorophyll-*a* and K490) were performed in ArcGIS and was set up to run semi-automatically using the *Model Builder* application to create a tool to extract the data.

**3.3.3.2.2 Chlorophyll-*a* concentration (March - September)** The Chlorophyll-*a* data were also obtained from NEODAAS and were generated from the MERIS satellite with a spatial resolution of 1 km. The data set consists of 1467 weekly composite images (layers) for a period of 9 years between 2002 and 2010. All data were received in digital number format and required extraction to real-world values (range 0.01 - 66.8  $\text{mg m}^{-3}$ ) prior to use in the model.

**3.3.3.2.3 Sea surface temperature, SST (March - September)** SST ( $^{\circ}\text{C}$ ) data were also obtained from NEODAAS and were generated from the AVHRR (Advanced Very High Resolution Radiometer) sensor on-board NOAAs (National Oceanic and Atmospheric Administrations) satellite platform with a spatial resolution of 1.1 km. The data set consists of 1804 weekly composite images (layers) for a period of 11 years between 2000 and 2010. All data were received in digital number format and required extraction to real-world values (range -3 to 22.5 $^{\circ}\text{C}$ ) prior to use in the model.

**3.3.3.2.4 Depth** Depth data were obtained from the CHARM habitat atlas via the Sextant portal [www.ifremer.fr/sextant/en/web/charm/geocatalogue](http://www.ifremer.fr/sextant/en/web/charm/geocatalogue) and consists of bathymetry plus mean sea level, considered to be of greatest ecological value, and supplied at a spatial resolution of 1  $\text{km}^2$  (Carpentier et al. 2009). The depth layer was created from a combination of bathymetric data derived from SHOM (Service Hydrographique et Oceanographique

de la Marine) navigation charts and mean sea level data estimated with the MARS (Model for Applications at Regional Scales) 3D hydrodynamic model (Le Roy and Simon 2003) the data were then interpolated using ArcMap in order to create continuous raster layers with a spatial resolution of 1 km<sup>2</sup>, before both data layers were summed using the raster calculator in ArcGIS to create a finalised depth layer (e.g. bathymetry plus mean sea level) (Carpentier et al. 2009).

**3.3.3.2.5 Bed shear stress** For the purpose of this model, bed shear stress was obtained from the CHARM habitat atlas via the Sextant portal and is based on the mean M2 tidal current on the bottom at a spatial resolution of approximately 8 km cell size which is estimated from the 2D hydrodynamic model of the north-west European shelf developed at Proudman Oceanography Laboratory (POL) (Carpentier et al. 2009). Bed shear stress was then calculated as a function of the maximum predicted tidal current and a bed friction coefficient (Carpentier et al. 2009). The raster layer was then interpolated to create a continuous layer of 1 km<sup>2</sup> resolution (Carpentier et al. 2009).

**3.3.3.2.6 Distance from the nearest coastline** A direct distance function was used to calculate the ‘Distance from the nearest coastline’ which represents the distance of every pixel to a shapefile of the land (UK and France). This data layer was created using the ‘Euclidean Distance’ tool in ArcGIS Spatial Analyst.

**3.3.3.2.7 Sea surface salinity** The data for sea surface salinity (Figure 3.4g) were downloaded from the International Council for Exploration of the Seas (ICES) website for surface data which is part of the ICES Oceanographic Data Centre <http://ocean.ices.dk/data/surface/surface.htm>. The data were download as CSV files which were imported into ArcGIS as XY feature layers. Once imported the relevant data points within the model location were extracted and a new point shapefile created. The individual point data shapefiles were then merged to create a single file, using the ‘Merge’ tool in ArcGIS. This point shapefile was then interpolated to a raster data layer (cell size [resolution] 0.009) using the ‘Inverse Distance Weighting (IDW)’ interpolation tool. The temporal data range of this data set varies from 1891 to 2012.

**3.3.3.2.8 Sediment** The data layer on seabed sediments used in this model was obtained from a digitised version of the ‘Larsonneur Map’ (Larsonneur et al. 1979). This layer was originally obtained in vector format and so was converted in ArcGIS to a Raster. Only the four main categories of seabed sediments (pebble, gravel, sand and mud) were included.

#### **3.3.4 Non-spatial analysis**

A non-spatial analysis of the data was performed prior to running the model in MaxEnt. This was done by extracting the data for each of the predictor variables to each of the sample points in ArcGIS. The extracted data were then exported to Excel (Microsoft, 2010) and the range and averages for each variable assessed to provide an indication of the values within which spawning occurs in the English Channel. These data were then compared with the data produced as part of the model output.

#### **3.3.5 MaxEnt pre-run settings (Step iii.)**

##### **3.3.5.1 Background points**

In addition to the presence-only sample points that are provided for input to MaxEnt, a sample of ‘background points’ were also required by the software program to provide a summary of the environmental conditions within the landscape against which the observed presences can be compared. These background points are created by MaxEnt during model building and can include locations of sample points where the species is known to occur as well as unsampled areas where the presence of the species is undefined. MaxEnt was used to create 10,000 randomly selected background points.

As described in Section 3.1.4.2, the presence-only datasets like that used within this study are of unknown sampling method and the spatial bias associated with this dataset is unknown. Whilst the background data are drawn at random from the entire region, occurrence data are often spatially biased. Since spatial bias often results in environmental bias, spatial sampling bias can affect the quality and accuracy of the model produced (Phillips et al. 2009). For example, if locations are only sampled within the 0 to 10 m depth range then this may cause the model to overfit the data to this region of the study area. By providing MaxEnt with a set of background

data that has the same bias as the occurrence data, it is possible to correct this bias estimation (Phillips et al. 2009). However, accurate information about spatial bias is usually lacking, so explicit biased sampling of background sites may not be possible, but by providing MaxEnt with a bias density map that contains a grid of cells that are weighted according to the degree of sampling bias that it is expected occurred within that cell (e.g. Dudík et al. 2005) it is possible to correct the bias at least in part.

For this purpose, a bias file was created and uploaded prior to running the model. The bias grid was created in ArcGIS and provided MaxEnt with a grid that contains a value for each cell in the modelling extent (in a similar format to the environmental data) following the methodology of Tingley and Clements (2011). The bias file is used by MaxEnt during training and must contain only positive values with the ratio of values in any two cells representing the relative sampling effort. The bias density map was uploaded as an optional bias file within the pre-run settings of MaxEnt.

#### **3.3.5.2 Random test percentage**

To evaluate model accuracy MaxEnt was used to randomly partitioning the original dataset into two independent data sets. The *training dataset* is used to build the model and comprises 75 % of the sample points (163 records). The *test dataset* comprises 25 % (54 records) of the data and is used to test the models accuracy (Pearson 2010). MaxEnt was used to partition the original dataset randomly into two, prior to building the model (Pearson 2010). This partitioning was done in the pre-run settings by specifying the ‘random test percentage’ as 25, thereby informing the software that 25% of the sample points should be set aside for use in the test dataset.

In addition, MaxEnt has the capacity to run multiple model generations from the same dataset within a single processing session, since each MaxEnt models are nondeterministic, each run (or replicate) will have a slightly different output, setting replicates will therefore allow an average model of all the replicates (as well as a model for each individual replicate) to be created, as well as the standard deviation in order to provide an approximation of the variability within the replicate set. A set of 15 replicates was considered sufficient to reduce any spurious effects from model. The random seed option was used to allow a different training and test partition to be



created for each run. The user is also required to specify which of three sampling techniques they wish to be used for the replicate runs: *crossvalidation*, which partitions the dataset into a number of equal sections and for each run one replicate is used for the test dataset and the remaining for the training dataset; *Bootstrapping*, which is sampling with replacement, which means that for each replicate run, the test data were sampled from the dataset with replacement so that the same point may be selected multiple times within each test dataset; *subsampling*, is sampling without replacement, which means that for each replicate run, the test data were sampled from the dataset without replacement so that each point may be selected only once within each test dataset. For the purpose of this study subsampling was used as the replicate run type.

#### 3.3.5.3 Other settings

For the purpose of this MaxEnt model, the remaining settings for the maximum number of iterations, which defines the time the model has to converge (in the form of number of iterations) and the regularisation, which controls the amount of smoothing that can occur within the model, were left at their default values of 500 and 1 respectively.

#### 3.3.6 MaxEnt model output and evaluation (Steps iv. and v.)

##### 3.3.6.1 Predictive habitat suitability maps

The main output from MaxEnt is a predictive distribution map. Version 3.0 and above of MaxEnt has a feature which transforms the exponential function into a logistic function to represent probability of presence, it is scale independent and is calibrated so that a typical presence point has a value of 0.5 on a scale of 0 to 1 (represented using a linear scale) Phillips (2008). The predictive distribution maps are therefore produced as a logistic output, where each cell has a probability estimate of between 0 and 1 that represents the likelihood (or probability) of *S. officinalis* spawning within that cell. A predicted probability close to 0 indicates that the environmental conditions within that cell are not suitable for spawning, whilst a predicted probability close to 1 indicates that the environmental conditions are suitable for spawning. A logistic output map is produced for each individual replicate with average, median, minimum, maximum and stan-

dard deviation maps also produced based on the results from the entire replicate set. The output maps are saved by MaxEnt in ascii format and can be viewed in ArcGIS following conversion to raster format. The predictive maps use colour to reflect the predicted probability that the environmental (and physical) conditions are suitable across the predetermined geographic location. The warmer colours (e.g. red) indicate a high probability that the conditions at that location are suitable for cuttlefish spawning to occur, whilst the cooler colours (e.g. blue) indicate a low predicted probability that the conditions at the location are suitable for cuttlefish spawning to occur (Phillips 2010).

To aid model interpretation, a binary map was produced to distinguish between ‘suitable’ and ‘unsuitable’ areas. This was done by setting a decision threshold above which the output is considered to be a prediction of presence (or suitable habitat). A wide range of approaches have been employed for setting decision thresholds (Liu et al. 2005) and to date there are no set methods to ascertain the most appropriate method and in general this will depend on the objective of the maps. MaxEnt computes a range of threshold values as part of the model output that include the minimum training presence logistic threshold, the 10 % training presence logistic threshold and the equal training sensitivity and specificity threshold. For this study a liberal approach was taken with the 10 % minimum threshold used to define the minimum probability value for suitable habitat. Using this threshold, suitable habitat is defined using 90 % of the data that was used to develop the model, if it was certain that the data used to create the model was error free then a minimum threshold of a lower value (e.g. 5 %) could be used. The threshold value produced in the MaxEnt output file is then used to adjust the model classification within ArcGIS so that the final map produced has only two classifications, representing unsuitable and suitable habitat.

#### **3.3.6.2 Analysis of Variable contributions**

Another output of MaxEnt is an estimation of the percentage contribution and permutation importance for each of the predictor variables. This allows the user to analyse which variables are contributing the most to the creation of the model and are therefore important for determining the distribution of the modelled species. During training of the MaxEnt model an assessment

is made as to which of the predictor variables are contributing most to the fitting of the model. This is done by monitoring the changes in gain that are made during each step of the MaxEnt algorithm when the coefficients for a single feature are modified, the increase in gain is then assigned to the environmental variable or variables that the modified feature depends on. At the end of the training process the total values for each environmental variable are then converted to percentages to provide an estimation of the contribution of each variable to the model. These values of percentage contribution are heuristically defined (dependent on the particular pathway used by the algorithm to obtain the optimal solution) and may vary between replicate runs. An average value is therefore taken across the entire replicate set for use in the final analysis.

During the training process a second measure of variable contribution is also calculated that is termed as permutation importance. In contrast to the percentage contribution value, the value for permutation importance is dependent only on the final MaxEnt model, not the pathway that the algorithm used to generate it. Permutation importance is determined for each predictor variable by random permutation of the values of that variable among the training points (both presence and background) and measuring the resulting decrease in training AUC. A large decrease in the training AUC indicates that the model depends heavily on that variable and a high permutation importance is generated.

#### **3.3.6.3 Jackknife**

In addition to the analysis of variable contributions (percentage contribution and permutation importance) that are calculated, it is also possible to determine the importance of each predictor variable to the model using a jackknife test that is performed within the MaxEnt software. The jackknife test provides two separate measures, firstly it tests the gain of the model when a single variable is excluded in turn and the model created using the remaining variables. Secondly it tests the gain of the model using only that variable in isolation. A model with all variables included is also run in order to provide a control gain with which to compare the results. The jackknife test therefore allows the user to determine the overall improvement in gain and loss of gain in a model when each individual variable is either included or excluded (Phillips and Dudík 2008). The results of these models are displayed in a series of three bar charts (training,

test and AUC) as part of the model output.

*Gain* is a term closely related to that of *deviance* which is commonly used in GLMs and GAMs to measure the goodness of fit (Phillips 2010). Within the context of MaxEnt, gain indicates how closely the model concentrates around the presence samples (Phillips 2010). At the beginning of the model run, the gain starts at 0 and increases during the run to an asymptote (Phillips 2010). In mathematical terms the gain is defined as the average log probability of the presence samples, minus a constant that makes the uniform distribution have zero gain (Phillips 2010). During model training MaxEnt calculates the contribution of each of the predictor variables to the model by measuring the gain. Whilst progressing through the MaxEnt algorithm, modifications to the single feature coefficients are made in order to increase the gain of the model, the variable that the feature depends on is then assigned this increase in gain. At the end of the training process, the gain assigned to each variable is then converted into percentages and the results presented in table format (Phillips 2010).

#### 3.3.6.4 Marginal response curves

The marginal response curves show how the MaxEnt prediction is affected by each of the predictor variables. This is done by displaying how the logistic prediction (y-axis) changes as each target predictor variable is varied (x-axis), whilst all other variables remain constant (at their average sample value).

## 3.4 Results

### 3.4.1 Non-spatial analysis

The data collated on egg cluster presence records indicate that within the English Channel, potentially suitable habitat areas for *S. officinalis* spawning occur within a set range of conditions for each of the predictor variables (Table 3.4). These include a weak bed shear stress, sea surface temperatures of 10 °C and above, shallow water depths of between 0 to 30 m, soft sediment types (e.g. sand and mud), a salinity of between 34.5 to 35.5, low Chlorophyll-*a* and K490 levels, and within close proximity of the coastline (2,000 to 12,000 m).

### 3.4. RESULTS

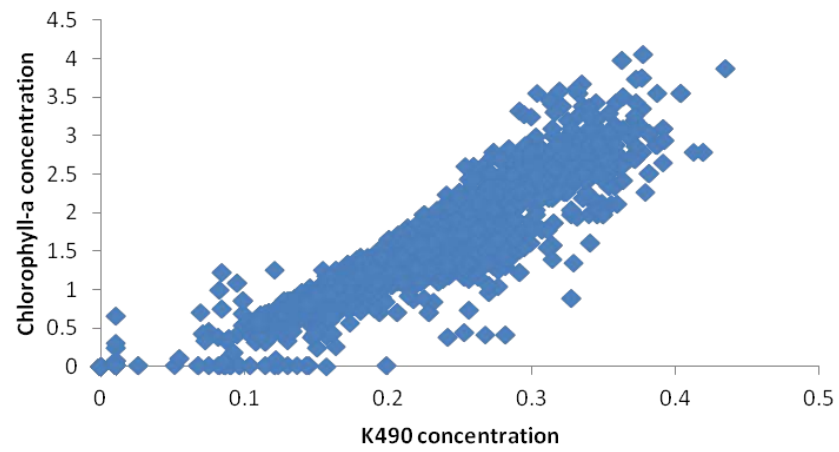
*Table 3.4:* Environmental limits for spawning in the common cuttlefish *S. officinalis* in the English Channel based on the outputs of the non-spatial model (NSM) and spatial model (SM) created within this study

Predictor variable	<i>S. officinalis</i> (NSM)	<i>S. officinalis</i> (SM)
Months	March to September	March to September
Bed shear stress	0.4 to 1.4 N.m <sup>-2</sup>	0.39 to 0.75 N.m <sup>-2</sup>
SST	10 to 19 °C	14 to 18 °C
Depth	0 to 30 m	4 to 23 m
Substrate type	sand and mud	-
Salinity	34.5 to 35.5	34.6 to 35.2
Chlorophyll- <i>a</i>	0.5 to 3.0 mg m <sup>-3</sup>	1.1 to 2.2 mg m <sup>-3</sup>
K490	0.1 - 0.4 m <sup>-1</sup>	-
Distance from coastline	2.0 to 120 km	2.2 to 121 km

#### 3.4.2 MaxEnt model

##### 3.4.2.1 Collinearity among predictor variables

From all the pairs of variables analysed using the Pearson's correlation coefficient, only two pairs of variables were found to have a value greater than the threshold values of between  $r = -0.7$  to  $0.7$ . After this level there is considered to be collinearity between the variable pair and one of the two correlated variables was excluded, as specified previously in Section 3.3.3.1. These pairs included chlorophyll-*a* and attenuation coefficient K490 (Figure 3.5) which had a Pearson's correlation coefficient of 0.95 and sediment type and bed shear stress which had a Pearson's correlation coefficient of 0.79. Attenuation coefficient K490 and Sediment type were both excluded from the MaxEnt model so as to avoid issues with collinearity. The results of all the Pearson's correlation coefficient analysis are presented in Table 3.5.



*Figure 3.5:* Correlation between Chlorophyll-*a* concentration and the attenuation coefficient (K490) within the English Channel

### 3.4. RESULTS

Table 3.5: Pearson correlation coefficient analysis among pairs of predictor variables

Predictor variable pairs	Pearson correlation coefficient	Above exclusion threshold
K490&SST	0.038	No
<b>K490&amp;Chl-<i>a</i></b>	<b>0.949</b>	<b>Yes</b>
K490&Sed	0.047	No
K490&Sal	-0.040	No
K490&Dist	-0.490	No
K490&Depth	-0.551	No
K490&Bstress	0.050	No
SST&Chl- <i>a</i>	0.046	No
SST&Sed	-0.017	No
SST&Sal	0.355	No
SST&Dist	0.184	No
SST&Depth	0.173	No
SST&Bstress	-0.124	No
Chl- <i>a</i> &Sed	-0.007	No
Chl- <i>a</i> &Sal	-0.106	No
Chl- <i>a</i> &Dist	-0.458	No
Chl- <i>a</i> &Depth	-0.520	No
Chl- <i>a</i> &Bstress	-0.016	No
Sed&Sal	0.089	No
Sed&Dist	-0.078	No
Sed&Depth	0.015	No
<b>Sed&amp;Bstress</b>	<b>0.791</b>	<b>Yes</b>
Sal&Dist	0.193	No
Sal&Depth	0.212	No
Sal&Bstress	0.045	No
Dist&Depth	0.517	No
Dist&Bstress	-0.082	No

#### 3.4.2.2 Presence maps

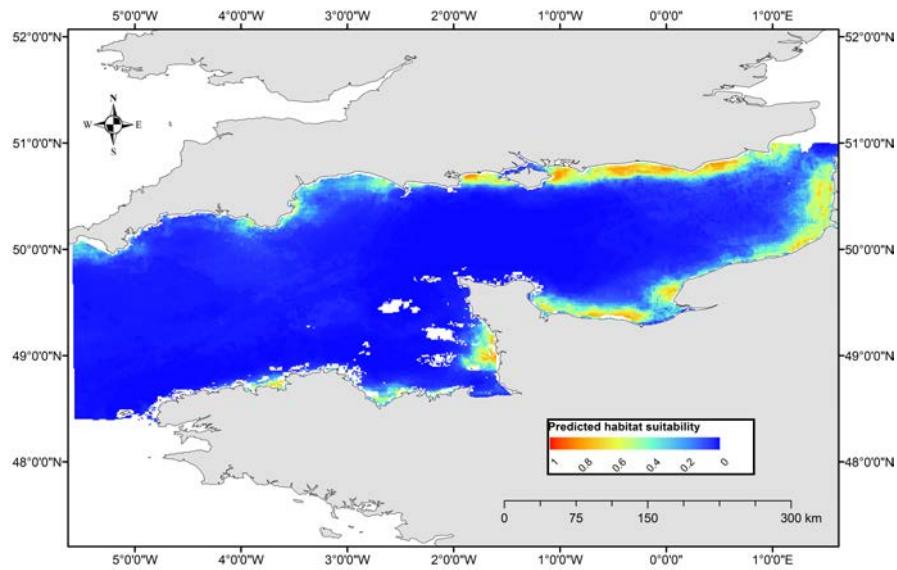
The presence maps created from the MaxEnt model (Figure 3.6) show the areas within the English Channel that have suitable conditions predicted for *S. officinalis* spawning. Figure 3.6a illustrates the average predictions (based on all 15 replicates) on a logistic scale from 0 to 1, with warmer colours (e.g. red (1)) showing areas with better predicted conditions than cooler colours (e.g. blue (0)).

Figure 3.6b illustrates the average predictions (based on all 15 replicates) following adjustment to the ten percentile training presence logistic threshold (0.182) with the output data divided into two categories: areas of suitable habitat (above 0.182) and areas of unsuitable habitat (below 0.182).

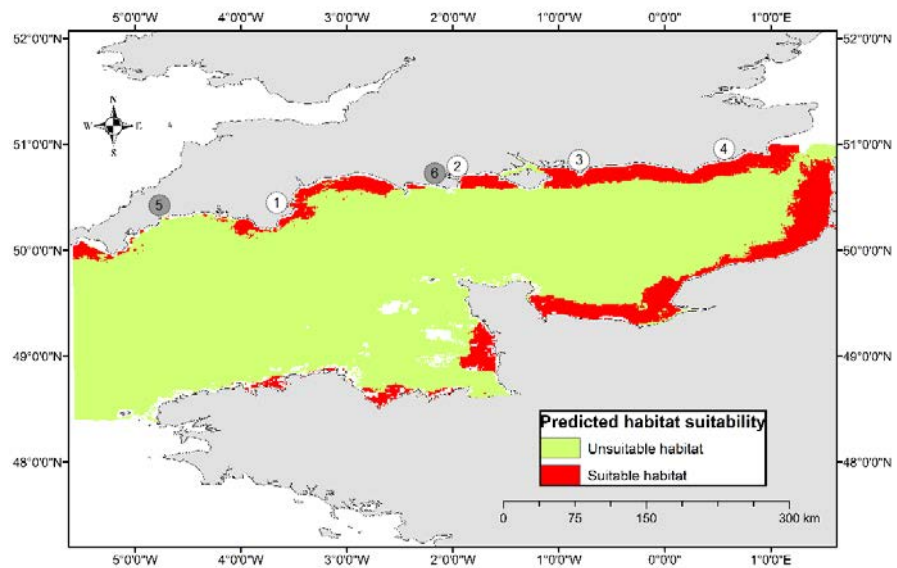
To investigate the prediction in more detail the U.K. coast is taken as an example. Here it is evident that a large portion of the inshore area has been predicted as suitable, with a larger proportion in the east of the Channel than the west (Figure 3.6b) for cuttlefish spawning to occur. The map highlights several areas along this coastline as suitable for spawning, where major inshore cuttlefish trap fisheries are known to occur (labelled as 1 - Torbay, 2 - Poole, 3- Selsey and 4- Hastings on Figure 3.6b). However, there are also several areas along this coastline which the map has highlighted as unsuitable for cuttlefish spawning and where major inshore cuttlefish trap fisheries do not occur (labelled as 5 - St Austell, 6- West Lullworth area, Figure 3.6b). In addition the entire offshore, deep water area in the centre of the English Channel has also been predicted as unsuitable for cuttlefish spawning.



### 3.4. RESULTS



(a)

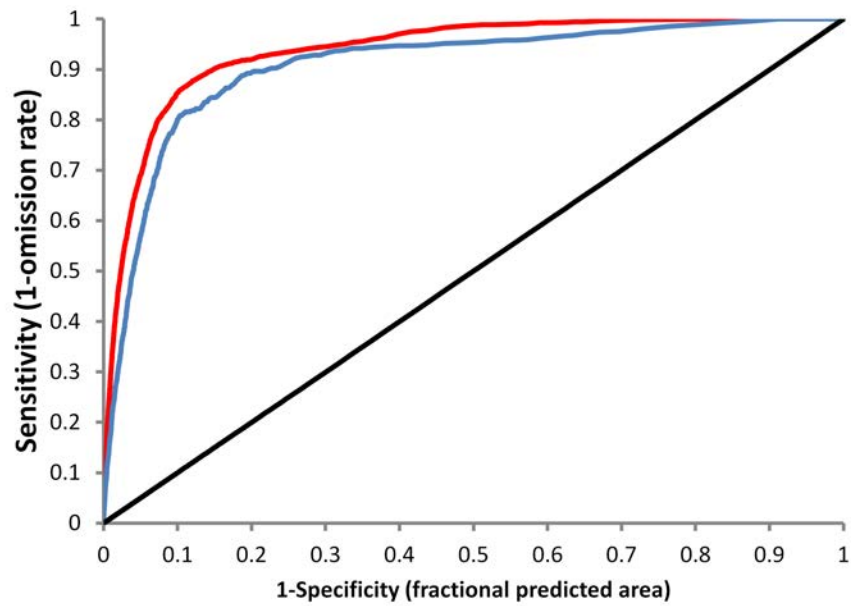


(b)

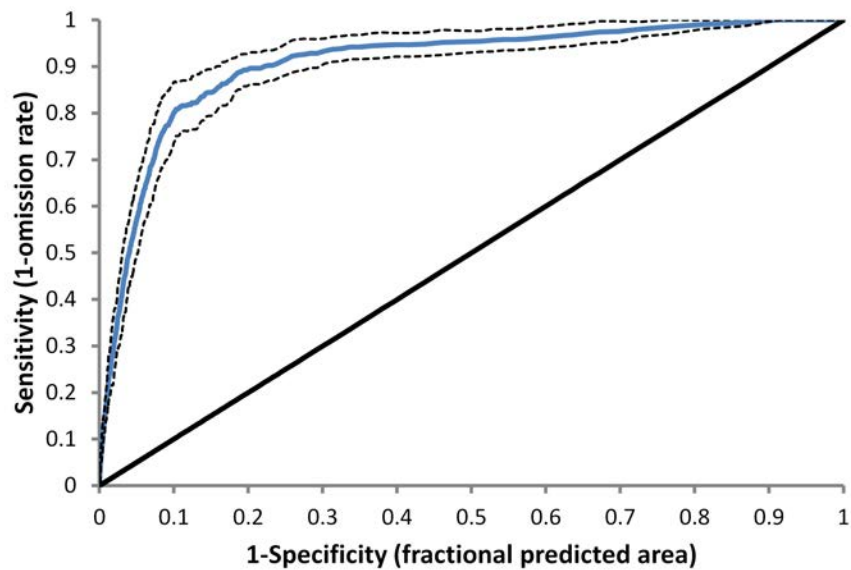
Figure 3.6: Predicted habitat suitability map for *S. officinalis* spawning distribution within the English Channel. (a.) Logistic output, presence predicted from 0 to 1. Warmer colours (e.g. red and orange) indicate a high probability that the conditions at that location are suitable for spawning to occur. Cooler areas (e.g. blue and green) indicate a low predicted probability that the conditions at the location are suitable for spawning to occur. (b.) Binary output, predictions were classified into two categories 'unsuitable habitat' and 'suitable habitat' using a threshold of 0.182 as specified by the ten percentile training presence logistic threshold. The map highlights several areas along the UK coastline as suitable (1 - Torbay, 2 - Poole, 3- Selsey and 4- Hastings) and unsuitable (5 - St Austell, 6- West Lullworth area) for spawning

#### 3.4.2.3 Model evaluation

**3.4.2.3.1 Area under the receiver operating characteristics curve (AUC)** Figure 3.7a illustrates the receiver operating characteristics (ROC) curves for both training and test data. The red line represents the fit of the model to the original training data, whilst the blue line represents the fit of the model to the testing data and is a good indicator of the models predictive power. The area under the ROC curve (AUC) value (averaged over 15 replicates) for the training data was 0.938 (SD  $\pm$  0.005) and the AUC value for test data was 0.909 (SD  $\pm$  0.017) (Figure 3.7a), which is higher than by chance (AUC =0.5). Figure 3.7b illustrates the ROC curve (averaged over 15 replicates) for the test data, plus and minus one standard deviation, in order to indicate the variability. According to the assessment in Table 3.2 both the training and test AUC values indicate excellent predictive ability of the model.



(a)



(b)

Figure 3.7: Receiver operating characteristic (ROC) curve averaged over 15 replicate runs for both training and test data. (a.) ROC curve for both training and test data. Training data (red line) has an area under the ROC curve (AUC) of 0.938; Test data (blue line) has an AUC of 0.909; Random prediction (black line) has an AUC of 0.5. (b.) ROC curve for test data, with 1 standard deviation shown (black dotted lines)

**3.4.2.3.2 Predictor variable importance** The relative contributions of the predictor variables to the MaxEnt model are indicated in Table 3.6. The variable with the highest percentage contribution was distance from coastline with 27.5 %, closely followed by depth (23.9 %), chlorophyll-*a* concentration (22.6 %) and bed shear stress (20 %). However, whilst distance from coastline, depth and bed shear stress all have accompanying high permutation importance (36.5 %, 23.8 % and 33.6 % respectively), chlorophyll-*a* has the lowest permutation importance of all the variables with a value of only 1.4 %.

The remaining two variables (sea surface salinity and sea surface temperature) both have a low percentage contribution (4 % and 1.9 % respectively) and a low permutation importance (2.5 % and 2.3 % respectively).

Table 3.6: Relative contributions of the predictor variables to the MaxEnt model

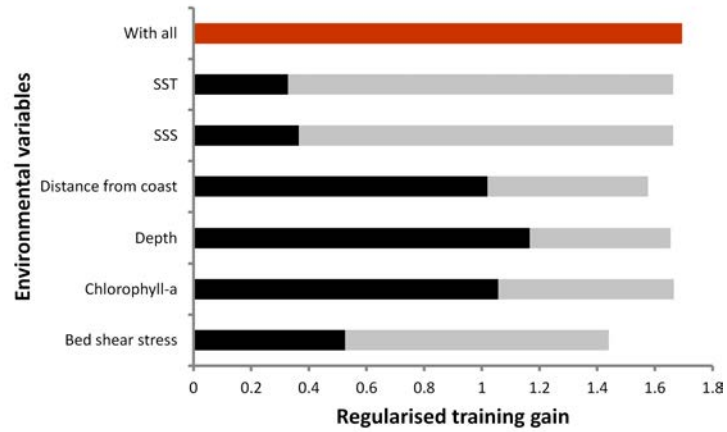
Variable	Percentage contribution	Permutation importance
Distance from coastline	27.5	36.5
Depth	23.9	23.8
Chlorophyll- <i>a</i>	22.6	1.4
Bed shear stress	20	33.6
Sea surface salinity	4	2.5
Sea surface temperature	1.9	2.3

**3.4.2.3.3 Jackknife** The results of the jackknife test are displayed within a series of three bar charts (Figure 3.8). In Figure 3.8a, the predictor variables with the highest regularised training gain when used in isolation were depth (gain = 1.17), chlorophyll-*a* (gain = 1.06) and distance from coastline (gain = 1.02) (longest black bars in Figure 3.8a), indicating that in isolation, these variables provide the most useful information for predicting the presence of *S. officinalis* spawning, with a good fit to the training data. In addition, the predictor variable that decreases the training gain most when removed from the model was bed shear stress (shortest grey bar in Figure 3.8a), indicating that this variable may contain the most information that is not present within the other variables.

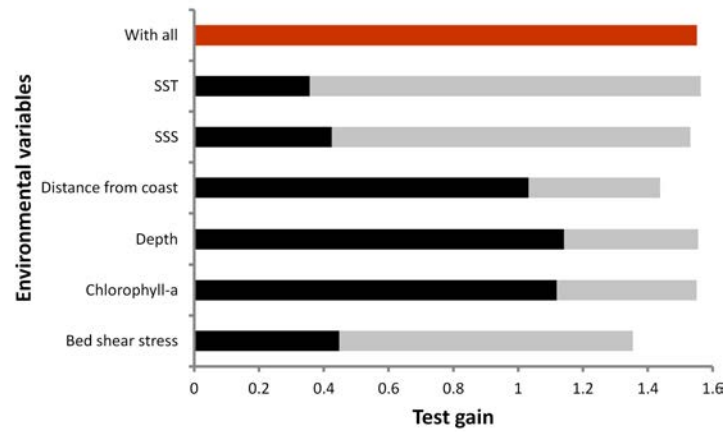
### 3.4. RESULTS

---

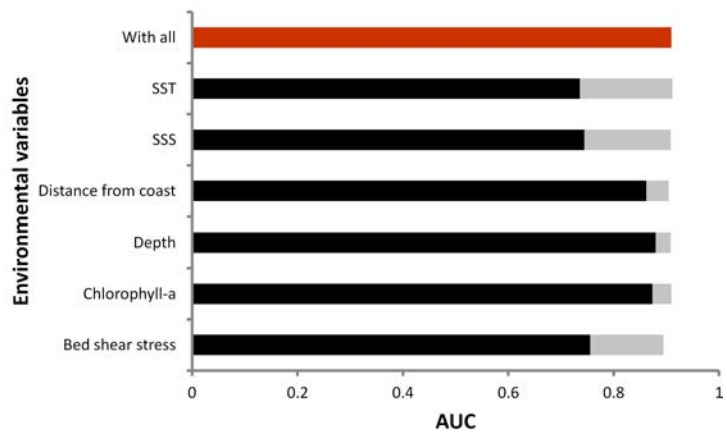
A comparison of the three bar charts is useful for exploring the model further, for example for this model, depth, chlorophyll-*a* and distance from coastline have the highest gain under both training and test conditions, when used in isolation, suggesting that these predictor variables might be the most transferable between models.



(a)



(b)



(c)

Figure 3.8: Jackknife of (a) regularised training gain, (b) test gain and (c) AUC, for predicted spawning distribution of *S. officinalis* within the English Channel. The black bars represent model gain using only that variable and the grey bars represent the effect of removing that variable from the model, the red bar indicates the total gain for the model with all variables.

**3.4.2.3.4 Marginal response curves** The marginal response curves (Figure 3.9) give an indication of the range of values that have the highest and lowest predicted probability of suitable conditions. For depth the highest response in terms of predicted probability of suitable conditions is between 3 and 26 m and declining from this point onwards. For chlorophyll-*a* the highest response in terms of predicted probability of suitable conditions falls between 1.0 and 2.5 mg.m<sup>-3</sup> whilst the lowest response falls between 2.5 and 5.0 mg.m<sup>-3</sup>. For distance from coastline the highest response is between 2.2 and 121 km from the coastline, after which the response again declines. For bed shear stress the lowest response in terms of predicted probability of suitable conditions falls between 1.5 and 3.0 whilst the highest response falls between 0.5 to 1.5, indicating a preference for areas with weaker bed shear stress. For sea surface salinity the marginal response curve is difficult to interpret, although there is a change in response between approximately 33 to 35. The marginal response curve for sea surface temperature is also difficult to interpret but again there is a change in response pattern between 14 and 18 °C. A summary of the highest response values for each predictor variable are also presented in Table 3.4.

### 3.4. RESULTS

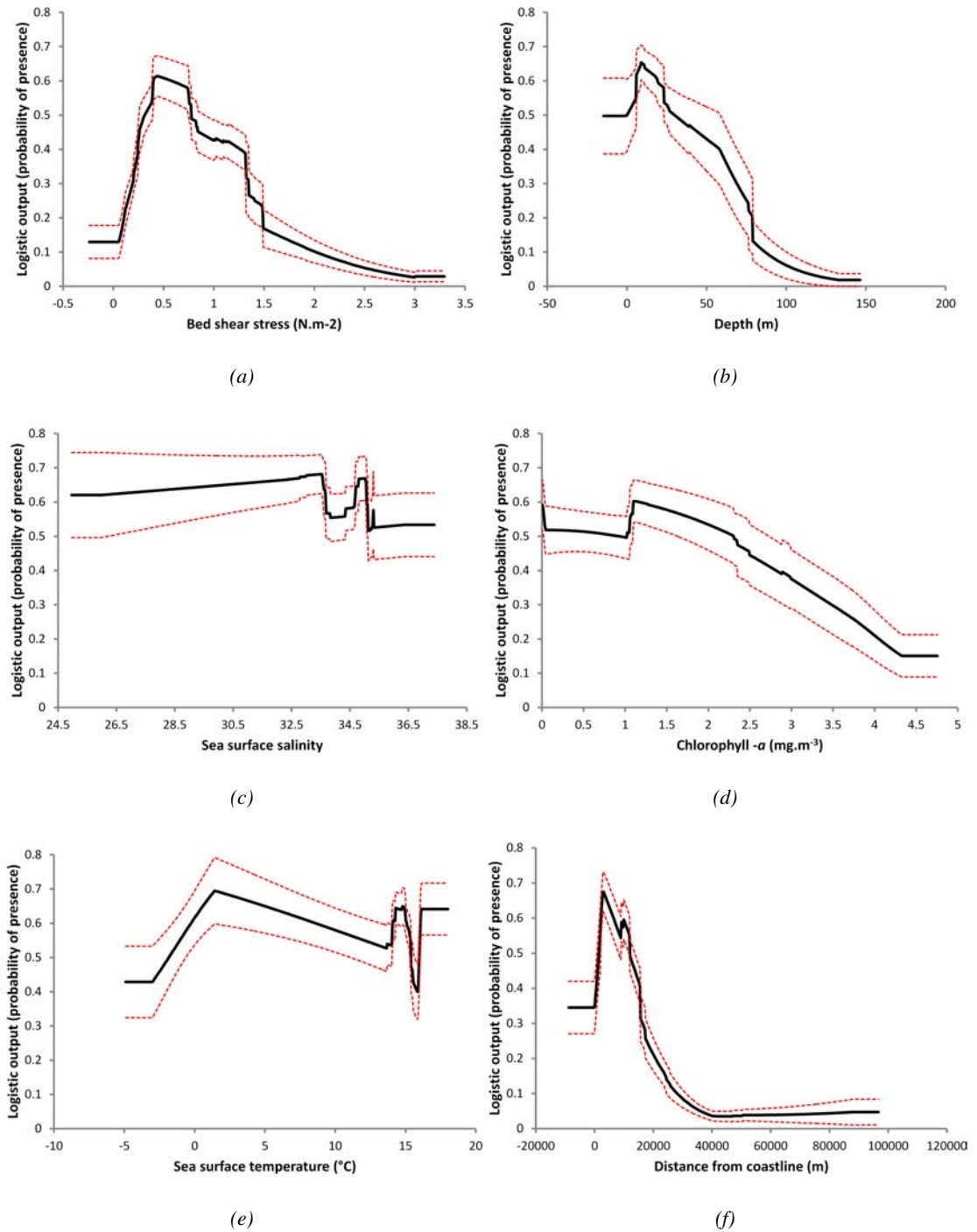


Figure 3.9: The average marginal response curve (black line) for each predictor variable, with standard deviation (dotted red lines) displayed (a) Bed shear stress, (b) Depth, (c) Salinity, (d) Chlorophyll-a, (d) Sea surface temperature, (e) distance from coastline. The curves indicate how the logistic prediction (y-axis) changes as each target environmental variable is varied (x-axis), with all other variables remain constant (at their average sample value).



## 3.5 Discussion

The aims of this study were to describe the potential spawning distribution of *S. officinalis* within the English Channel and to understand the influence of environmental and physical conditions on this predicted distribution pattern. Although a few studies have already begun to investigate the topic of spatial and temporal distribution patterns of this species (e.g. Wang et al. 2003; Carpentier et al. 2009; Valavanis et al. 2002), the present study differs from these in a number of ways. Firstly, previous studies (e.g. Wang et al. 2003; Carpentier et al. 2009; Valavanis et al. 2002) have focused on the use of occurrence records from all life stages of the target species, with data sourced from commercial fisheries landings (Wang et al. 2003; Valavanis et al. 2002) or targeted research surveys (e.g. Carpentier et al. 2009). In contrast, this study makes use of readily available presence-only data sets from a range of sources including both archival and ad-hoc records. In addition, only the occurrence records from a key life stage (spawning), with presence records of benthic egg clusters were utilised as a means to represent a true measure of spawning. Secondly the type of methodology differs from previous studies which use either geographic information systems to assess correlations with environmental characteristics (Wang et al. 2003; Valavanis et al. 2002) or presence-absence techniques such as GLMs and GAMs (e.g. Carpentier et al. 2009). In contrast, this study uses a novel presence-only species distribution modelling technique known as MaxEnt, a method that has not been used previously for studying this species.

### 3.5.1 Spawning distribution of *S. officinalis* within the English Channel

The model predicted areas suitable for *S. officinalis* spawning in coastal areas on both sides of the English Channel, with a predominance of suitable habitat predicted in the eastern part and a smaller fraction, of more discrete sites, predicted in the western part. Areas where important targeted coastal trap fisheries for *S. officinalis* exist were all identified by the model as suitable areas for spawning, included: Torbay, Exmouth, Poole, Selsey, Eastbourne and Hastings on the UK coast and Agon-Countainville, Langrune-sur-mer on the French coast (Figure 3.6b).

#### 3.5.1.1 Western and Eastern English Channel

The MaxEnt model predicted a larger proportion of suitable spawning habitat in the Eastern English Channel relative to the Western English Channel. There are several possible theories as to why this spatial distinction in the model prediction might have occurred. The first is based on sample selection bias, which indicates a slight predominance of samples in the Eastern Channel compared with the Western Channel, however a discussion on bias is made separately (Section 3.5.3.3). Another possible explanation for the higher fractional predicted area of suitable spawning habitat in the eastern part of the Channel compared with the western part may be the innate difference in hydrodynamic and physical conditions which vary distinctly between the two areas, as outlined in (Dauvin 2012) and summarised in Table 3.7. For example, in the western part of the Channel, the hydrological and oceanographic conditions are mainly dominated by the input of water from the Atlantic; whilst in the eastern part, the large fresh water input from the Seine estuary plays an important role in dictating the conditions, particularly on the French coast (Dauvin 2012). The sediment in the Western Channel is generally coarser than that found in the Eastern Channel with a decrease in the benthic species from west to east (Pawson 1995). The Western Channel is known to account for approximately 63 % of the English Channel, covering a total area of 56,452 (Stanford and Pitcher 2004), however, despite its size, there are still many aspects of its features that remain unknown. For example, whilst several studies have studied the benthic macrofaunal assemblages within the Eastern Channel (e.g. Sanvicente-Añorve et al. 2002), a detailed study regarding the relationships of these assemblages within the Western Channel has yet to be undertaken (Araujo et al. 2005). Further research is therefore required to elucidate the true nature of the differences between these two areas.

The fisheries that exist within the English Channel have also been studied in some detail with ecosystem models exploring the interactions between a variety of aspects including economic, technical, biological and trophic (Araujo et al. 2005). One such model, produced by (Stanford and Pitcher 2004) for the whole English Channel, lead its authors to postulate that the significant distinctiveness that exists between the two areas (Eastern and Western Channel) would certainly warrant their study as two separate models. This distinctiveness is manifested not

### 3.5. DISCUSSION

*Table 3.7:* General characteristics of the Western and Eastern basins of the English Channel, highlight the similarities and differences between these two area (Adapted from Dauvin (2012))

Oceanographic characteristic	Western basin	Eastern basin
Mean depth	80 m	50 m
Maximum depth	174 m	100 m
Major estuary	Absent	Seine
Water clarity	High	Low
Maximum tidal range	13.2 m	8 m
Maximum bottom current speed	10 knots	5 knots
Maximum bottom temperature	17°C	20°C
Minimal bottom temperature	8°C	4°C

only in the physical and hydrodynamic features of the areas but also in the ecology and fisheries, with examples of fish stocks confined to single side of the Channel, or that exhibit very different long-term trends between the two sides (e.g. sole, *Solea solea*) (Araujo et al. 2005). In addition, for the commercial species Atlantic cod *Gadus morhua* the distribution and abundance trends of this species between the two areas has led to its management as two separate stocks with the Western Channel cod treated as part of the Celtic Sea Stock and the Eastern Channel cod as part of the North Sea Stock (Araujo et al. 2005). The evidence that suggests the Eastern and Western Channel are different enough, both hydrodynamically and ecologically, to be considered, managed and modelled as two separate entities is growing. For the purpose of *S. officinalis* a more in depth study incorporating sample points across the entire range of the lifecycle (e.g. adults, juveniles and eggs) will be required in order to investigate whether the distribution of this commercial fishery resource is sufficiently different between these two areas to require separate consideration.

#### 3.5.2 Species-habitat relationship

The internal validation procedure of the MaxEnt model indicates that it performs well in terms of predictive ability (test AUC =0.909), and identifies three variables as being most relevant for predicting the spawning distribution of *S. officinalis*, these are depth, chlorophyll-*a* concentration and distance from coastline, with bed shear stress providing additional useful information. As indicated in Table 3.4, the MaxEnt model predicts similar suitable conditions as predicted

by the non-spatial analysis, with cuttlefish spawning occurring between the months of March to September, in shallow (4 to 23 m depth), inshore (2.2 km to 121. km distance from the coastline) areas, at warmer temperatures (between 14 to 18 °C, SST), within a moderate range of salinity (34.6 to 35.2), in areas of weaker bed stress (0.39 to 0.75 N.m<sup>-2</sup>) and with relatively low Chlorophyll-*a* concentrations (1.1 to 2.2. mg m<sup>-3</sup>). These conditions will be discussed in more detail individually.

#### 3.5.2.1 Depth

Within this model, depth was found to be an important factor in determining the spatial distribution of spawning in *S. officinalis*, with a relatively high percentage contribution (23.9%) together with a relatively high percentage permutation importance (23.8%). In addition, the jackknife test indicated that this variable, when used in isolation, was the most important in terms of both training gain (1.17) and test gain (1.14) when averaged over all 15 replicates. This suggests that of the predictor variables it might be the most important and the most transferable between models, its transferability is likely given the static nature of depth as a long-term factor, when compared with the temporally changing variables such as SST or Chlorophyll-*a* concentration. The marginal response curve indicated that the values for depth that predicted the best conditions suitable for spawning were between 4 and 23 m, which is congruent with what is known about the species life-history traits, with migration of spawning adults to the shallow coastal waters of the English Channel in Spring (Boletzky 1983; Boucaud-Camou and Boismery 1991), as well as with data collated from other populations of *S. officinalis* within the eastern Mediterranean (Valavanis et al. 2002), which suggests that cuttlefish spawning occurs at depths  $\leq 50$  m. English Channel fisheries data for this species also supports this assertion with deep water, offshore trawling ceasing during the summer period (from March onwards) when both adult and sub-adult cuttlefish have moved inshore (Dunn 1999).

However, the sample point data for egg clusters that were collected for the model, indicate that a small proportion of egg clusters have been located in the centre of the English Channel (Figure 3.3). A study by Challier et al. (2005a) that investigated trends in recruitment of *S. officinalis* within the English Channel indicated that although the majority of recruitment occurred in au-

tumn, some recruitment was found to occur throughout the year. One of the explanations that the authors made to account for these findings was that as spawning takes place from spring through summer, when spawning adults occur both offshore (prior to or during their migration) and inshore (following migration), that eggs could potentially be laid in both shallow, warm waters as well as deeper, cold waters (Challier et al. 2005a). As a result of the temperature dependent rate of embryogenesis in this species, eggs spawned in deeper, colder waters would take longer to develop and hatch later than those spawned inshore (Challier et al. 2005a). Whilst it is entirely possible that some spawning does occur in the deeper waters of the Channel as evidenced both by the findings of Challier et al. (2005a) and the location of cuttlefish egg clusters within the presence-only dataset used for model construction (Figure 3.3), the model constructed in this study indicates that spawning predominately occurs in shallow waters. In addition, the benthic nature of cuttlefish spawning, requires that females have access to a range of benthic supports to attach their eggs to, in order that the eggs receive adequate aeration and water circulation, such structures are likely to be limited in some deeper offshore waters of the Channel where gravel and pebble sediments dominate (e.g. Figure 3.4h). Although some structures which are suitable for spawning (e.g. *Hydroids* and *Porifera* spp.) may occur in these deeper offshore waters, such spawning (if apparent) is likely to be limited in contrast to shallow inshore spawning, possibly occurring only under certain environmental conditions or situations.

#### 3.5.2.2 Chlorophyll-*a*

Within this model, chlorophyll-*a*, was found to be an important factor in determining the spatial distribution of spawning in *S. officinalis*, with a high percentage contribution (22.6 %) but a low percentage permutation importance (1.4 %). In addition, the jackknife test indicated that this variable was one of the two most important in terms of training gain (1.06) and test gain (1.12), when used in isolation. The marginal response curve also indicated that the values of chlorophyll-*a* concentration that predicted the best conditions suitable for spawning was between 1.1 and 2.2 mg m<sup>-3</sup>.

Whilst the sea surface concentration of chlorophyll-*a* is not likely to directly determine the distribution of spawning in this nekto-benthic species, which attaches its eggs to structures that

radiate from the seabed, the role of chlorophyll-*a* has been shown in other studies to provide a useful indicator of primary productivity (Pierce et al. 2002). Chlorophyll-*a* concentration is often used as a proxy for primary production (Friedland et al. 2012), which may in turn related to food availability and abundance, and has also been found to be positively correlated with fisheries yield in some ecosystems (e.g. Friedland et al. 2012). These factors are believed to be important in cephalopod distribution and in particular for the distribution of paralarvae and hatchlings (e.g. Vidal et al. 2010). A similar relationship was found between the distribution of *Octopus vulgaris* and chlorophyll-*a* in the Mediterranean, when modelled with MaxEnt (Hermosilla et al. 2011), although this study was not focused on spawning distribution in particular.

A study by Smyth et al. (2010) which investigated the environmental conditions in the Western English Channel state that typical background chlorophyll-*a* concentrations for this area are around 1 mg.m<sup>-3</sup> throughout the year. The authors also note that whilst in winter (October to March) coastal areas appear to be characterised by higher chlorophyll-*a* concentrations, that this elevation could actually be caused by an artefact of increased suspended particulates and dissolved organic matter which causes an increase in the levels recorded by the satellite (Smyth et al. 2010). This, combined with the colinearity of the attenuation coefficient (K490) (representative of turbidity) and chlorophyll-*a* concentration that was recorded in this study and required the removal of one of these variables from the model building process, could indicate that cuttlefish use areas with low chlorophyll-*a* concentration (which would also represent low concentrations of K490) as these areas have a lower turbidity than areas with higher chlorophyll-*a* concentrations.

The English Channel is subject to spring blooms where chlorophyll-*a* values increase in the English Channel within June, July and August (Smyth et al. 2010). Creating a long-term average for the chlorophyll-*a* satellite data smoothes out point events and lessens their impact. As such, the impact of variations both spatially and temporally in primary production relating to the availability of food within these inshore coastal areas might have been reduced within this model and alternative ways to incorporate these long-term, variable datasets need to be investigated in the future.

#### 3.5.2.3 Distance from the coastline

Within this model, distance from coastline was found to be an important factor in determining the spatial distribution of spawning in *S. officinalis*, with a high percentage contribution (27.5%) and the highest percentage permutation importance (36.5%). In addition, the jackknife test indicated that this variable was one of the three most important in terms of both training (1.02) and test (1.03) gain. The marginal response curve also indicated that the values of distance from the coastline that predicted the best conditions suitable for spawning, were between 2.2 and 121 km from the nearest coastline.

Within the English Channel, *S. officinalis* undertake seasonal migrations, from the deeper offshore waters where they spend the winter months, to the shallow, inshore waters in the spring and summer, when mature adults spawn. The results of the model are congruent with this aspect of their life cycle, predicting the area between 2.2 and 121 km from the coastline to be the most suitable for spawning to occur within. A study undertaken by Valavanis et al. (2002) which developed a marine information system for cephalopod fisheries in the eastern Mediterranean found that *L. vulgaris* and *S. officinalis* selected areas to spawn that were closer to the coast when the coastline was rocky and sharp and further away from the coast when the coastline was smooth and sandy. Future analysis using information on the composition of the coastline could be of interest to see if a similar effect is observed in the English Channel.

#### 3.5.2.4 Bed shear stress

Bed shear stress, was found to be an important factor, within this model, for determining the spatial distribution of spawning in *S. officinalis*, contributing to the model the most information that was not contained by any other variable producing the lowest training (1.44) and test (1.35) gain value when excluded from the model during the jackknife test. In addition, the percentage contribution to the model (20 %) from this variable was relatively high with a corresponding high value for permutation importance (33.6%). The marginal response curve for this variable indicated that the values of shear bed stress that predicted the best conditions suitable for spawning were between 0.39 to 0.75 N. m<sup>-2</sup>, indicating a preference for areas with weaker shear bed stress, such as sheltered bays with fine sandy sediment.

As part of the CHARM II project, a distribution model of all life stages of *S. officinalis* was created in the eastern English Channel for the two months of July and October. The results of the July model, which occurs during the spawning season, indicated similar results to those reported in this study, suggesting that the species is tied to areas of weak bed shear stress (Carpentier et al. 2009). Such areas are often found in sheltered bays and are defined by the presence of fine sand and mud as a result of the associated weak currents (Dauvin 2012). For *S. officinalis* which are benthic spawners, the importance of shear bed stress as a factor may be two-fold, to begin, the task of attaching eggs to a structure, which is performed by the female using her tentacles to ‘tie’ each individual egg around the structure, by means of a basal ring. This is a complex task that may be best achieved in areas where the currents are low and thus the effect of water movement, on both the structure and the spawner may be reduced. In addition, in areas of weak bed stress, the currents are reduced and the areas are defined by finer sediments which have a higher degree of retention in such conditions, the type of sediment present will additionally affect the type of structures available for spawning and may be important in determining the degree to which a site is utilised for spawning, with cuttlefish spawning grounds thought to occur predominantly in sandy areas (Nixon and Mangold 1998).

#### 3.5.2.5 Salinity

As a predictor variable, sea surface salinity appears to contribute minimally to the model with a low percentage contribution (4 %) and permutation importance (2.5%). This suggests that within this area salinity is not a determining factor in the spawning distribution of *S. officinalis*. In addition, the jackknife values for this variable in isolation were also low for both training (0.36) and test (0.42) gain, with the marginal response curve indicating a change in the pattern of response with a sub-peak at 34.6 to 35.2. However, generally the curve indicates a suitability across the entire range of salinity values (e.g. 25 to 37). This suggests a large degree of tolerance to variations in salinity, a result that is supported by the model produced in CHARM II, which also indicates a large tolerance to salinity conditions for all life stages of this species in the eastern English Channel during the month of June (Carpentier et al. 2009).

Within the literature a salinity of 28 or greater has been considered as optimal for spawning



grounds of *S. officinalis* (Paulij et al. 1990a; Boucaud-Camou and Boismery 1991; Mangold-Wirz 1963) with salinity shown to be an important factor for successful embryonic development (Paulij et al. 1990a). The benthic nature of spawning in this species means that the eggs are fixed to a variety of substrata on the sea floor, rendering them stationary and subject to any fluctuations in salinity that may occur at the spawning site (e.g. river outflow, rainfall etc.). A study by Paulij et al. (1990a) which investigated the effects of salinity on the embryonic development of *S. officinalis* eggs in the Delta found that at a salinity of 28.7 or less, the developmental rate of embryos was significantly reduced, whilst at a salinity of 22.4 or below, malformed embryos were found. The authors suggest these effects of reduced salinity may be a result of the increased osmotic stress experienced by the developing embryo which causes large energy demands, thereby reducing the energy reserves available for successful development (Paulij et al. 1990a). However, it has been observed that if individuals are slowly acclimatised to salinity changes, it is possible for ELS of *S. officinalis* to survive for some time at lower salinities (e.g. 18 or 19) (Boletzky 1983; Paulij et al. 1990a).

The effects of salinity on embryogenesis and hatching are important and confirm *S. officinalis* niche as an essentially marine species, unsuited to spawning in brackish water (Palmegiano and d'Apote 1983). This means that in order to maximise the survival potential of eggs and hatchlings, sexually mature females must select a spawning site with a suitable salinity, in an area where fluctuations (e.g. river input, rainfall runoff etc.) are minimal. As such the area within the English Channel which is most affected by salinity variations, and for which this factor may be of more importance in determining spawning distribution at a local scale, is the area in the eastern Channel near to the Seine Estuary, where fresh water inflow creates a desalinated corridor parallel to the French coast, known as the coastal river (Dauvin 2012), however further investigation would be required to determine if annual or seasonal salinity variations in this area are great enough to limit spawning. In addition, the mechanism by which cuttlefish are able to assess the salinity of the ambient water is still not well known, and requires additional study to determine if it is essential process by which spawning females determine site selection.

#### 3.5.2.6 SST

SST also appears to contribute minimally to the model, with the lowest percentage contribution (1.9 %) and a low permutation importance (2.3 %). In addition, the jackknife value for this variable, in isolation, was also the lowest for both training (0.33) and test (0.36) gain. The marginal response curve for this variable indicates that the best predicted conditions for habitat suitability for temperature are between 24 to 18°C. This is consistent with what is known about the life cycle of this species which suggests that spawning adults migrate inshore to spawn when the water temperature is around 12°C (Boucaud-Camou and Boismery 1991) and that in general temperatures of between 9.5 – 20°C are considered optimal for this species (Mangold-Wirz 1963).

There are several reasons why SST may not have factored as an important variable in predicting suitable spawning areas for *S. officinalis* within the English Channel. Given the benthic nature of spawning in this species, it could be that sea bottom temperature (SBT) might be a more useful predictor variable, however, this data is harder to obtain and a study by Wang et al. (2003) indicated that in the English Channel these two variables were highly correlated. The other issue may be the temporal limitations of the data layer which is a long-term median of March to September between 2000-2010. By averaging the data many of the temporal patterns that are evident in the data during these time periods (Figure 3.10), as well as inter-annual differences, may not be evident. Whilst a previous study within the English Channel, has shown SST to affect the extent of cuttlefish migrations, with an expansion of their distribution more northerly in warmer years and shifting further south in cooler years (Wang et al. 2003). However, the authors suggest that whilst this indicates a positive correlation between local abundance of *S. officinalis* and SST during the spawning season, it is difficult to determine whether this reflects a causal link or not (Wang et al. 2003), but highlights the need to incorporate temporal temperature variability into the model.

### 3.5. DISCUSSION

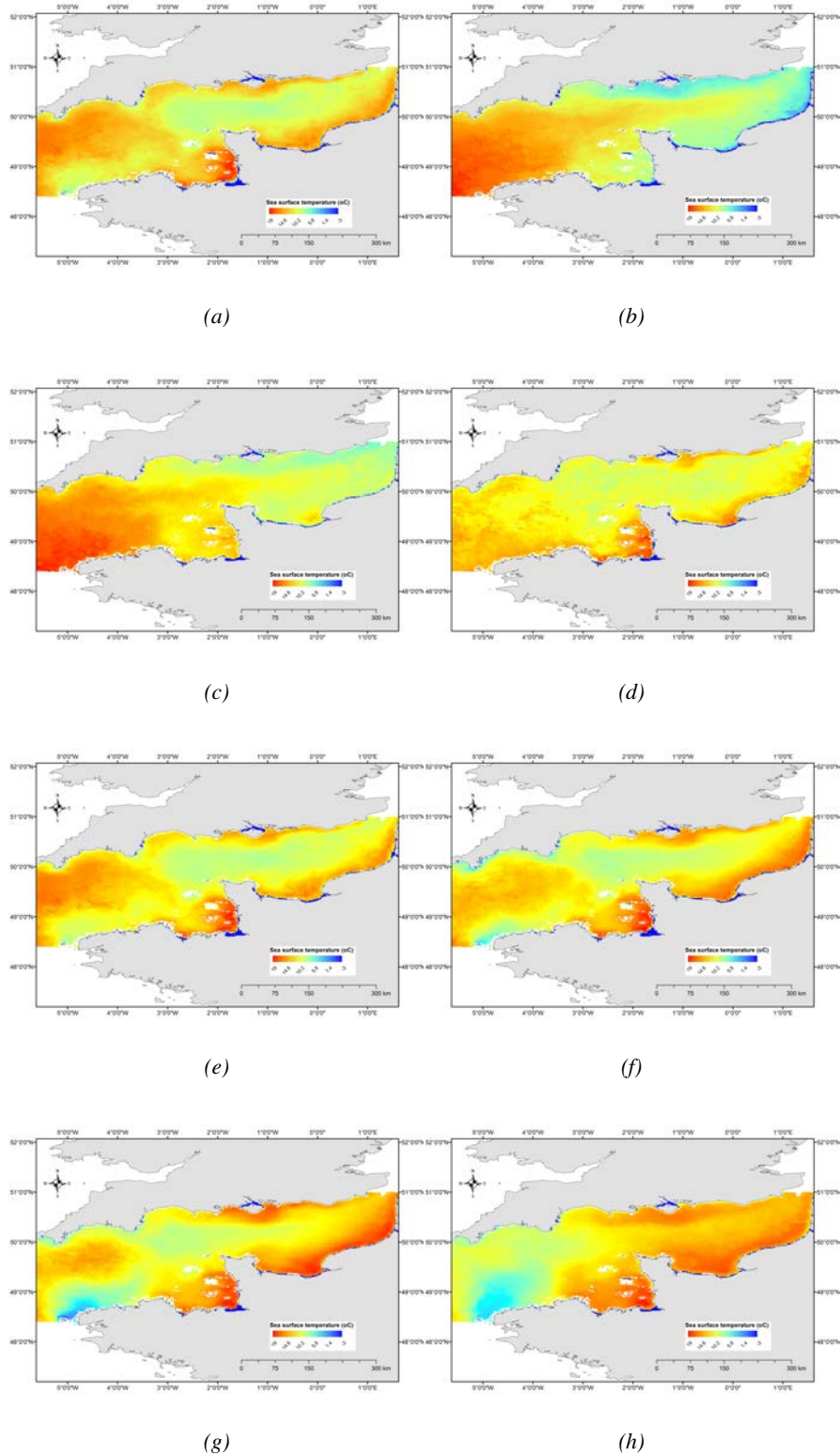


Figure 3.10: Maps showing the data for SST: (a) long-term average (March to September), (b) Long-term average March (c) Long-term average April, (d) Long-term average May, (e) Long-term average June, (f) Long-term average July, (g) Long-term average August, (h) Long-term average September

#### 3.5.3 Data limitations

A model is a means of simplifying or approximating a complex reality using the most accurate data and information available. It must therefore be noted that any model will always incorporate at least a degree of inaccuracy by its very nature, with the quality of the model produced depending heavily on the quality of the data and methods available for its constructions (Wilson et al. 2009). However, despite this caveat, SDMs are useful tools that allow an assessment of species distribution to be made, providing important information on the way that a species interacts and responds to environmental conditions.

##### 3.5.3.1 Long-term averages

In terms of the predictor variables, one of the other limitations of the data, is the inability to incorporate the information on temporal variability of these factors, such as SST or Chlorophyll-*a*. For these factors conditions can change on daily, monthly and annual scales. A large portion of this temporal information is therefore lost when the data layers are averaged to produce the long-term median. Long-term averages were used in this study to allow a large-scale model of spawning distribution to be created using remotely sensed satellite data. One solution to this problem would be to create models for different months within the spawning season or for different years, where the data are combined into monthly or annual median composites, rather than the long-term composite used in this model. This type of modelling would also be of interest given the large inter-annual variations in recruitment (and associated landings) that result from variability in annual environmental conditions. There is the potential that large variability in the quantity and location of predicted suitable spawning habitat may occur between years as well. However, the major restriction on this approach is the lack of sample point data available across these smaller timescales, with the original dataset of 163 points, reduced significantly when broken down by individual months or years across a 10 year timescale.

##### 3.5.3.2 Spatial resolution and data availability

One of the biggest limitations of the data is the lack of availability of detailed habitat maps, indicating the type and distribution of habitats within the Channel. Whilst, high quality habitat

data does exist for several restricted areas of the Channel, is it a long way off being created for the entire extent of the study area and highlights an important data limitation in this study. For example, a large area of the Channel was predicted by the model as being potentially suitable for spawning, in terms of the conditions outlined by the predictor variables. However, the benthic nature of spawning in this species with eggs being individually attached, by means of the basal ring, to structures that radiate from the seabed, mean that spawning can only occur within predicted areas that also contain suitable spawning structures onto which eggs can be laid. The lack of suitable habitat maps currently restricts the integration of such data into the model.

The spatial resolution of some of the data layers is also a limitation on the model, for example, the data layer for sediment is high quality at a large scale across the entire Channel, but when it is investigated at a finer geographic scale the detail is lacking. For example, the area in the west part of the Channel around Lyme Bay is labelled entirely as 'mud' (Figure 3.4h), whereas in reality this area is actually composed of a variety of sediment types, but the low resolution of the data does not reflect these intricacies with sufficient depth. For regional areas where higher resolution data exists (e.g. high resolution sediment data and/or high resolution marine habitat maps), local models could be created at a finer scale. This would allow additional detail to be input into the model and may enable a better resolve of suitability for spawning to be achieved. However, in order for such modelling to be undertaken suitable sample point data for cuttlefish eggs would also need to be available at this regional scale.

#### **3.5.3.3 Sample selection bias**

The issue of sample selection bias is another limitation of the presence-only dataset used within this study. Whilst, such issues are known to have a greater impact on models derived from presence-only data relative to those derived from presence-absence data, absence datasets are not without their own sets of issues and bias. when sampling bias is known, it can be addressed, but one of the biggest issues within presence-only datasets is simply that this bias is unknown. Several new techniques are emerging to deal with bias in these models, including target group sampling for background data (Phillips et al. 2009) and creating bias map files (Dudík et al.

2005). In this study a bias density map was used to guide the model with relation to the sampling bias within the presence-only dataset. However, a thorough and comparative examination of different bias mitigation techniques and the effects that each has on model output and model performance would provide useful information on the degree to which sampling bias within these datasets impacts this and other such models.

#### 3.5.3.4 Data errors:

Errors may also occur within both the sample point dataset and predictor variable data layers from a range of sources, which for the sample points can include misidentification of species, transcriptional errors or lack of geographic detail (Phillips et al. 2006). The data obtained from DAASH which is a MEDIN standard data archive centre has already undergone rigorous checks to ensure as far as possible that all these issues are addressed. In addition, records from bibliographic and fisheries survey sources are considered to be of high quality as these are often studies performed by experts in the field of study, and will likely have undergone peer-review. The occurrence records submitted to us by the general public, where possible are included with photographic detail in order that the record can be validated for correct species identification and geographic coordinates, along with a description of the dive location are also obtained to try and limit any sources of error. Prior to use in the model, the sample point dataset were plotted spatially in ArcGIS and checked visually for any obvious transcriptional errors.

In terms of the sources of error that may exist within the predictor variable data layers, these could include, the initial choice of data layers, correlation among variables, issues with resolution, interpolation of lower-resolution data, loss of information due to amalgamation of temporal series data, errors in data manipulation or data processing (Phillips et al. 2006). To eliminate issues with correlation among variables, a Pearson correlation analysis was performed and for any pairs of variables with a correlation of  $r \geq 0.7$ , one variable from the pair was excluded. In terms of errors in data manipulation and processing, all data layers and calculations were checked and interpolation avoided where possible.

#### 3.5.4 Conclusions

Despite the increase in landings of cuttlefish within the English Channel by UK fishing vessels over the last 30 years, there exists no directed cuttlefish management for this fishery. Given the extensive coverage of the fishery, which occurs across a wide range of habitats and almost all areas of this species life cycle, and targets nearly every life stage; a need for basic information of the distribution of spawning locations and habitats (both at the broad and finer-scale) is identified within the English Channel. The results of this study have begun to address this knowledge gap by providing the first data available on predictive spawning habitat suitability across the entire area. Such data will enable an evaluation of the important spawning location for *S. officinalis* and could feed into potential future management measures for this species, or in an assessment of the need to protect inshore spawning habitats.

In addition, the results of this study provide ample support for the use of MaxEnt as a tool for modelling the distribution of cephalopod species. With the main predictor variables of depth, chlorophyll-*a* concentration, distance from the coastline and bed shear stress, aligning with the known life history traits of this species. As a tool, MaxEnt has been useful to assess how environmental and physical variables are related to spawning distribution of *S. officinalis* within the English Channel and can be used to generate valid distribution models for this species given the limited data available on cuttlefish egg cluster presence.

Further study and investigation is required to properly assess the degree that the spatial bias within the presence-only dataset may contain and how this may affect the predictive ability of the model and which mitigation measure proves the most valid for this model. Such biases must be considered during interpretation of any presence-only model predictions, and continued research into the problems related to bias for both MaxEnt and other presence-only modelling methods will hopefully provide a better understanding and improved methods to help reduce the effects of sample selection bias within these models.

## Chapter 4

# Observations of natural spawning substrates on the UK and French coastlines of the English Channel

### 4.1 Introduction

Although Chapter 3 has provided predictive maps of potential spawning habitats/locations within coastal areas of the English Channel, large gaps in our knowledge and understanding of the quantity and composition of cuttlefish spawning habitats within these inshore areas still exist.

In many species, migratory patterns like those exhibited by *S. officinalis* (see Chapter 2; Section 2.2.2.3) have evolved so that spawning adults can deposit their eggs in a habitat in which the ecological and environmental conditions are optimal (spatially and/or temporally) for survival and growth of their offspring (e.g. Dodson 1997; Pierce et al. 2008). At spawning, *S. officinalis* females deposit their eggs on structures attached to the seabed, which means that developing embryos remain at the site of spawning (see Chapter 2; Section 2.2.3.3.2 and 2.2.3). As such, the location (oviposition site) that mothers ‘select’ to lay their eggs can dramatically affect offspring performance and fitness by determining the local environment and conditions in which their offspring will develop (Marshall et al. 2008), yet specific details of the structural components and substratum types of these inshore spawning habitats is lacking.

In 1983 Boletzky wrote that *S. officinalis* eggs are generally laid in shallow water (e.g. < 40 m) and are attached to any oblong object with a diameter of around 1 cm (Boletzky 1983). Each egg is attached by the female who uses her tentacles to manipulate the basal ring of the



egg's gelatinous envelope around the support to fix it in place (Boletzky 1983). Eggs are found attached to natural structures such as plants or sessile animals as well as to artificial structures such as submerged trees, cables or fishing nets (Boletzky 1983). Blanc (1998) undertook a qualitative analysis of the spawning structures used within the east and west area of the Gulf of Morbihan which is located in the northern part of the Bay of Biscay. In this study Blanc found a total of twelve different natural spawning structures (Table 4.1), 6 in the west and 8 in the east (Blanc 1998). This present study will undertake a qualitative survey of spawning structures on both the UK and French coastline of the English Channel in order to determine the key habitats and structures used by *S. officinalis* for spawning.

*Table 4.1:* A list of natural spawning supports for *Sepia officinalis* found by Blanc (1998) in a survey of sites in the west and east of Morbihan Bay situated in the northern part of the Bay of Biscay.

West	East
<i>Spirographis spallanzanii</i>	<i>Spirographis spallanzanii</i>
<i>Sabella pavonina</i>	<i>Sabella pavonina</i>
<i>Hypoglossum woodwardii</i>	<i>Sargassum muticum</i>
<i>Laminaria saccharina</i>	<i>Soleria chordalis</i>
<i>Zostera</i> sp.	<i>Zostera</i> sp.
<i>Cladophora pellucida</i>	<i>Gracilaria multipartita</i>
	<i>Gracilaria verrucosa</i>
	<i>Dictyota dichotoma</i>

The English Channel is not homogeneous across its extent and is known to vary in habitat, sediment, oceanographic and hydrodynamic conditions (e.g. East vs West English Channel Araujo et al. 2005). The general ecology and range of habitats that occur also change rapidly over small scales and may affect spawning intensity at a given site. As such, it is thought that spawning intensity will vary both spatially and temporally across the English Channel coastline. Patterns of spawning in benthic species like *S. officinalis* can be described directly through natural observations of spawning areas. *In situ* observations will help to better understand the

range of habitats and structures used for spawning and the factors and processes that influence variability in spawning patterns. Once the range of spawning structures has been determined, it would be useful to understand whether spatial or temporal differences between patterns of spawning intensity occur as a result of the quality or presence of habitat available and whether that is affected by depth or the type of structures present. Information on habitat requirements for spawning and the characteristics of spawning habitats and locations (e.g. preferred spawning structures and depth range) is important (Valavanis et al. 2004b). Within the two French spawning sites information on spawning within three depth strata was used to analyse whether a preferred depth range occurred within these spawning sites.

In terms of the availability of structure types and spawning patterns, seagrass beds, for example, are considered highly productive nursery areas with the potential to provide diverse and abundant sources of prey items and the structural complexity to provide shelter from predation to juveniles of many species (e.g. Jackson et al. 2001). Seagrasses are also recognised for their capacity to modify currents (e.g. Fonseca et al. 1982) and promote sediment deposition (e.g. Ginsburg and Lowenstam 1958). This provides a sandy substrate, which is useful for ELS to bury themselves, low wave exposure, which may reduce egg loss during embryonic development, shallow depth or local warming, which may decrease embryonic development time. If key nursery areas can be identified for juvenile cuttlefish then this may help direct future conservation and management strategies for this species.

The spatial arrangement of seagrass beds can vary from a single rhizome or group of shoots (e.g. cm to m), to a patch or patches (e.g. m) and up to entire seagrass landscapes (e.g. m to km) (e.g. Olsen and Sand-Jensen 1994). Within a seagrass landscape the context of the bed will also be constrained by the level of patchiness or heterogeneity of the patches contained within it (e.g. Kotliar and Wiens 1990). Seagrass beds are spatially and temporally dynamic and are sensitive to both natural and anthropogenic disturbance (e.g. Den Hartog 1987). In areas where seagrass is used as a spawning structure the scale of the spatial arrangement will affect the quantity of structures available for spawning and therefore the maximum spawning intensity possible. The fragmentation of heterogeneity of a seagrass landscape is known to

display a strong relationship with the physical characteristics of the area (e.g. wind-generated wave dynamics and tidal currents) Fonesca and Bell (1998); Frederiksen et al. (2004); Fonesca et al. (1983). The implications for such larger-scale spatial patterns will be explored within this study to examine the role of the heterogeneity of seagrass landscapes in influencing the patterns and intensity of spawning within seagrass beds in Torbay, Devon (Turner et al. 1999). One means of quantifying the differences in spatial variation of the seagrass beds between sites is to undertake a 'Fractal Dimension' analysis which enables a measure of transect heterogeneity to be made (e.g. Jackson et al. 2006). The relationship between spawning patterns and seagrass fractal dimension was examined to test the hypothesis that seagrass patch characteristics are important in explaining variations in spawning patterns within seagrass beds. In addition, to understand the use of this habitat not just spatially, but temporally, time series analysis was undertaken to investigate patterns in spawning both within a season (e.g. March, April, May, June) but also between seasons (e.g. 2011 and 2012).

##### 4.1.1 Aims and objectives

The overall aim of this study was to survey important spawning areas and habitats, for *S. officinalis*, within the English Channel and to investigate the different structures within these areas that are used for spawning, during a series of subtidal and intertidal surveys. The objectives of this study were to:

- Provide a qualitative assessment of the range of structures used for spawning by *S. officinalis* within the English Channel using *in situ* observations of natural spawning habitats.
- Assess whether differences in spawning patterns occur between different depth strata.
- Assess whether differences in spawning patterns occur between different structural strata.
- Investigate the temporal patterns of spawning within seagrass beds at the UK coastal site of Torbay:

Compare egg densities between months and years.

Analyse variability in cluster size between months and years.

Analyse spatial dynamics of seagrass beds between years (fractal dimension).

Analyse spawning characteristics among individual seagrass plants.

## 4.2 Methods and Materials

### 4.2.1 Study sites

Subtidal surveys were conducted at study sites along the English Channel coastline to obtain the data for both qualitative and quantitative analysis of the natural spawning structures used by *S. officinalis* within these inshore waters. Surveys were restricted to shallow coastal areas, due to both the safe limit for scientific diving and pre-existing knowledge of the lifecycle of this species, which indicates that spawning adults migrate to the shallow inshore areas of the English Channel to spawn. Five study sites were selected from areas that supported active inshore cuttlefish trap fisheries during the spring and summer, and incorporated sites in both the Eastern and Western English Channel. The sites selected were:

#### 4.2.1.1 Torbay

Torbay is situated in Devon off the south-west coast of England, in the Western English Channel (Figure 4.1). The maximum spring tidal range at Torbay is around 4 m (Herbert et al. 2007), describing a mesotidal environment (Woodroffe 2003). Torbay is exposed to the east and sheltered from the prevailing west and south-west winds, it has relatively weak tidal streams, but is vulnerable to wind and wave action from the east (Forster 1955). The area is relatively shallow, reaching depths of about 20 m in the centre of the bay, where muddy sediment dominates (e.g. Larssonneur et al. 1982), further inshore areas of sand and reef are also found (McBreen et al. 2011). The reef features in this area are in discrete formations around the bay, mainly associated with headlands and coves and are known to support rich species that typify reef habitat, including hydroids, algae, sponges and corals (Natural England 2010). The site also contains a number of seagrass beds, with surveys by Torbay Coast and Countryside Trust's (TCCT) 2006 seagrass project indicating that there are at least 80 hectares of seagrass meadows in Torbay (TCCT 2006).

### 4.2.1.2 Selsey

Selsey is situated in West Sussex off the south-east coast of England, in the Eastern English Channel (Figure 4.1). The maximum spring tidal range at Selsey Bill is 4.5 m (Cope 2005), describing a mesotidal environment (Woodroffe 2003). The inshore region within the study area is relatively shallow (< 30 m) although the seabed does slope gently to about 60 m depth off Selsey Bill. The sediment in the area is a mix of sand, mud and rocky reef (McBreen et al. 2011) which supports a diverse range of flora and fauna.

### 4.2.1.3 Poole Bay

Poole is situated in Dorset off the central south coast of England, in the Eastern English Channel (Figure 4.1). The maximum spring tidal range at Poole Bay is approximately 2 m, which is among the lowest in the English Channel (Pingree and Maddock 1977) and describes a microtidal environment (Woodroffe 2003). The area is also known to experience the unusual tidal phenomena of a double high and low water (Pingree and Maddock 1977). Within Poole Bay, the Studland Bay area contains extensive seagrass (*Zostera marina*) beds (156 hectares, of which 61 hectares is considered sparse seagrass)(Jackson et al. 2012) and additionally the Poole Bay area is known to contain both maerl and sabellaria reefs as well as artificial and natural patch reefs (Collins 2007). The small patch reefs within the Bay are known to support a mixture of brown (e.g. *Dictyota dichotoma*) and red (e.g. *Calliblepharis ciliata*) algae at shallower depths (below 10 m) and only red algae at deeper depths (above 10 m) (Collins 2007). In addition the area is also known to be the eastern-most extent within the English Channel for pink sea fans (*Eunicella verrucosa*) (Collins 2007).

### 4.2.1.4 Agon-Coutainville

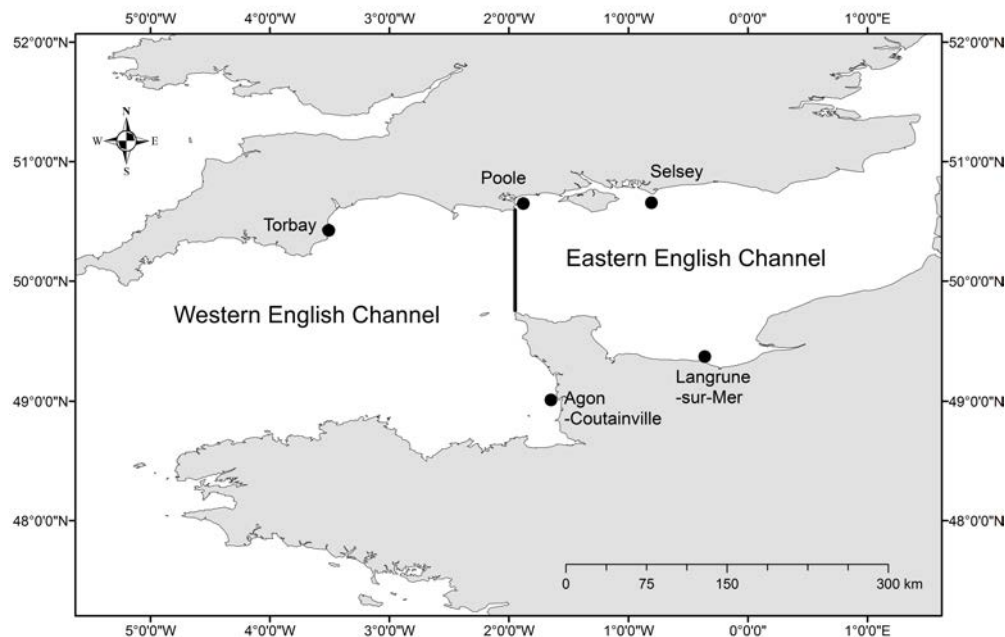
On the French coast the Cotentin peninsular divides the English Channel into two basins (east and west) (Figure 4.1). For the purpose of this study, sites were chosen on either side of the Cotentin peninsular. Agon-Coutainville is situated in the region Basse-Normandie off the north-west coast of France, in the Western English Channel (Figure 4.1). The maximum spring tidal range for the area is up to 14 m, which is among the highest in the world, describing a megatidal

system (Lefebvre et al. 2009). The substrate in the area is generally classified as medium to coarse sand with areas of rock (Lefebvre et al. 2009). The west coast of Cotentin is generally exposed to the currents of the North Atlantic drift, which run eastward and are then deflected north-ward along the coast from the Baie du Mont Saint Michel (Lefebvre et al. 2009). The riverine watersheds that emerge on the west coast are relatively small (approximately 929 km<sup>2</sup>) compared to the east coast (Lefebvre et al. 2009)

### 4.2.1.5 Langrune-sur-Mer

Langrune-sur-Mer is situated in the region Basse-Normandie off the north-west coast of France, in the Eastern English Channel (Figure 4.1). The maximum spring tidal range is approximately 8 m, describing a macrotidal environment. (Lefebvre et al. 2009). The substrate on the east coast is generally of a smaller grain size than on the west, with fine and muddy sand predominating in inshore areas (Lefebvre et al. 2009). The Baie de Seine is sheltered from prevailing winds and currents. The larger riverine watershed (approximately 4383 km<sup>2</sup>) on the east of Cotentin does however leave the area vulnerable to large terrestrial inputs and fluctuations in salinity (Lefebvre et al. 2009).

## 4.2. METHODS AND MATERIALS



*Figure 4.1:* English Channel Study Sites. The locations of each study site are indicated on the map and include: Torbay, Poole and Selsey on the U.K. coast and Agon-Coutainville and Langrune-sur-Mer on the French coast. The vertical black line indicates the split between the Eastern and Western basins of the English Channel as proposed by Pomerol (1977)

### 4.2.2 Survey methods

A variety of methods, using SCUBA, were used to obtain data for both the quantitative and qualitative analysis of natural spawning structures. During this research, five study sites along the UK and French coast were surveyed (Torbay, Selsey, Poole Bay, Agon-Coutainville and Langrune-sur-Mer). As specified in Section 4.2.1, the variation in hydrological conditions among study sites was high, with tidal systems ranging from megatidal at Agon-Coutainville to microtidal at Poole Bay and additionally large differences in current regimes were also evidenced. Therefore, a single unified survey method was not considered suitable for standardised use across all study sites. Whilst every effort was made to keep the survey method as standardised as possible, methodological changes were required among sites to allow surveys to be undertaken safely. These methods included 50 m<sup>2</sup> circular belt transects (Figure 4.2 a), 100 m<sup>2</sup> line belt transects (Figure 4.2b), which were used in areas with low currents and timed global

positioning system (GPS)-tracked drift transects (Figure 4.2 *c*), which were suitable for areas with strong currents. A brief description of each method is provided:

### **4.2.2.1 Circular transect (radius of 4 m, area of 50.3 m<sup>2</sup>)**

A distance line was extended out to 2 m to define the limits of the first circular sweep and a weighted positional marker placed to mark the start of the transect. Rotating in a clockwise direction, information from the transect was recorded (in a 1 m belt either side of the line). On return to the positional marker, the rope was then extended to 4 m and a second circular sweep undertaken in an anti-clockwise direction (Figure 4.2*a*).

### **4.2.2.2 Line belt transect (area of 100 m<sup>2</sup>)**

A 50 m distance line (marked off in 5 m sections) was extended in a pre-determined direction and information recorded from the transect in a belt of 1 m either side of the central transect line (Figure 4.2*b*).



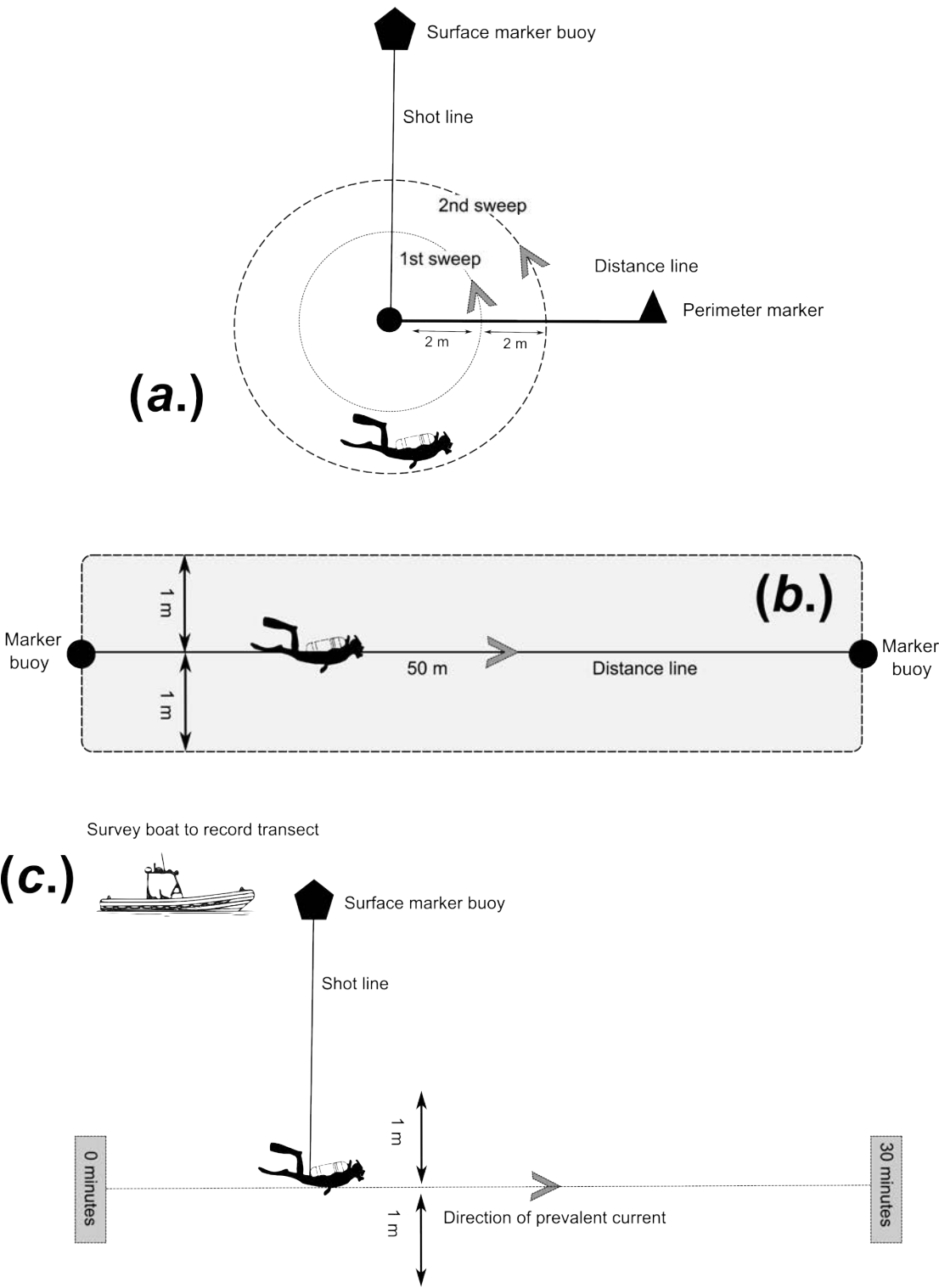


Figure 4.2: Survey methods: (a.) Circular belt transect, (b.) Line belt transect, (c.) GPS-tracked drift transect

### 4.2.2.3 GPS-tracked drift transect (15 minutes; variable length)

Transects were completed in the direction of running currents. A surface marker buoy was used to denote the location of the survey divers, enabling a boat (with a GPS unit) to follow and record the transect path from the surface. Geographic coordinates of the survey track were matched to egg cluster observations which the dive pair recorded using a time stamp from a dive watch synchronised to the GPS. This allowed both the search area and the positions of egg cluster observations to be recorded (Figure 4.2c).

### 4.2.3 Survey design

At all sites stratified random sampling was used to obtain the start locations of transects. This was done using ArcGIS (Esri, v.9.3) to construct a grid that overlaid the study site. Each square within the grid was assigned a unique identifying code and then a random subset of squares were selected using a random number generator in Excel (Microsoft Ltd, 2007) to obtain random start locations for transects.

#### 4.2.3.1 Qualitative assessment of natural spawning structures

For this study three methods described in Section 4.2.2 were utilised to obtain data for qualitative analysis. Surveys were conducted between 2010 and 2012 and took place between April and July, to align with the cuttlefish spawning season. On the UK coast all surveys were subtidal, whilst on the French coast, due to the large tidal range both subtidal (SCUBA) and intertidal (walking) surveys were undertaken.

#### 4.2.3.2 Comparison of spawning strata (depth)

A comparison of spawning among different depth strata was undertaken at Agon-Coutainville and Langrune-sur-Mer in June 2011. Transect start points were randomly assigned within three depth strata (0-5 m, 5-10 m and 10-15 m). Five surveys were undertaken at each depth stratum at Agon-Coutainville and between three and nine surveys at Langrune-sur-Mer. All surveys were completed using timed (15 minutes) GPS-tracked drift transects. This methodology was used due to the large currents experienced in the area, which prevented divers from undertaking stationary surveys such as those proposed for use in the UK (e.g. circular or line belt transect).

### 4.2.3.3 Comparison of spawning strata (structures)

A comparison of spawning between two strata with different structure types, seagrass beds (seagrass stratum) and mixed seaweed habitat (mixed stratum) was undertaken. A preliminary trial within Torbay was conducted in May and July 2010, where the study area was delimited by the natural geographic boundary of the Bay and within the area of the 10 m depth contour (Figure 4.3a). Four seagrass and four mixed stratum sites were surveyed at Torbay in May 2010 and five seagrass and five mixed stratum sites in July 2010. For each survey site, three replicates were undertaken using 50 m<sup>2</sup> circular belt transects. The main study was undertaken in June 2011 at two study sites (Torbay and Poole Bay) to compare spawning patterns between seagrass and mixed stratum. The study site for the Torbay area was altered for the main study in 2011, with the new study area extending from Hollicombe Head around the headland at Hope's Nose and on to Babbacombe Bay (Figure 4.3c). This was done to better reflect the study area at Poole Bay which extended from the Branksome reef around the headland at Handfast point and onto Ballard Pinnacle (Figure 4.3d). For both sites the study area was restricted to within the 10 m depth contour. Transect start points for all surveys (2010 and 2011) were randomly assigned within the two strata. In June 2011, our seagrass and four mixed stratum sites were surveyed at each study site, with eight replicates at each stratum site. All surveys were completed using 100 m<sup>2</sup> line belt transects, although due to adverse diving conditions, it was not always possible to undertake a full set of replicates.

## 4.2. METHODS AND MATERIALS

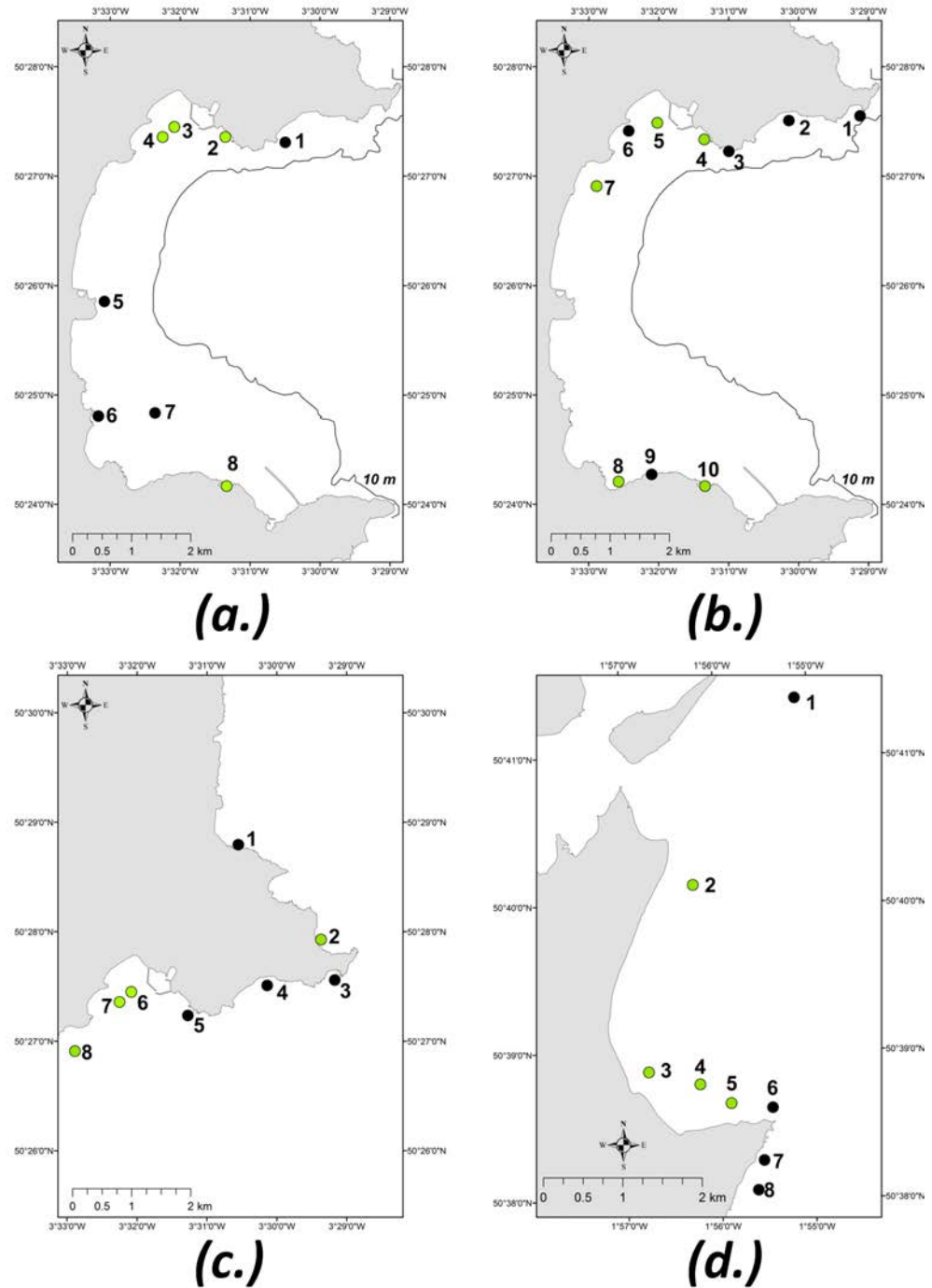


Figure 4.3: Study sites for comparison of natural spawning structures with subtidal survey sites marked. (a.) Subtidal survey sites Torbay May 2010, (b.) Subtidal survey sites Torbay May 2010, (c.) Subtidal survey sites Torbay June 2011 and (d.) Subtidal survey sites Poole June 2011.

### 4.2.3.4 Temporal analysis of spawning in seagrass beds

A comparison of spawning patterns and intensity within a season (May, June and July) was undertaken at two seagrass beds (Millstones Bay and Torre Abbey Sands) between 2010 and 2012. The extents of the seagrass beds were delimited according to the results of the 2006 TCCT seagrass project (TCCT 2006). Transect start points were randomly assigned within each seagrass bed, however, due to the dated nature of the available seagrass maps, a drop-down video camera was also used to verify the presence of seagrass prior to the deployment of transects. If seagrass presence could not be verified at a transect start position, an alternative location was selected. At Millstones Bay and Torre Abbey Sands four replicates were undertaken at each site in 2010 (May and July) using 50 m<sup>2</sup> circular belt transects and eight replicates undertaken at each site in 2011 and 2012 (May, June and July) using 100 m<sup>2</sup> line belt transects, although due to adverse diving conditions, it was not always possible to undertake a full set of replicates.

### 4.2.4 Data collection

Data were recorded on template data sheets which were printed on waterproof paper. For all studies a general description of the habitat was made for each transect. When encountered, the presence of egg clusters was recorded together with details of the attachment structure. A predetermined scale was used by all divers for collection of data on egg number per cluster whilst underwater (Egg number: <10; 10-30; 30-100; 100-1000).

In addition, for the temporal analysis of spawning in seagrass beds (Torbay 2011 and 2012) video recordings of the transect were made in order to enable calculation of the fractal dimension of the seagrass within each transect. At UK sites photographic records of egg clusters were also made to ensure that diver estimation of egg cluster size could be validated. In 2011 at Torbay, data were also collected on the position and length of egg clusters on individual seagrass plants to assess how these structures are specifically utilised by cuttlefish for spawning.

### 4.2.5 Data analysis

As a result of the categorical scale used (Section 4.2.4), the datasets collected within this study failed to meet the assumptions of normality and so non-parametric tests were used for statistical

analysis. Two non-parametric tests were used to analyse the data within this study and a brief description of each is provided.

### 4.2.5.1 Kruskal-Wallis test

The Kruskal-Wallis test was performed using the 'Analyse-It' (Analyse-It Software Ltd, version 2.20) add-in for Excel (Microsoft, 2007). The null hypothesis for the test ( $H_0$ ) was that there is no difference in the response variable (e.g. medians of the groups: egg cluster size, egg density etc.) as a result of the explanatory variable (e.g. depth, structure type, year etc.). When using a Kruskal-Wallis test to analyse data from more than three groups, each with more than five samples per group then the calculated  $H$  statistic should be treated as Chi-Square and interpreted using the Chi-Square critical value table at the appropriate degrees of freedom. For an alpha value of 0.05 the decision rule for this test states that if the calculated value was greater than the tabled value then the null hypothesis ( $H_0$ ) was rejected. If the calculated value was less than the tabled value then the null hypothesis ( $H_0$ ) was accepted.

### 4.2.5.2 Mann-Whitney U test

The Mann-Whitney U test was performed using the 'Analyse-It' (Analyse-It Software Ltd, version 2.20) add-in for Excel (Microsoft, 2007). The  $H_0$  for the test was that there is no difference between the medians of the two groups. The Mann-Whitney U-Test was used to undertake two tailed tests at an alpha value of 0.05. The output of this test was assessed using a  $Z$  table distribution and evaluated using both a  $Z$  and  $p$ -values.

### 4.2.5.3 Qualitative assessment of natural spawning structures

**4.2.5.3.1 Characteristics of natural spawning structures** The data collected from all sites were pooled and a list of all the different spawning structures produced. A short description for each individual spawning structure was produced that included a brief discussion of the number of egg clusters located on each structure type and at which sites, the size of egg clusters and any noticeable spawning patterns. The variety of physical characteristics of all the spawning structures was assessed and a short summary produced.

**4.2.5.3.2 Variability in egg cluster size** A box plot was created to display the minimum and maximum numbers of eggs laid per cluster on each type of spawning structure in order to visually analyse the data set for patterns. A Kruskal-Wallis test was then performed to test whether or not the difference observed was significant. Prior to analysis any group with fewer than five samples was excluded from the test. The  $H_0$  for the test was that there is no difference in the number of eggs laid per cluster (*Response Variable*) on different types of spawning structure (*Explanatory Variable*). For an alpha value of 0.05 the decision rule for this test stated that if the calculated value was greater than the tabled value (alpha = 0.05, DF 10, Chi-Square = 18.31) then the null hypothesis ( $H_0$ ) was rejected. If the calculated value was less than the tabled value then the null hypothesis ( $H_0$ ) was accepted.

### **4.2.5.4 Comparison of spawning strata (Depth)**

For each transect an egg density (eggs per m<sup>2</sup>) was calculated. The distributions of the populations of egg densities (*Response Variable*) were compared between depth strata (0-5 m, 5-10 m and 10-15 m) (*Explanatory Variable*). This was done at each site and then for both sites pooled. Analysis was undertaken using a Kruskal-Wallis test. The  $H_0$  for the test was that there is no difference between the distributions of egg densities from transects within different depth strata.

### **4.2.5.5 Comparison of spawning strata (Structures)**

The number of egg clusters and egg density per m<sup>2</sup> were calculated and the data from both sites pooled by stratum (seagrass and mixed). The data were then compared between sites using a Mann-Whitney U Test. The  $H_0$  hypotheses for the test was that there is no difference between the median number of egg clusters or median egg density (*Response Variables*) collected from seagrass and mixed strata (*Explanatory Variable*).

### **4.2.5.6 Temporal analysis of spawning within seagrass beds (Torrey 2010-2012)**

**4.2.5.6.1 Egg density** The density of eggs per m<sup>2</sup> was calculated for each site by dividing the total number of eggs recorded at a site by the total area of the site surveyed by transects. This was done by year and by month for Millstones Bay and Torre Abbey Sands. From these

density calculations an estimate of the total numbers of eggs at each site was then calculated by multiplying the estimated egg density for a site by the known area of seagrass (Millstones Bay 15,500 m<sup>2</sup> and Torre Abbey Sands 595,000 m<sup>2</sup> TCCT2006). Finally the potential number of females spawning at each site was estimated by dividing the total number of eggs for each site by the average potential fecundity of a female (2,000 eggs e.g. Hanley et al. (1998)).

**4.2.5.6.2 Variability in the number and size of egg clusters** Variability in cluster size was plotted graphically with standard error, in order to assess whether any variability in cluster size was evident between months and/or between years. Egg cluster size was compared between years using a Mann-Whitney U test in order to determine whether a significant difference existed in the size of egg clusters between the years 2011 and 2012. The  $H_0$  for the test was that there is no difference between the median number of eggs laid per cluster (*Response Variables*) in transects from 2011 and 2012 (*Explanatory Variable*).

**4.2.5.6.3 Fractal dimension** Fractal dimension was used to measure the heterogeneity of seagrass in the transects and was estimated from video recordings that were completed during dive surveys for each transect. Fractal dimension was calculated by measuring the presence or absence of seagrass along the transect across a range of increasing resolutions ( $R$ ) (1 m, 5 m, 10 m, 50 m). For each resolution, the cumulative length ( $L$ ) of sections with seagrass present were measured. The fractal dimension was then obtained by regressing  $\log(L)$  on  $\log(R)$  (Jackson et al. 2006). Transects with a fractal dimension of zero can be considered as homogeneous (e.g. seagrass is continuously present along the transect), whilst transect heterogeneity is reflected by dimensions closer to one (e.g. seagrass within the transect is fragmented and patchy). This method allows quantitative information on the spatial patterns of the seagrass within each transect to be assessed and considered in terms of cuttlefish spawning patterns. A Mann-Whitney U test was used to test if a difference existed between the fractal dimension of seagrass transects in 2011 and 2012. The  $H_0$  for the test was that there is no difference between the medians of the fractional dimensions of seagrass transects between the two years.

**4.2.5.6.4 Seagrass analysis** In order to investigate the characteristics of one of the predominant spawning structures (for Torbay and Poole) in more detail, an assessment of the lengths



and positions of egg clusters on *Z. marina* plants was undertaken in Torbay during 2011. In order to examine the data visually, a box plot of seagrass length was created using R software (RGui, version 2.1.2.0) with the data grouped by month and a box plot of the height of the egg cluster on the seagrass plant measured from the seabed to the base of the egg cluster. In addition, basic univariate statistics (e.g. mean, minimum, maximum of seagrass and egg cluster length measurements) were calculated and compared to analyse patterns of spawning on *Z. marina* plants.

### 4.3 Results

#### 4.3.1 Qualitative assessment of natural spawning structures

##### 4.3.1.1 Characteristics of natural egg laying structures

A total of 15 different types of spawning structure were recorded in this study. The height of these structures varied between 20 and 800 cm and the widths of sections used for egg attachment varied between 0.6 and 15 mm. A summary of the key attributes of these structures and the patterns of spawning observed are presented in Table 4.2, whilst photographic examples of spawning structures are presented in Figures 4.4, 4.5 and 4.6. The patterns of spawning observed varied between structures, for example *Chorda filum* is composed of cylindrical fronds which are formed from hollow tubes of approximately 0.6 mm in diameter and which grow up to 8 m in height (Bunker et al. 2010). Whilst the diameter of fronds is small, observations of egg laying on this species showed that multiple fronds were grouped together in order to achieve a suitable size for egg attachment (Figure 4.7) with eggs observed attached to large portions of the total length of this structure. A different type of structural composition and pattern of egg attachment was observed in *Halidrys siliquosa*. This species reaches heights of between 30 and 120 cm and has compressed fronds (< 1 cm wide) with air bladders attached. It grows as a bushy structure with thalli which attach via a strong discoid holdfast (Bunker et al. 2010). The rigid nature of this structure enables it to support multiple egg cluster to be attached to the branches of this plant with coverage of large areas (Figure 4.5c). In the angiosperm *Z. marina* which grows up to 2 m in height and can form large meadows or beds, with egg attachment observed on both the stem and to groups of leaves (Figure 4.6a). In addition to plant structures,

#### 4.3. RESULTS

---

egg laying was also observed on sessile animals, for example, *Sabella pavonina* which is a polychaete worm that grows up to 30 cm in height and 4 mm in width and can form small forests (Wood 2007). Eggs were observed attached to the tubes of the worms and their rigid nature supported attachment along the entire length of the tube (Figure 4.6c).

Table 4.2: A table indicating the characteristics of natural spawning structures recorded during qualitative subtidal and intertidal surveys (2010-2012)

Species	Type	Structure			IT/ST	FR/UK	Cluster	
		Max depth	Height (cm)	Width (mm)			median size	Total recorded
<i>C. crispus</i>	seaweed (R)	24	22	xx	IT	FR	20	188
<i>C. filum</i>	seaweed (B)	xx	800	0.6	IT	FR	200	1
<i>D. ligulata</i>	seaweed (B)	9	200	2-7	ST	FR	75	3
<i>D. sanguinea</i>	seaweed (R)	30	25	xx	ST	UK	50	1
<i>F. lumbricalis</i>	seaweed (R)	12	30	2	IT/ST	FR	35	6
<i>F. serratus</i>	seaweed (B)	xx	60	20	IT	FR	75	41
<i>Gymnogongus</i> sp.	seaweed (R)	xx	10	xx	IT	FR	35	6
<i>H. siliquosa</i>	seaweed (B)	xx	120	10	ST	UK	35	30
<i>Nemertesia</i> sp.	hydroid	xx	25	xx	ST	FR	150	16
<i>Porifera</i> sp.	sponge	xx	xx	15	ST	FR	50	9
<i>S. chordalis</i>	seaweed (R)	5	20	2	ST/IT	FR	40	3
<i>S. latissima</i>	seaweed (B)	30	150	xx	ST	FR	35	4
<i>S. muticum</i>	seaweed (B)	xx	200	xx	ST/IT	FR	20	116
<i>S. pavonina</i>	fan worm	xx	30	4	ST	UK/FR	75	82
<i>Z. marina</i>	seagrass	9	200	xx	IT/ST	UK/FR	10	1007

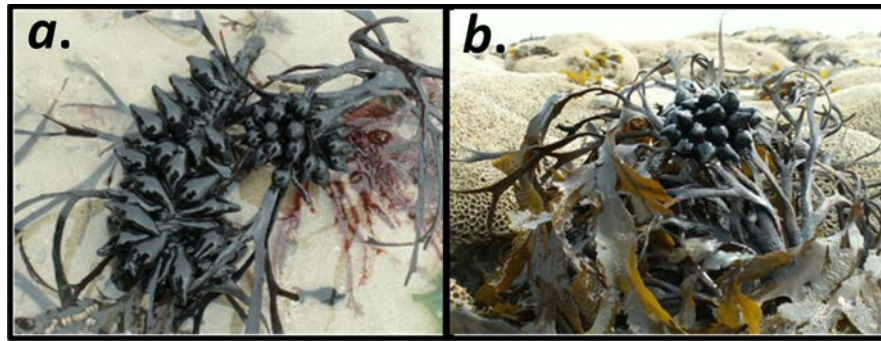


Figure 4.4: Examples of egg clusters laid on spawning structures intertidally. (a.) *Chondrus crispus*, (b.) *Fucus serratus*. Photographs courtesy of University of Caen

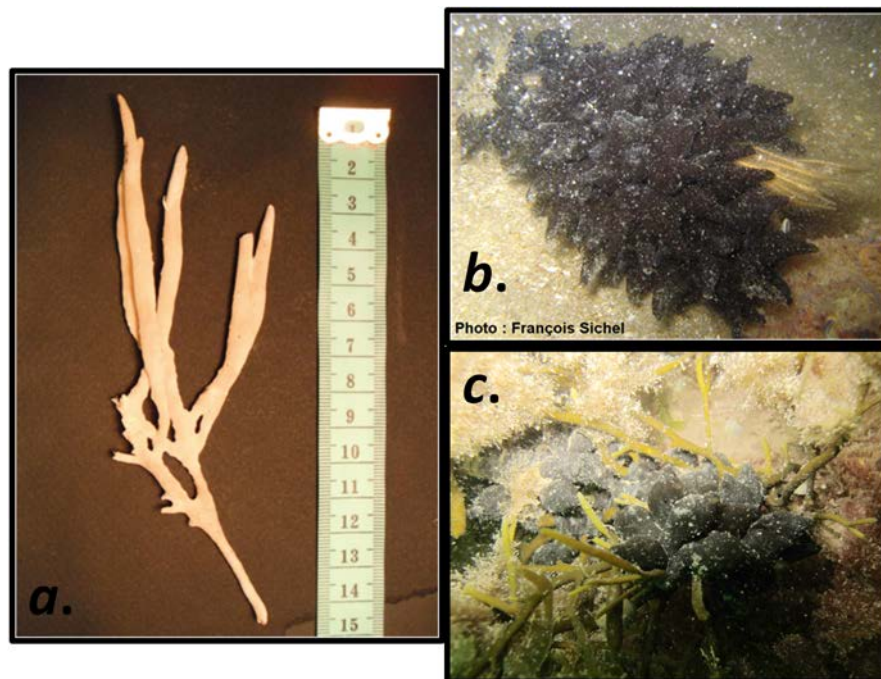


Figure 4.5: Examples of egg clusters laid on subtidal spawning structures. a. a piece from a *Porifera* sp. on which eggs were found, (b.) *Nemertesia antennina* (Photograph by François Sichel) and (c.) *Halidrys siliquosa*. Photographs a and b courtesy of University of Caen

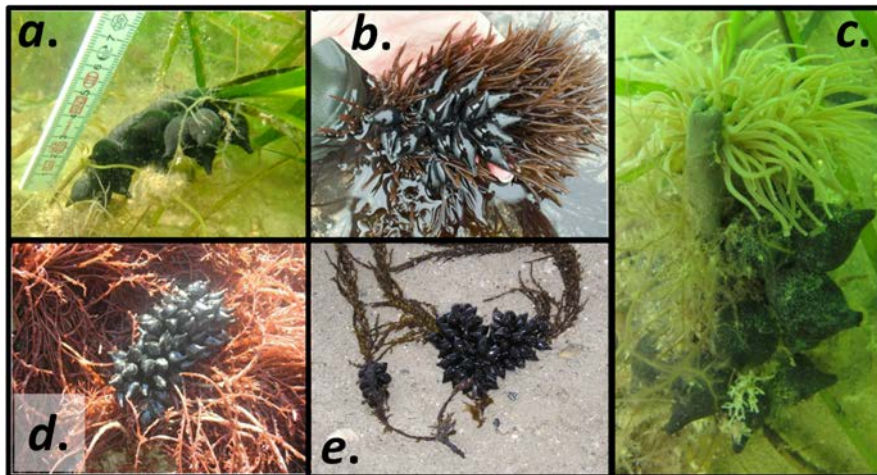


Figure 4.6: Examples of egg clusters laid on spawning structures both subtidally and intertidally. a. *Z. marina*, (b.) *Furcellaria lumbricalis*, (c.) *S. pavonina*, (d.) *Solieria chordalis* and (e.) *Sargassum muticum*. Photographs b, d and e courtesy of University of Caen

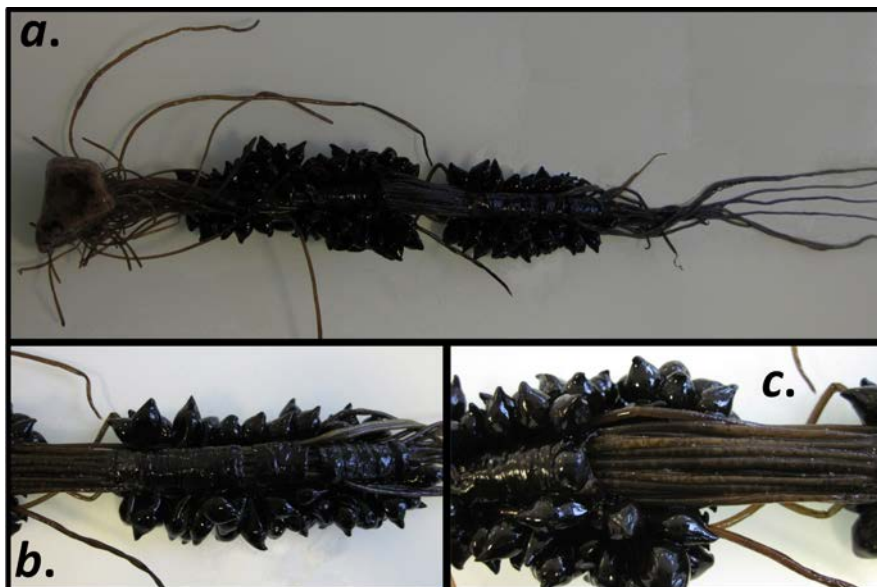


Figure 4.7: *C. filum* with cuttlefish eggs attached. (a.) egg cluster on *C. filum*, (b.) and (c). close up of egg attachment, illustrating the use of multiple fronds to obtain a suitable diameter for egg attachment. Photographs courtesy of University of Caen

#### 4.3.1.2 Structure use among sites

The diversity and type of spawning structures utilised was found to vary among sites. On the UK coast only three different types of spawning structure (Table 4.3) were identified across the three study sites (*Z. marina* 997 egg clusters, *H. siliquosa* 30 egg clusters and *S. pavonina* three

#### 4.3. RESULTS

egg clusters) with *Z. marina* providing the highest numbers of egg clusters recorded for UK sites . On the French coast a total of twelve different spawning structures were identified with the most egg clusters (188) recorded on *C. crispus*. The most diverse range of structures was identified at Agon-Coutainville (Table 4.3) with ten different types of spawning structure identified within the subtidal and intertidal ranges. *Z. marina* plants recorded the highest number of egg clusters when pooled across all sites (UK and France) and across all years with 1007 egg clusters recorded in total.

Table 4.3: A table indicating the structures, with *S. officinalis* egg clusters attached, recorded during qualitative subtidal and intertidal surveys and listed by study site

Torbay	Selsey	Poole Bay	Agon-Coutainville	Langrune-sur-Mer
<i>Z. marina</i>	<i>H. siliquosa</i>	<i>Z. marina</i>	<i>S. muticum</i>	<i>S. latissima</i>
<i>H. siliquosa</i>	<i>D. sanguinea</i>	<i>S. pavonina</i>	<i>F. lumbricalis</i>	<i>Porifera</i> sp.
			<i>S. chordalis</i>	<i>Nemertesia</i> sp.
			<i>S. pavonina</i>	<i>S. muticum</i>
			<i>D. ligulata</i>	
			<i>Nemertesia</i> sp.	
			<i>C. crispus</i>	
			<i>C. filum</i>	
			<i>F. serratus</i>	
			<i>Gymnogongrus</i> sp.	

##### 4.3.1.3 Variation in cluster size among natural structures

Figure 4.8 shows the variation in egg cluster size (number of eggs per cluster) with structure type. By comparing the variation in egg cluster size indicated in Figure 4.8 with the size, diameter and location of egg attachment illustrated in Figure 4.9 a few key points can be highlighted. For example in structures such as *S. pavonina* where the entire length of the tube (up to 30 cm) can be utilised for egg laying (Figure 4.9) the box plot indicated a relatively high median, range and maximum number of eggs per cluster. A similar pattern is shown for *C. filum* where almost

#### 4.3. RESULTS

the entire length of the structure (up to 800 cm) can be utilised for egg laying (Figure 4.9b). In contrast for structures such as *S. latissima* where only a small fraction of the total structure (e.g. Stipe) is available for egg attachment (Figure 4.9l) the box plot indicated a relatively low median, range and maximum number of eggs per cluster. In order to test whether the difference in egg cluster size among structures was significant a Kruskal-Wallis test was performed. The results of this test indicated a significant difference among structure type in the numbers of eggs laid per cluster ( $H = 431.49$ ,  $DF = 10$ ,  $P < 0.0001$ ). The following structure types were excluded from the analysis as they contained fewer than five samples per group: *Solieria chordalis*, *Desmarestia ligulata*, *Algae Z*, *C. filum*, *Delesseria sanguinea* and *Saccharina latissima*.

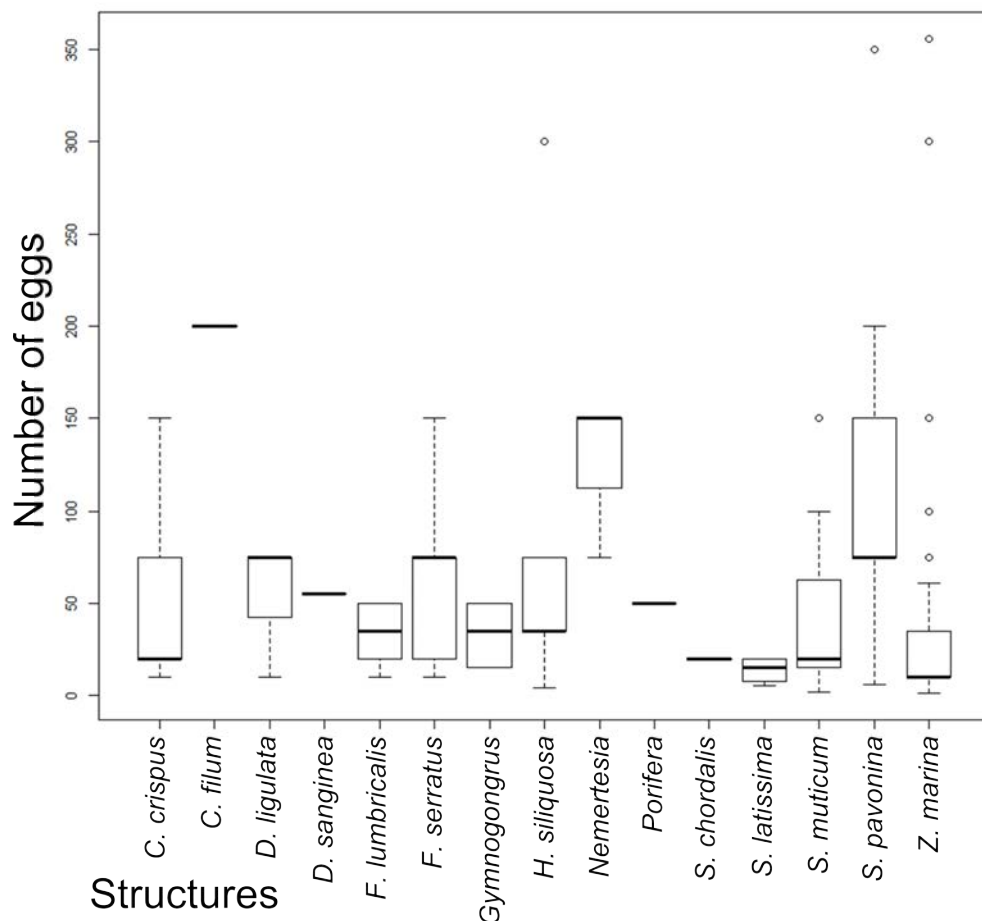


Figure 4.8: A box plot showing the variation in egg cluster size (numbers of eggs per cluster) among natural spawning structures



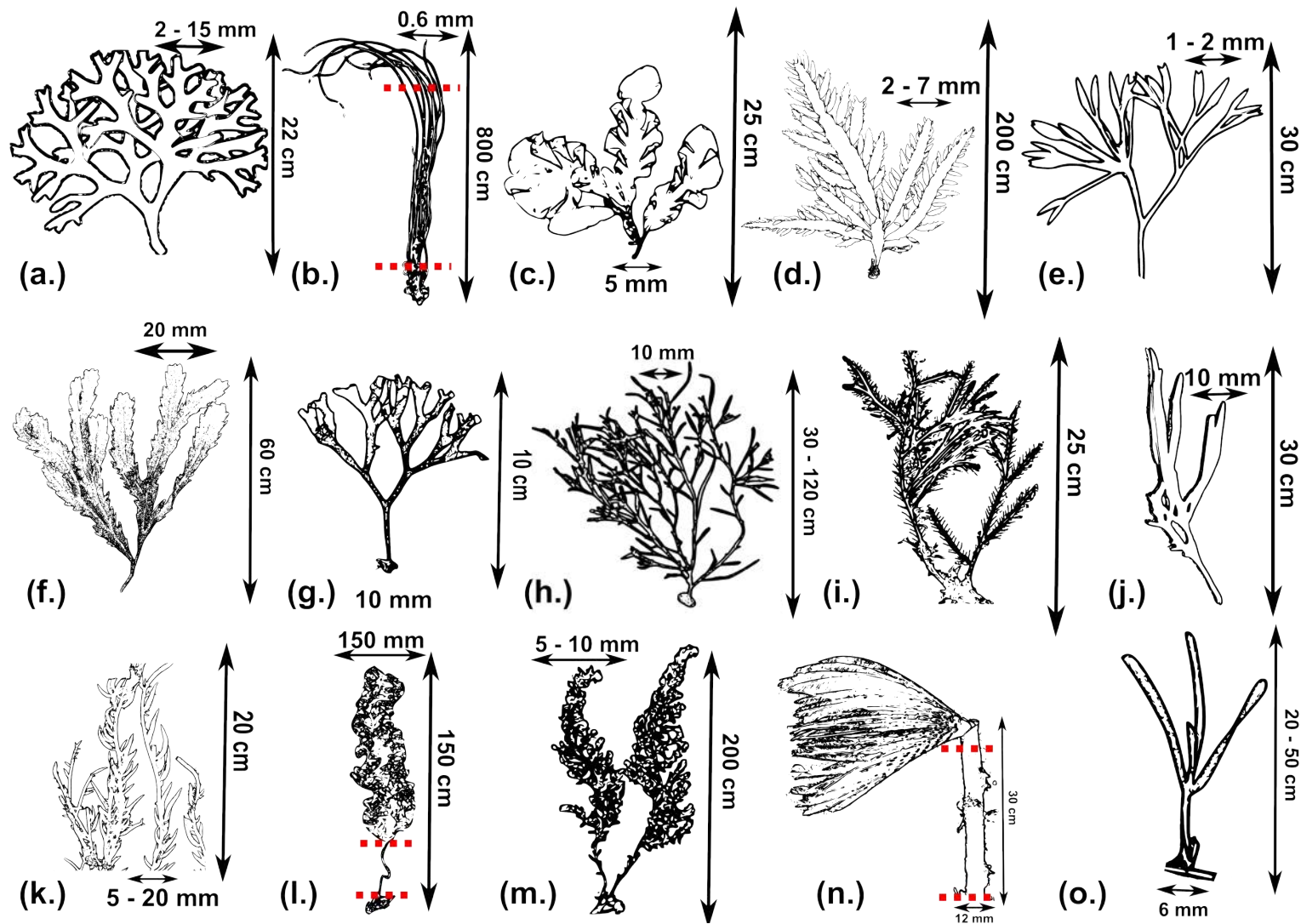


Figure 4.9: Diagrams illustrating the natural structures to which egg clusters have been found attached. (a.) *C. crispus*, (b.) *C. filum*, (c.) *D. ligulata*, (d.) *D. sanguinea*, (e.) *F. lumbricalis*, (f.) *F. serratus*, (g.) *Gymnogongus* sp., (h.) *H. siliquosa*, (i.) *Nemertesia* sp., (j.) *Porifera* sp., (k.) *S. chordalis*, (l.) *S. latissima*, (m.) *S. muticum* (n.) *S. pavonina*, (o.) *Z. marina*. Red dotted lines show examples of the rough area limits of egg attachment for a structure.



4.3.2 Comparison of spawning strata (depth)

The analysis of depth strata showed no significant difference in median egg densities at Agon-Coutainville (Kruskal-Wallis:  $H = 0.87$ ,  $DF = 2$ ,  $p = 0.6482$ ) at Langrune-sur-Mer (Kruskal-Wallis:  $H = 2.09$ ,  $DF = 2$ ,  $p = 0.3509$ ) or for both sites pooled (Kruskal-Wallis:  $H = 2.13$ ,  $DF = 2$ ,  $p = 0.3451$ ). Visual inspection of the dataset for Agon-Coutainville (Figure 4.10) indicated a difference between the types of spawning structures used within different depth strata. For example, *S. pavonina* was predominately used in the 0-5 m and 5-10 m depth stratas. *Nemertesia* sp. was predominately used within the deeper 10-15 m stratum.

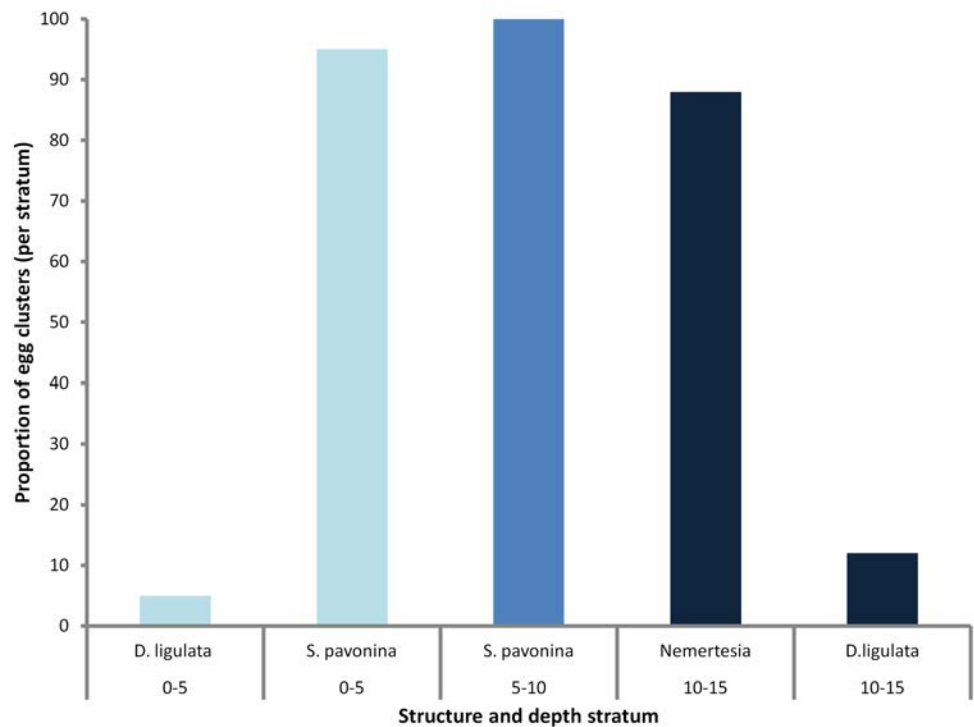


Figure 4.10: A graph showing the proportion of egg clusters recorded within each depth stratum by spawning structure type and is independent of the area surveyed (Agon 2011)

4.3.3 Comparison of spawning strata (structures)

In May 2010 a total of 147 egg clusters were recorded across all sites surveyed within Torbay (Appendix A). Egg clusters were recorded at all four seagrass sites attached to *Z. marina* plants. However, no egg clusters were recorded at any of the four mixed structure sites where seaweeds

#### 4.3. RESULTS

---

were present. Seaweeds recorded as present within mixed substrate sites included short faunal turf and mixed red and brown seaweed species.

During July 2010, ten sites (five seagrass and five mixed) were surveyed within Torbay with four replicates at each site (excluding Torre Abbey Sands where adverse weather conditions forced the survey to be terminated for safety reasons), with a total of 45 egg clusters recorded across all sites (Appendix A). Egg clusters were recorded at all five seagrass sites attached to *Z. marina* plants. However, no egg clusters were recorded at any of the five mixed structure sites where seaweeds were present. The results of these two studies indicated that a significant difference existed between the spawning pattern of these two strata within Torbay, with eggs only recorded attached to *Z. marina* plants within the seagrass stratum.

In June 2011 egg clusters were recorded at all seagrass sites in Torbay (Appendix A). Egg clusters were also recorded attached to a steel rope at Outer Millstones and to the brown seaweed *H. siliquosa* at Babbacombe Bay, there were no egg clusters recorded at the remaining mixed substrate sites. Across the eight sites surveyed at Torbay, a total of 173 egg clusters was recorded.

In June 2011 egg clusters were recorded at two of the four seagrass sites at Poole Bay (Appendix A), where eggs were attached to both *Z. marina* and *S. pavonina*. Only a single egg cluster was recorded within the four mixed substrate sites attached to a ghost trap within the site at Handfast Point. Structures recorded as present during surveys of the area included *S. muticum*, *H. siliquosa*, Kelp (*Laminaria hyperborea*), short faunal turf and large quantities of red foliose algae. Over the eight sites a total of 100 egg clusters was recorded at Poole Bay.

The replicates undertaken at both sites were pooled by strata and a Mann-Whitney U-Test performed to compare the distributions of the two groups. This was done to compare egg clusters and number of eggs observed within transects. For both situations the results indicated a significant difference between the two distributions, with the mean ranks indicating that seagrass beds were found to have significantly more eggs and egg clusters per transect than mixed seaweed habitats (Table 4.4).

### 4.3. RESULTS

*Table 4.4:* Results of the Mann-Whitney U test for differences between seagrass and mixed strata (Poole and Torbay, June 2011). MR = Mean Rank.

Condition	Test	Z value	MR Seagrass	MR mixed	Significance
No. egg clusters	Mann-Whitney U	3.18	59.30	44.56	0.0015
No. eggs	Mann-Whitney U	2.78	57.91	45.23	0.0055

#### 4.3.4 Temporal analysis of spawning within seagrass beds (Torbay 2010-2012)

##### 4.3.4.1 Egg density

The egg densities for each month and year are presented in Table 4.5 for Millstones Bay and Table 4.6 for Torre Abbey Sands. The data for 2010 are presented for both sites, although it should be noted that a different methodology was used to collect data for this year. At Millstones Bay the highest egg density recorded was in May 2010 (11.3 eggs per m<sup>2</sup>) and the lowest egg density recorded was in May 2012 (0.025 eggs per m<sup>2</sup>). Egg densities at this site were lower in 2012 (for all months) compared to 2010 or 2011 (Table 4.5). At Torre Abbey Sands the highest egg density recorded was in July 2011 (3.9 eggs per m<sup>2</sup>) and the lowest egg density recorded was in June 2012 (0.008 eggs per m<sup>2</sup>). Egg densities at this site were lower in 2012 (for all months) compared to 2010 or 2011 (Table 4.6).

*Table 4.5:* Temporal variation in mean egg density at Millstones Bay seagrass site (2010-2012). Estimates of total eggs calculated for the total area of Millstones Bay (15,500 m<sup>2</sup>). Estimates of potential spawning females calculated using the total number of eggs at a site divided by an average fecundity of 2,000

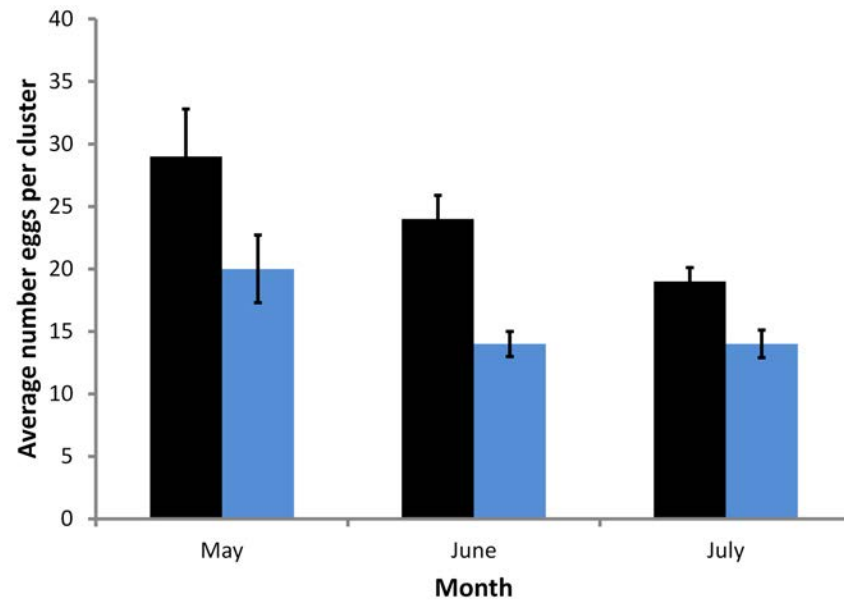
Year	Egg clusters			Egg density			Total eggs at site			Potential spawning females		
	May	June	July	May	June	July	May	June	July	May	June	July
2010	117	-	23	11.3	-	1.4	175150	-	21700	88	-	11
2011	8	116	29	0.3	3.7	1.4	4650	57350	21700	2	29	11
2012	20	77	77	0.025	0.1	0.1	388	1550	1550	1	1	1

*Table 4.6:* Temporal variation in mean egg density at Torre Abbey Sands seagrass site (2010-2012). Estimates of total eggs calculated for the total area of Torre Abbey Sands (595,000 m<sup>2</sup>). Estimates of potential spawning females calculated using the total number of eggs at a site divided by an average fecundity of 2,000

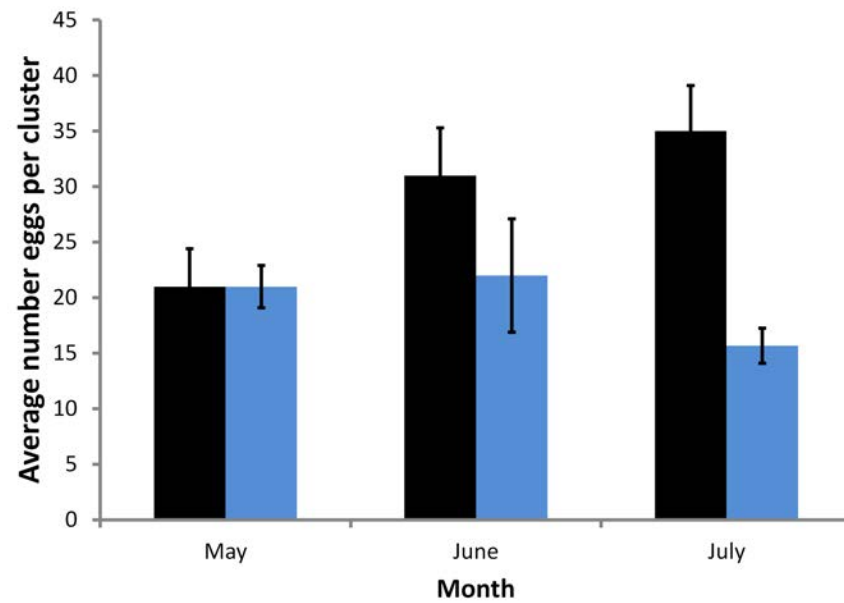
Year	Egg clusters			Egg density			Total eggs at site			Potential spawning females		
	May	June	July	May	June	July	May	June	July	May	June	July
2010	19	-	12	2.8	-	3.8	1666000	-	2261000	833	-	1131
2011	29	67	74	0.9	2.9	3.9	535500	1725500	2320500	268	863	1160
2012	56	6	44	0.07	0.008	0.055	41650	4760	32725	21	2	16

##### **4.3.4.2 Variability in the number and size of egg clusters**

A reduction in the size of egg clusters (number of eggs per cluster) was recorded between 2011 and 2012 at both Millstones Bay and Torre Abbey Sands (Figure 4.11). A Mann-Whitney U-Test found a significant difference between egg cluster size at both of these sites in 2011 and 2012, with the mean ranks indicating larger egg clusters in 2011 (Table 4.7).



(a)



(b)

Figure 4.11: Variability in egg clusters size at for 2011 (black bars) and 2012 (blue bars) with standard error displayed. (a.) Millstones Bay: A significant difference was found between egg cluster size in 2011 and 2012 ( $Z = -4.06$ ,  $p < 0.0001$ ) with larger egg clusters recorded in 2011 (mean rank = 181.77) than in 2012 (mean rank = 148.37), (b) Torre Abbey Sands: A significant difference was found between egg cluster size in 2011 and 2012 ( $Z = -3.24$ ,  $p = 0.001$ ) with larger egg clusters recorded in 2011 (mean rank = 149.38) than in 2012 (mean rank = 121.05)

### 4.3. RESULTS

Table 4.7: Results of Mann-Whitney U tests for differences between egg cluster size at Millstones Bay and Torre Abbey Sands between 2011 and 2012. MR = Mean Rank.

Condition	Z value	MR 2011	MR 2012	Significance
MB Months 2011 v Months 2012	- 4.06	181.77	148.37	0.0001
TAS Months 2011 v Months 2012	- 3.24	149.38	121.05	0.0001

#### 4.3.4.3 Fractal dimension

The results of the Mann-Whitney U test for differences between the fractal dimension of the transects in 2011 and 2012 (Appendix A) at Millstones Bay showed a significant difference between years ( $Z = 3.98$ ,  $p < 0.0001$ ). The mean rank indicated that there was a greater degree of fractal dimension (fragmentation of the seagrass bed) of the seagrass transects in 2012 (mean rank = 27.59) compared to 2011 (mean rank = 12.58). The results of the Mann-Whitney U test for differences between the fractal dimension of the transects in 2011 and 2012 at Torre Abbey Sands showed a significant difference between years ( $Z = 4.92$ ,  $P < 0.0001$ ). The mean rank indicated that there was a greater degree of fractal dimension (fragmentation of the seagrass bed) of the seagrass transects in 2012 (mean rank = 30.79) compared to 2011 (mean rank = 12.21).

#### 4.3.4.4 Seagrass analysis

A box plot of seagrass length was created to allow a visual comparison of the data between months (Figure 4.12). In order to assess whether a significant difference existed, a Kruskal-Wallis test was performed using the data grouped by month. The results indicated that a significant difference did exist between the height of *Z. marina* plants among different months ( $H = 35.81$ ,  $DF = 2$ ,  $p < 0.0001$ ). To assess where this difference lay post-hoc tests were performed for each pair of months using a Mann-Whitney U test, the results indicated that there was a significant difference in seagrass height among all months (Table 4.8). The mean ranks indicated that the seagrass was highest in June, followed by July and then May and the  $p$  values indicated that the difference was least significant between June and July (Table 4.8).

### 4.3. RESULTS

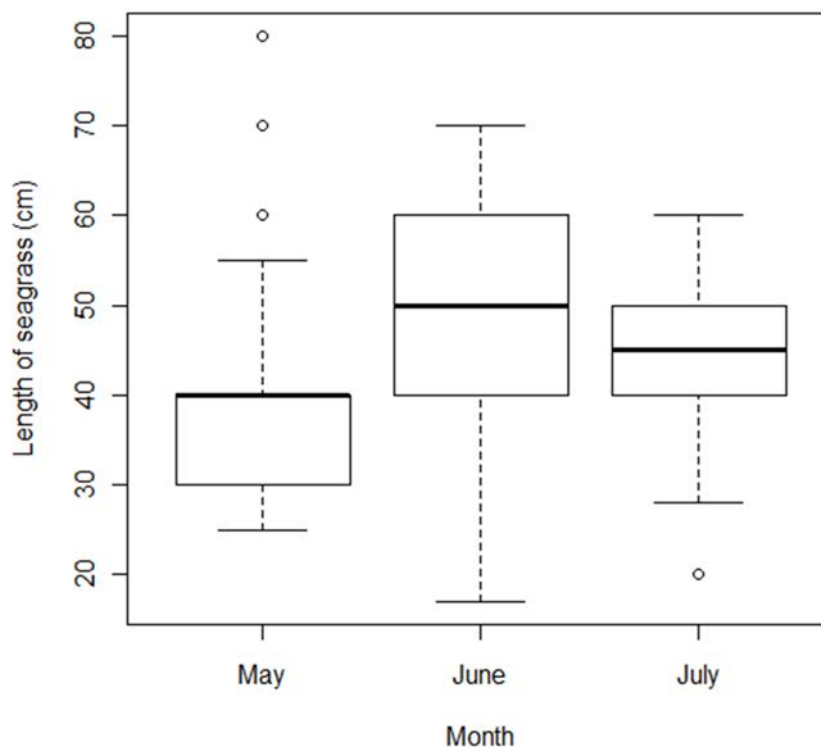


Figure 4.12: A box plot showing the variation in seagrass length (cm) in May, June and July in Torbay (2011)

Table 4.8: Results of Kruskal-Wallis and Mann-Whitney U tests for differences between seagrass height among the months of May, June and July at Torbay (2011). MR = Mean Rank.

Condition	Test	Result	MR May	MR June	MR July	Significance
May, June & July	Kruskal-Wallis	H = 35.81	-	-	-	p < 0.0001
May & June	Mann-Whitney U	Z = -5.37	74.63	153.63	-	p < 0.0001
May & July	Mann-Whitney U	Z = -3.80	38.76	-	62.68	p < 0.0001
June & July	Mann-Whitney U	Z = 3.12	-	172.25	134.05	p = 0.001

Observations on the distance (height) of the egg cluster up the *Z. marina* plant (as measured from the seabed to the base of the egg cluster) indicated that an average height of 6.4 cm (n = 361, SDDV = 3.19) was observed with a minimum height of zero cm (i.e. the egg cluster was laid at the very base or bottom of seagrass plant) (Figure 4.13a) and a maximum height of 20 cm from the base or bottom of the seagrass plant (e.g. Figure 4.13b). The average length of the



cluster from the top of the seagrass plant was 32.3 cm ( $n = 361$ , SDDV = 11.8) with a minimum of 1 cm and a maximum of 65 cm. The average percentage length of the seagrass plant that was covered by the egg cluster was 19.2 % ( $n = 361$ , SDDV = 10.3) with a minimum of only 2 % (Figure 4.13c) and a maximum of 75 % although in this latter case the plant was generally not able to remain erect and subsided under the weight of the eggs (Figure 4.13d).

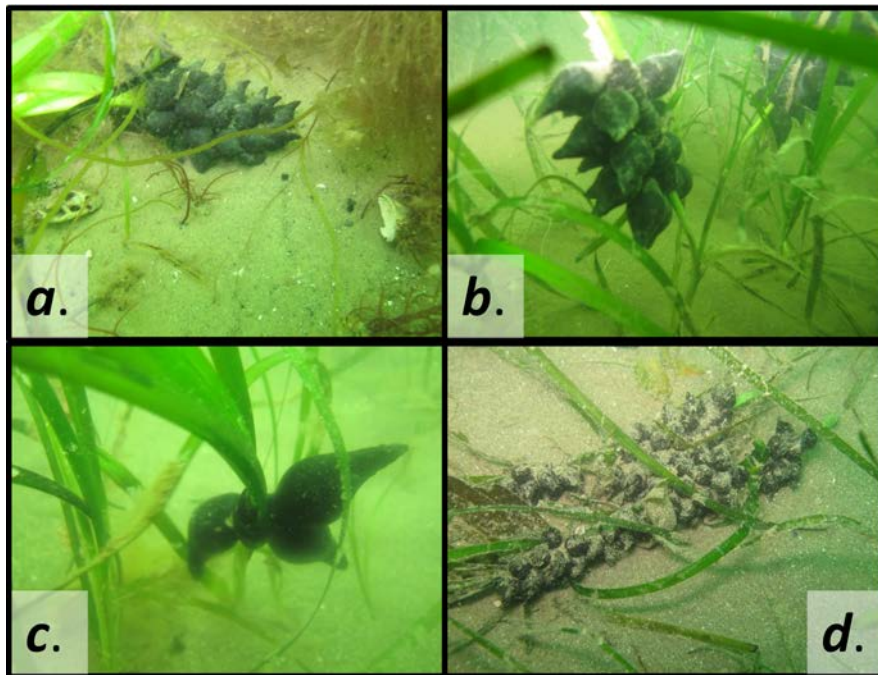


Figure 4.13: Photographs showing eggs attached (a.) at the base of a *Z. marina* plant, (b.) eggs attached higher up *Z. marina* plants, (c.) showing a small percentage coverage of egg cluster to *Z. marina* plants and (d.) showing a large percentage coverage of egg cluster to *Z. marina* plants

## 4.4 Discussion

### 4.4.1 Qualitative assessment of natural spawning structures

The range of different spawning structures identified in this study indicated that within the English Channel *S. officinalis* was not confined to a specific spawning structure or habitat. A total of 15 different spawning structures were recorded across the two coasts, whilst it would appear that the use of a structure for spawning is likely to be constrained by a maximum diameter or width, to allow the basal rings of the eggs to be securely attached around the structure. As previously indicated in the literature, which suggests a maximum diameter of 1 cm described

as suitable for spawning structures (Boletzky 1983). However, within this study a minimum diameter or width was indicated to be less important, with cuttlefish adapting structures with smaller diameters by clumping or aggregating multiple leaves or thallus until a suitable diameter or width is achieved. This was observed for both *C. filum* on the French coast and *Z. marina* on the UK coast and indicated that spawning could occur across a wider range of spawning structures than has previously been suggested. As seen in Section 4.3.1.1 and Figure 4.9, the characteristics of these structures are varied, however what drives the criteria and processes behind selection of suitable structures by females remains unknown.

##### **4.4.1.1 Spawning structures among sites**

As seen in Table 4.3 the types and range of structures utilised varied among study sites. The qualitative nature of this analysis does not allow for an assessment of these differences. However, it is of interest to note that intertidal spawning is prevalent at least one of the study sites (Agon-Coutainville) where the tidal range is one of the largest in the world. This large tidal range could allow cuttlefish within the area to access a wider range of structures across these different depth ranges. That cuttlefish eggs are capable of surviving in intertidal conditions is also of interest, with a recent study estimating hatching rates from eggs collected from the intertidal zone to be as high as 73 % (Safi, pers. comm.). This suggests that hatching rates are not detrimentally affected by the regime of daily exposure to air and/or the fluctuations in ambient conditions that accompany these changes from subtidal to intertidal (e.g. water temperature, light intensity and oxygen saturation), although the duration of embryogenesis or size at hatching may vary in eggs hatched intertidally when compared to those hatched from the subtidal range, which may subsequently affect survival or recruitment rates. In addition, the synchronicity of timing for hatching of eggs laid in the intertidal range would need to correspond with subtidal conditions for the hatchlings to have any chance of survival.

##### **4.4.2 Comparison of spawning strata (depth)**

The results of this study indicated that there was no apparent difference in median egg densities recorded within three depth strata (0-5, 5-10 and 10-15 m) at either Agon-Coutainville or Langrune-sur-Mer. It is recognised that this study only included only two sites both situated

on the French coast and so interpretation of these results cannot be extrapolated to the wider extent of the Channel. The effect of depth on the presence, distribution and composition of faunal and floral communities may be a co-factor in any study investigating depth related changes in spawning patterns and intensity. Further research to assess exactly how deep spawning occurs at each site could provide a better understanding of the relationship between depth and spawning intensity, however, the practicalities of safe limits for scientific diving restrict the use of SCUBA techniques for such surveys, indicating that a remotely operated vehicle may be required to complete depth surveys of this kind.

##### **4.4.3 Comparison of spawning strata (structures)**

In 2010, studies were undertaken within Torbay (delimited by the extent of the bay) to assess spawning patterns between two different strata, seagrass beds and mixed seaweed habitats. During these surveys (completed in May and July), it was found that within the geographical extent of this Bay, eggs were only recorded attached to *Z. marina* plants within seagrass beds. Despite the presence of a variety of seaweed species (e.g. *C. filum*, *S. muticum*, *S. latissima* and red foliose algae) within the mixed seaweed stratum that are known to be utilised as spawning structures at other study sites, no egg clusters were recorded within this substratum. Whether these results indicate a ‘preference’ for seagrass as a spawning structure/habitat within this study site remains to be determined. Seagrass has often been cited in the literature as providing important nursery areas for a variety of commercial marine species, providing food and relative safety and protection for vulnerable ELS (e.g. Jackson et al. 2001). However, demonstrating that a higher density of eggs exist within a habitat does not provide conclusive evidence of the nursery role of that habitat (Beck et al. 2001). For that, additional evidence showing increased growth rates or survival of juveniles or successful movement to adult habitats would also be required as specified by Beck et al. (2001) in their ‘Nursery-role’ hypothesis.

In 2011, studies were undertaken within Poole Bay and Torbay, although for this study the geographic extent of the Torbay study site was adjusted to include the area from Torbay to Babacombe Bay (which extends outside of the geographic extent of the Bay). Within the Torbay study site egg clusters were recorded at all four seagrass sites, but within the mixed substratum

only at one site on *H. siliquosa* (Babbacombe Bay), although a single egg cluster was recorded at Outer Millstones but attached to a section of steel rope. At Poole Bay, eggs were located on natural structures only within the seagrass stratum (although a single egg cluster was recorded at Handfast Point attached to a ghost trap). A significant difference between the numbers of eggs and the numbers of egg clusters recorded within each stratum was demonstrated with seagrass dominating in both categories. As was the case in the 2010 study, a variety of seaweed species (e.g. *H. siliquosa*, *S. muticum*, *S. latissima* and red foliose algae) were recorded within the mixed stratum that are known to be utilised as spawning structures at other study sites. A number of theories can be proposed to account for the difference in spawning patterns that was observed within these two strata: (1) a 'preference' for seagrass structures/habitats exists at this sites, as it provides an appropriate ecological environment for the development and survival of ELS (e.g. food and shelter); (2) in contrast to seaweed sites, the hydrodynamic conditions within seagrass areas (e.g. reduced current flows and reduced exposure), may provide better conditions for spawning making it easier for female cuttlefish to attach eggs to structures within sheltered areas. The mechanisms for spawning site and spawning structure/habitat selection need to be investigated in further detail and an investigation into the effect of exposure or current strength on patterns of spawning intensity now made.

#### 4.4.4 Temporal analysis of spawning within seagrass beds (Torbay 2010-2012)

The results of this study highlighted the potential effects that changes in the spatial dynamics of seagrass beds, which were demonstrated to be an important spawning habitat within the UK study sites at Poole Bay and Torbay, can have on cuttlefish spawning patterns and intensity. Changes in the spatial dynamics of seagrass beds can occur for a variety of reasons that include both anthropogenic and natural disturbances. Seagrass beds are legislated for under several agreements which include being listed as a priority species in the UK Biodiversity Action Plan (Maddock 2008) and as a threatened habitat under the OSPAR agreement (Tullrot 2009). Despite this, in many areas these beds are still damaged as a result of anthropogenic (e.g. pollution, fishing activity and recreational boat anchoring) or natural disturbance (e.g. from physical factors such as wind and wave exposure) (Maddock 2008; Tullrot 2009).

In 2012 the subtidal survey data from Torbay indicated a significant increase in the fractal dimension (spatial heterogeneity) of sampled seagrass beds compared to the 2011 subtidal survey data. Fractal dimension is used to represent the degree of fragmentation (or spatial heterogeneity) within a seagrass bed, which is described by a combination of the area of seagrass cover, patch size and the distance between patches (Jackson et al. 2006). The spatial dynamics of seagrass beds are known to be influenced by a range of factors including natural disturbance (e.g. wind-generated wave dynamics), which can affect both the development of the bed and its heterogeneity or patchiness (e.g. Turner et al. 1999; Robbins and Bell 1994). This change in fractal dimension (or spatial heterogeneity) of the sampled seagrass beds could be caused by anthropogenic (e.g. pollution) or natural disturbance (e.g. storms). Torbay is an easterly facing bay and is therefore vulnerable to easterly winds, during the spring of 2012 Torbay was exposed to higher levels of easterly winds in April 2012 (Figure 4.14) in the month prior to surveys being conducted. The resultant damage from the easterly winds to the seagrass habitat in the area may have affected the growth, health and extent of the seagrass beds within Torbay. This is reflected in the levels of fractal dimension within transects compared from 2011 and 2012, with significantly higher levels of fractal dimension in 2012 transects indicating a higher level of fragmentation. The numbers of eggs per cluster and egg density were also significantly different between 2011 and 2012 with larger egg clusters and higher densities of eggs recorded in 2011 than in 2012. The results of this study indicate that as the fractal dimension of seagrass transects increased (e.g. an increase in the fragmentation of the seagrass landscape) the density of cuttlefish eggs laid at a site decreased. This could indicate a link between egg laying patterns and status and fragmentation of seagrass beds, such that events that cause variation in the spatial dynamics of seagrass beds (e.g. wind-generated wave dynamics from storms) may also operate to produce differences in the spawning patterns of *S. officinalis* within these areas both spatially and temporally. By developing a better understanding of this relationship, information required for management of this important habitat and commercial fishery resource can be provided.

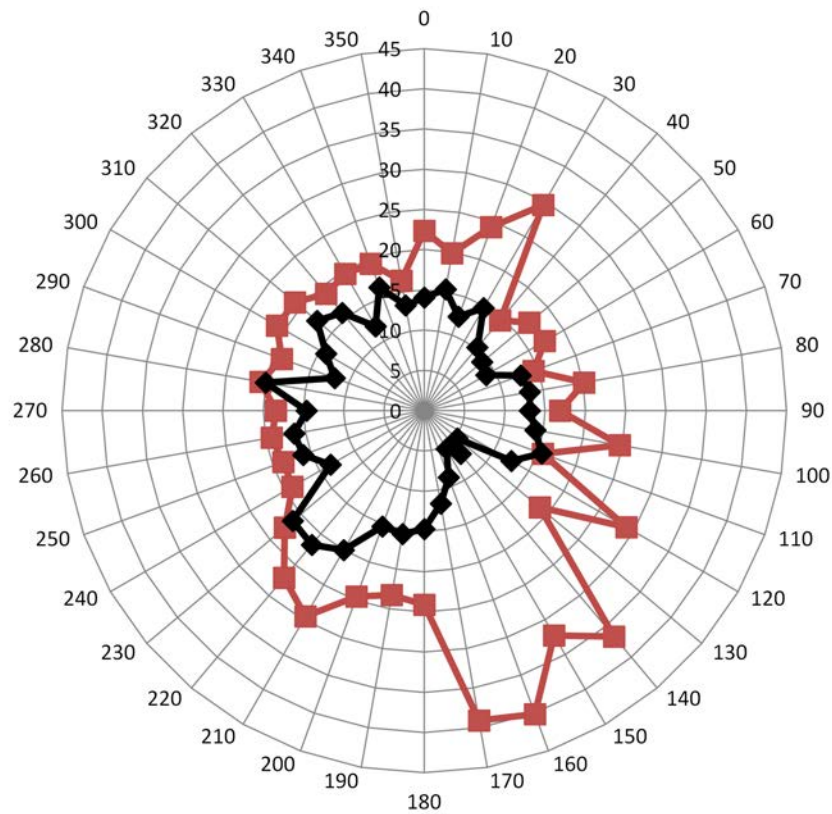


Figure 4.14: A wind rose showing the mean wind speed and direction for April 2011 (black line) and April 2012 (red line) (UKMO 2012)

At Millstones Bay where the seagrass bed covers an area of approximately 15,500 m<sup>2</sup> (TCCT 2006) the total numbers of eggs laid (estimated per month) ranged from 388 (May 2012) to 175,150 (May 2010) from this data an estimated number of spawning females present at the site (per month) was calculated to range from one (May 2012) to 88 (May 2010) (Table 4.5). At Torre Abbey Sands where the seagrass bed covers a larger area of approximately 595,000 m<sup>2</sup> (TCCT 2006), the total numbers of eggs laid (estimated per month) ranged from 4,760 (June 2012) to 2,320,500 (July 2011), whilst the respective estimates for numbers of spawning females (per month) ranged from two and 1,160 (Table 4.6). This temporal variation may also be linked to changes in the spatial dynamics of the seagrass beds between years.

#### 4.4.5 Selection Strategies

The results of this study have indicated that female *S. officinalis* can lay their eggs on a wide variety of erect fauna and flora. At different sites, the patterns and use of these structures varied,

in part this may be due to the availability of the structures (e.g. the presence/absence of a structure at each site) but, at the UK study sites of Poole and Torbay spawning was predominately associated with *Z. marina* plants within seagrass beds even though alternative structures, identified as suitable for spawning at other survey sites or locations, were present (e.g. *H. siliquosa* and *S. latissima*). Whether this indicates a ‘preference’ for *Z. marina* as a spawning structure at these locations has yet to be determined. Further investigation at sites where eggs were found to be present and where eggs were not found to be present among both seagrass and mixed seaweed strata would be of interest to assess whether additional hydrodynamic or biotic factors may influence spawning intensity or absence of spawning at different survey sites.

Given the results of this study it would appear that the pattern of spawning substrate selection by female *S. officinalis* is not ‘random’ (i.e. spawning on the first suitable substrate encountered), but to the same degree it would appear that neither do they exhibit a ‘specificity’ for a particular spawning structure. An intermediate spawning selection strategy would better suit the patterns of spawning observed within this study, with the potential for *Z. marina* to provide a ‘preferred’ spawning structure that may, at least at the study sites of Poole and Torbay, be used preferentially, but not to the complete exclusion of other structures.

A habitat ‘preference’ is often assumed when animals are found to associate with a particular habitat or spawning substrate, suggesting that they have actively ‘selected’ that habitat from a variety of suitable habitats encountered (Rosenzweig 1981). Habitat preference can be defined as ‘the ratio of the use of a habitat over its availability, conditional on the availability of all habitats to the study animal’ (Aarts et al. 2008). It is expected that animals will ‘select’ or ‘prefer’ a spawning habitat which confers advantages for reproductive success and/or survival of embryos and ELS (e.g. Levins 1968). To enable organisms to demonstrate an active ‘selection’ for a habitat, then some sort of selection/choice process is required (e.g. sensory selection or natal imprinting). This kind of selective/preferred spawning behaviour for a spawning structure or substrate is already known to occur in some species of cephalopod, for example Sauer et al. (1992) demonstrated that for *L. vulgaris reynaudii* fine grain sand was the preferred spawning substrate, a specific grain size may be preferable to form a good anchoring for the eggs, as

suggested by Augustyn (1990).

Within the current literature there is only one main example of a cephalopod species demonstrating specificity in spawning structures (*S. apama* Hall and Hanlon 2002). It is far more common to find examples of cephalopod species which lack specificity and utilise multiple spawning substrates (Moltschaniwskyj and Pecl 2003). Moltschaniwskyj and Pecl (2003) state that in both South Australia and Tasmania, the southern calamary species *Sepioteuthis australis* displays a lack of specificity for spawning substrates, with eggs observed to be attached to a wide variety of both natural substrates (e.g. attached to *Amphibolis antarctica*, macrophyte algae or embedded in sand) and artificial substrates. The same would appear to be true for *S. officinalis* spawning observed within this study, with a variety of spawning structures identified. In addition, other authors have also observed *S. officinalis* spawning on a wide variety of natural (e.g. seagrass, algae, hydroids, crabs) (e.g. Boletzky 1983; Clark 2007) and artificial (e.g. cuttle pots, ropes, sticks) (e.g. Clark 2007).

#### **4.4.6 Data limitations**

##### **4.4.6.1 Methodological variation**

One of the biggest limitations of the three year dataset that was collected as part of this research, across both the French and UK coastline, was the different methodologies used for both the design and implementation of the surveys at different study sites. This has significantly reduced the validity of this dataset to be analysed quantitatively as a whole, using all study sites, which had been the intention at the outset of this research. The reasons for the differences in methodology are two-fold. Firstly at the outset of the project, publically available benthic habitat and sediment maps were originally intended to be used to stratify the study sites into three strata (by habitat type) for the surveys. However, the data available were not at a high enough resolution in the <10m zone to enable stratification to this level and alternative stratification methods had to be used, according to the data available at each site. Secondly, a wide variety of hydrodynamic conditions existed among the survey sites. The large tidal currents exhibited on the French coast meant that divers found they were unable to use the standard methodology that had been designed in the UK using 50 m<sup>2</sup> circular belt transects to undertake the surveys. This resulted



in the use of two separate methods being used in 2010 to complete the surveys. In the UK the standard methodology of 50 m<sup>2</sup> circular belt transects was adhered to, whilst in France a timed GPS-tracked drift transect was the only methodology found to be suitable and safe for use in the water conditions. In 2011 a meeting between UK and French divers was arranged to try and find a standard methodology that could be used on both coasts. Discussions were resolved for UK divers to use an alternative methodology that used line belt transects of 100 m<sup>2</sup> as a means of trying to make the two survey methods as similar as possible. However, in practice these methods were still very different, whilst UK line belt transects covered a set area (100 m<sup>2</sup>) the French method used a set time (15 minutes) which meant the area covered varied considerably between transects (e.g. 383 m<sup>2</sup> to 2045 m<sup>2</sup>) depending on the rate and direction of the current, among other factors. In addition, UK sites had been divided into two broad habitat based strata, seagrass beds (seagrass stratum) and mixed seaweed habitats (mixed stratum) by overlaying seagrass habitat maps produced by (TCCT 2006) in 2006 on to the study site to produce a seagrass stratum and labelling the remainder of the area as mixed seaweed stratum, with large areas of bare sand excluded from the stratum following a broad-scale drop-down camera survey. However, at the French sites, no such habitat delineation existed and the large variable area covered by transects reduced the ability of the divers to survey specific habitat. The area was instead divided by depth strata for which the data were easy to obtain and for which the large blocked areas created were well suited for the transect methodology used. The vast differences in environmental, physical, ecological and hydrodynamic conditions among the study sites had made it very challenging to produce a unified dataset for which robust, parametric statistical analysis could be produced. However, the undertaking of diving studies at the scale of the entire English Channel, with dive teams based in two different countries and with diving sites within both basins of the Channel was and still remains a vital goal in order to obtain the information that is necessary to manage this shared fishery resource at this larger scale. In addition, in many ways the diving research was a success despite the limitations of the dataset obtained at this larger scale. For example, the knowledge that female cuttlefish are able and indeed do spawn over this wide range of conditions is of interest in itself. That eggs are laid at sites with macrotidal regimes where the tidal range can reach up to 15 m (e.g. Agon-Coutainville) and

eggs can be exposed to the air on a daily basis, but also at microtidal sites where the tidal range is only 4 m and eggs are always subtidal, highlights the degree of plasticity and the complex range of patterns that exist within the spawning behaviour of this species.

##### 4.4.7 Conclusions

The patterns and intensity of spawning by *S. officinalis* within the inshore waters of the English Channel were shown to vary both spatially and temporally. Eggs were found attached to 15 natural structures across all study sites (UK and France). Whilst at the UK study sites only three separate spawning structures were identified, at the French study sites 12 separate spawning structures were described. At the UK sites of Poole Bay and Torbay, *Z. marina* was the dominant structure used for spawning, however whether this determines a ‘preference’ at these sites remains to be determined. The process by which selection of spawning structures or habitats may occur in female *S. officinalis* remains unknown. Whilst there was shown to be no significant difference in the numbers of eggs or egg clusters laid within different depth strata (0-15 m) at the French site of Agon-Coutainville, this site did support eggs that were laid on structures in the intertidal zone, increasing both the area and diversity of structures available for spawning. The results of this study also indicate that changes in the environmental, hydrodynamic or physical conditions at a site may affect the patterns and intensity of spawning, with interannual variation between years, depending potentially on the conditions encountered.



## Chapter 5

# Acoustic tagging for the study of adult and sub-adult *S. officinalis* in inshore waters

Parts of the work in this Chapter has been submitted for publication:

- Wearmouth, V.J., Durkin, O.C., Bloor, I.S.M., McHugh, M.J., Rundle, J. and Sims, D.W. (in press). A method for long-term electronic tagging and tracking of juvenile and adult common cuttlefish *Sepia officinalis*. *Journal of Experimental Marine Biology and Ecology*.
- Bloor, I.S.M., Wearmouth, V.J., Cotterell, S.P., McHugh, M.J., Humphries, N.E., Jackson, E.L., Attrill, M.J. and Sims, D.W (submitted). Movements and behaviour of European common cuttlefish *Sepia officinalis* in English Channel inshore waters: first results from acoustic telemetry. *Journal of Experimental Marine Biology and Ecology*.

### 5.1 Introduction

Management measures to support and maintain a sustainable fishery require a thorough understanding of the temporal and spatial use of habitats across the lifecycle of a species. Hence, a detailed knowledge of the spatial ecology (distribution in time and space) of this species, in particular the movements, migrations and behaviour of key life stages such as spawning adults, is both ecologically and commercially important for ensuring sustainable management of this population in the English Channel.

What is currently known about the free-living movements of this species is generally based upon mark and recapture studies (e.g. Boucaud-Camou and Boismery 1991; Ezzedine-Najai

et al. 1997), fisheries data (e.g. Dunn 1999; Denis and Robin 2001; Wang et al. 2003), or the occurrence of beaks or other hard parts in predator stomach samples (e.g. Morte et al. 1997; Salman et al. 2001). From the basis of these studies, a consistent life cycle has been described for *S. officinalis* which is punctuated by migratory behaviour. Nevertheless, the exact migratory routes have yet to be identified and the locations of inshore spawning grounds are often only inferred, at a large scale, from the known presence of commercial trap fisheries for cuttlefish. The exact habitats utilised by this species and the range of movements and interactions that occur within or between these inshore spawning grounds remain unknown. Specifically, information on how long sexually mature adults spend in these inshore spawning areas and whether they remain at a single specific site, indicating a degree of seasonal or short-term site fidelity, remains unknown.

Animal-borne electronic tags (acoustic and archival) provide useful tools by which we can advance our knowledge of the movements and behaviour of cuttlefish and other cephalopods. For example, Rigby and Sakurai (2005) used acoustic telemetry to study the movements and behaviour of the octopus species (*Enteroctopus dofleini*) in the inshore waters of Japan and found unusual vertical movements of individuals that represented tagged individuals scaling fish nets to attain easy access to the fish trapped within. Pecl et al. (2006b) also used acoustic telemetry in the inshore areas of Tasmania, Australia, to investigate the movements of the calamary squid species (*Sepioteuthis australis*) for spatial management, enabling the authors to assess the effectiveness of a closed area for the protection of spawners during the egg laying period. Acoustic and archival tags have also been used in combination in Australia to monitor the energetics and movements of the Australian giant cuttlefish (*S. apama*) within localised inshore areas (O'Dor et al. 2002; Aitken et al. 2005; Jackson et al. 2005). These studies all demonstrate the potential of acoustic telemetry to enable scientists to study the complex movement patterns of free-ranging cephalopods and to use this information to infer their behaviour and develop new insights into their spatial ecology.

Advances in acoustic telemetry technology and the increasing miniaturisation of electronic tags (Semmens et al. 2007), combined with the development of new tag attachment procedures for

*S. officinalis* (Wearmouth et al. 2012), have now made it possible for smaller marine invertebrates such as these to be tagged. These advances have enabled the fine scale movements and behaviours of *S. officinalis* to be monitored using traditional radio acoustic positioning systems, which can be temporarily deployed for the duration of the study. In addition, longer-term acoustic monitoring is also possible using static acoustic arrays, such as that developed and deployed by the Marine Biological Association in Whitsand Bay. This static system allows the departures, arrivals and occupancy times of tagged individuals within the area to be monitored in order to study site fidelity and spatial dynamics. Previous studies have used this array to monitor several fish species (e.g. small-spotted catshark, *Scyliorhinus canicula*, Jacoby et al. (2012)), but to date its use as a system to track inshore cephalopod species remains untested.

During the spring and summer months, sexually mature cuttlefish migrate inshore to spawning grounds in the shallow coastal waters of the English Channel, where they loosely aggregate to mate and spawn. Females then lay benthic egg clusters which are attached to upright structures that radiate from the seabed. During this critical key life stage this species are also the subject of focused fishing pressure, both from trawlers and nets during the inshore migration and from the cuttlefish trap fishery on the inshore spawning grounds themselves. One of the major issues currently associated with the trap fishery is the tendency of female cuttlefish to lay their eggs on the traps (both internally and externally), which are then often removed by fishermen mid or post fishing season during the cleaning process. Whilst a great deal of research has been undertaken in the laboratory to investigate aspects of this key life stage (e.g. Boletzky 1986b, 1987a, 1988, 1989; Forsythe et al. 1994), demonstrating that in captivity this species has a high degree of flexibility in its reproductive behaviour, and is capable of both semelparous (one oviposition event, at one location and dying shortly afterward (Fritz et al. 1982)) and unisexual intermittent (multiple oviposition events over a single breeding season, at a single or multiple spawning sites (Kirkendall and Stenseth 1985)) spawning patterns. In contrast, our limited knowledge on the *in situ* spawning behaviours and movements of these adult spawners within natural populations and habitats remains of concern for their future management, especially in light of the additional anthropogenic egg loss from the cuttlefish traps. It is hoped that electronic tagging may help provide additional insights into the dynamics of spawning movements and

behaviours of *S. officinalis* within natural populations, enabling such knowledge gaps to be shortened.

The aim of this study was to investigate the fine scale movements and behaviours of both adult and sub-adult cuttlefish within the inshore waters of the English Channel using two acoustic telemetry methods. Habitat use and site fidelity of spawning adults were assessed in real-time within a known spawning ground, whilst the movements and behaviours of sub-adult cuttlefish were also studied with the aim of determining whether sexually immature individuals exhibit site attachment to a specific area (e.g. seasonal site fidelity) and whether they return to the same geographic locations or areas (e.g. natal homing of regional philopatry) during subsequent years.

### 5.2 Objectives

1. To assess the feasibility of using electronic tags to study *S. officinalis* in their natural environment (i.e. validating the transference of novel tagging techniques from the laboratory to the field).
2. Monitor the movements of sexually mature adult cuttlefish within a known spawning site, in order to monitor their habitat use, assess the degree of seasonal site fidelity (if any) that occurs and to infer possible associated behaviours (e.g. reproductive patterns).
3. To determine whether sub-adult (Year 1) cuttlefish remain at a single coastal locality (e.g. seasonal site fidelity) during the inshore period or whether they move along the coastline to multiple localities.
4. Estimate how long sub-adult (Year 1) cuttlefish remain in inshore waters before making their return offshore migration to deeper waters.
5. To investigate diurnal cycles and activity patterns and whether sub-adult cuttlefish (Year 1) return to the same inshore areas as sexually mature adults (year 2) to breed and spawn (e.g. to investigate whether natal homing or regional philopatry occurs in this species).

## 5.3 Methods

### 5.3.1 Study location

#### 5.3.1.1 Torbay, Devon, U.K.

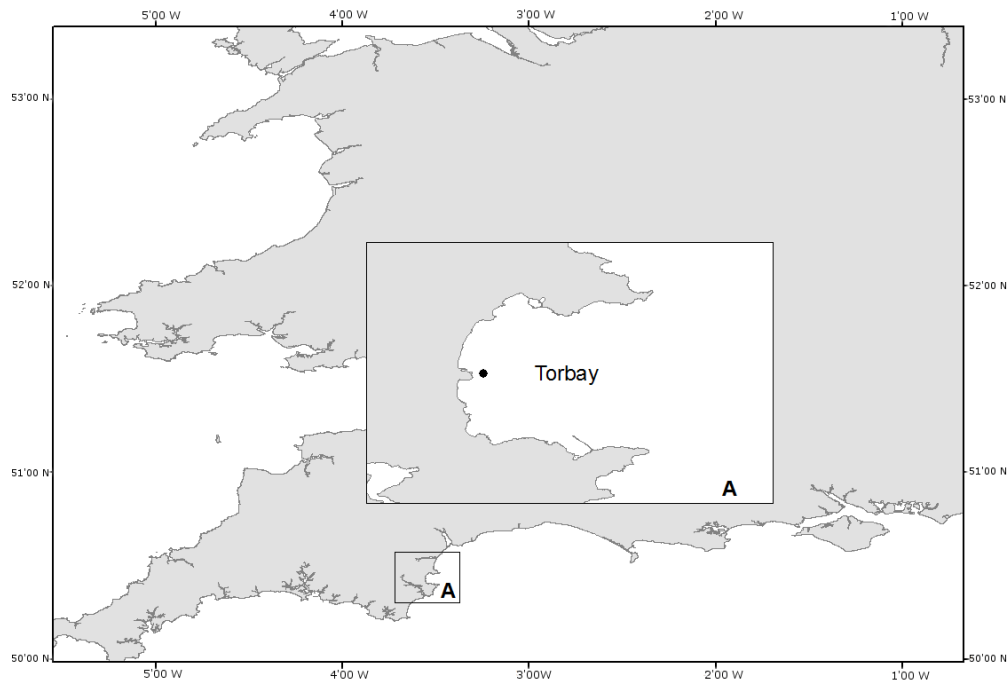
The Vemco radio acoustic positioning array (VRAP) array (operated with assistance from the Marine Biological Association's behavioural ecology group) was deployed in Torbay which is situated off the south coast of England, within the Western English Channel (Figure 5.1). The study area of Millstones Bay (50° 27.30'N; 03° 31.40'W) is a small embayment (Figure 5.2) within the Torbay area that encompasses a seagrass bed (*Zostera marina*) of approximately 1.5 hectares (15,500 m<sup>2</sup>) that is a known spawning ground for *S. officinalis* during the spring and summer. The water depth in the bay reaches approximately 10 m.

The sediment in the bay is varied with pebbles and boulders predominating in the intertidal zone, rapidly transitioning to sand in the near subtidal and then to silty-mud as the area deepens away from the shore. Large rocky outcrops also punctuate the bay with Millstones Rock on the west and Saddle Rock to the east. The water temperature within the bay was recorded during the study period using an *in situ* temperature sensor situated at approximately 5 m depth, and was found to average 12.7 °C ( $\pm$  SD 0.35).

#### 5.3.1.2 Whitsand Bay, Cornwall, U.K.

The static acoustic array (operated by the MBA's behavioural ecology group) is situated at Whitsand Bay (50° 20.40'N, 04° 15.40'W), Cornwall (UK). The study site (Figure 5.3) has a water depth of less than 30 m and is within 2 km of the coast. The substrate at the site is mainly 'soft ground' (e.g. fine sand, coarse sand and mud) although areas of gravel and broken shell are also present. Within the study area there are also two ship wrecks on the seabed, the HMS Scylla (< 28 m depth) and the James Egan Lane (< 24 m depth), which are located within Receiver 2 and Receiver 3 respectively (Figure 5.3).





*Figure 5.1:* Map of south-west England showing the location of the study site. The study site Millstones Bay is shown in inset A



*Figure 5.2:* A photograph depicting the layout of the study site Millstone bay with the seagrass bed situated within the embayment, a visual line of sight from Living Coast over the bay and Millstones Bay rock emerging from the water on the right-hand side.

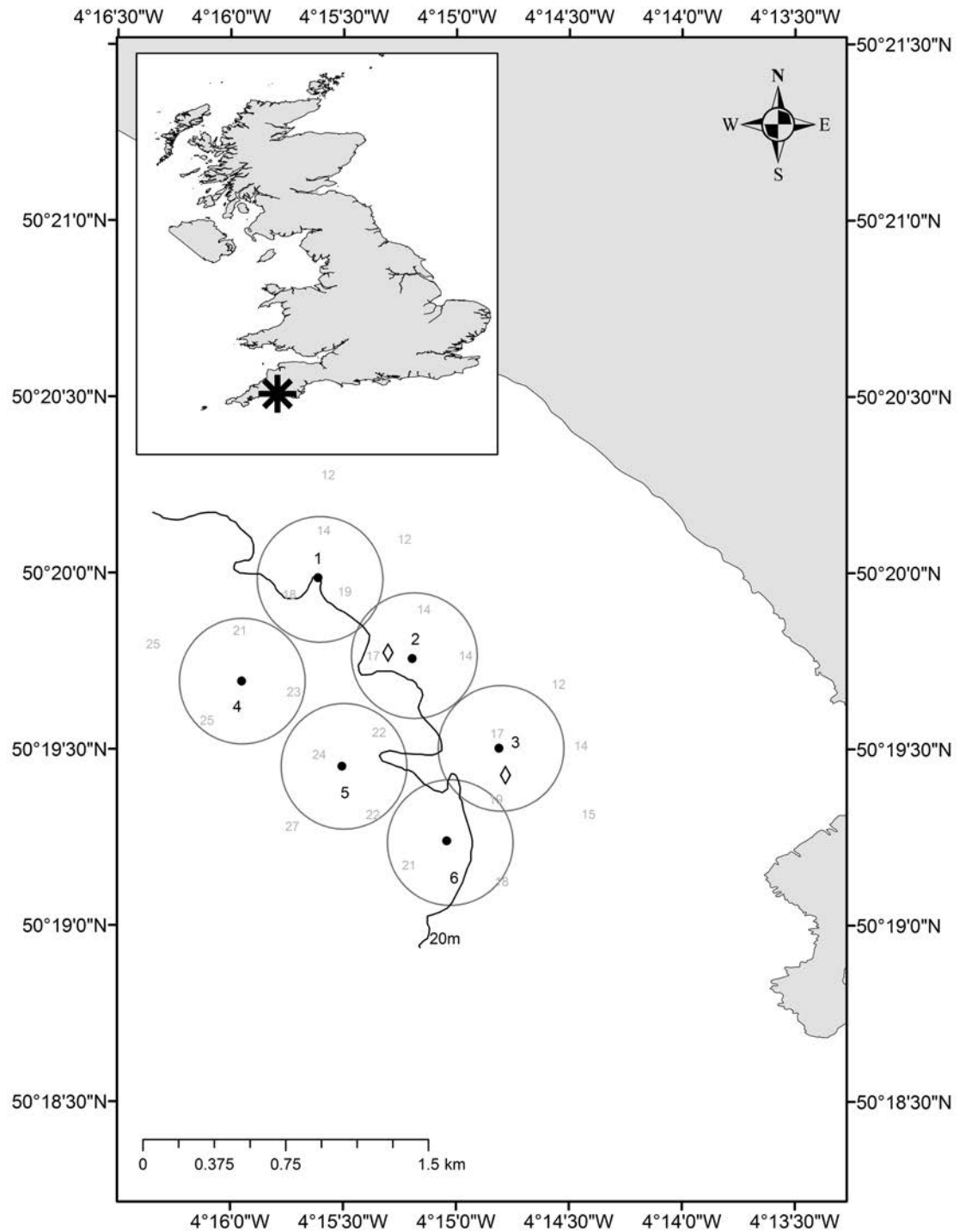


Figure 5.3: The location and spatial arrangement of the static acoustic array (6 VR3-UWM receivers) at Whitsand Bay, Cornwall, U.K. The grey area denotes land, black line denotes 20 m depth contour, black dots indicate locations of six receivers and larger grey circles indicate the approximate extent of each receiver (data from the MBA behavioural ecology group). Diamonds denote locations for two submerged wrecks.

#### 5.3.2 Study animals

Eight adult cuttlefish ranging in size from 170-205 mm dorsal mantle length (DML) were captured by standard commercial cuttlefish traps in Millstones Bay by the commercial fishing vessel *Our Wendy* during May 2011. On deck, animals were held in temporary storage units with clean seawater (12 – 13°C) for transfer back to the Living Coast laboratory where they were immediately placed in aerated, recirculating aquaria. At the laboratory individual animals were examined and excluded from tagging if they showed any external signs of damage or abnormal behaviour. Eight adults were tagged and re-released, into the centre of the array, on the same day as tagging.

Ten sub-adult cuttlefish (132 - 180 mm DML) were captured by short hauls of a demersal trawl (12 m otter trawl, cod-end mesh size 12 mm) in Whitsand Bay, by the research vessel *RV MBA Sepia* during summer 2011. On deck, animals were held in aquaria with a constant supply of clean seawater before being transferred to the MBA laboratory where they were held in aerated, recirculating aquaria for between three to four weeks prior to tagging. All tagged cuttlefish were released on 27<sup>th</sup> October 2011 (approximately two days after tagging) at 09:05h at the position 50° 19.549'N; 04° 15.251'W.

#### 5.3.3 Tagging methodology

All eight adult cuttlefish were fitted with continuous transmitters (24 mm long x 9 mm diameter and weighed 2.2 g in water, VEMCO V9-1L continuous transmitter, VEMCO, Halifax, Nova Scotia) which were surgically attached to the internal cuttlebone, as outlined in Section 5.3.5. Each transmitter operates at a unique frequency (63, 69, 72, 75, 78, 81, 84 kHz) to allow individual identification and functions as a 'position only' transmitter, with an expected battery life of approximately 20 days.

All sub-adult animals were fitted with a coded acoustic transmitting tag (29 mm long x 9 mm diameter and weighed 2.9 g in water; **V9-2L** coded tag, VEMCO, Halifax, Nova Scotia). Each coded transmitter operates at a frequency of 69 kHz with a nominal delay of 180s and had an expected battery life of approximately 738 days.

Acoustic transmitters were fitted into purpose-built harnesses prior to attachment to the internal cuttlebone. Each harness was constructed from a 10 mm long section of acrylic rod (17 mm diameter), with a nylon screw (3.8 mm) threaded into the base. A hole was then drilled through the centre of the acrylic section to enable the acoustic transmitter to be inserted and secured into place using a small amount of quick drying cyanoacrylate glue (Wearmouth et al. 2012). The harnesses were also printed with contact details for the MBA so that they can be returned.

#### 5.3.4 Sedation procedures

Adult cuttlefish were immobilised prior to tagging using a magnesium chloride seawater ‘bath’ (1.9 %  $\text{MgCl}_2$ ). The bath was prepared by mixing 300 g  $\text{MgCl}_2$  (Magnesium chloride hexahydrate 99 %;  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , Fisher Scientific, Loughborough, U.K.) dissolved in two litres of distilled water (13.04 %  $\text{MgCl}_2$ ). Sub-adult cuttlefish were immobilised using a higher concentration  $\text{MgCl}$  seawater ‘bath’ (3.35 %  $\text{MgCl}_2$ ) (Chapter 6, Section 6.3.3), prepared by mixing a stock solution of 600 g  $\text{MgCl}_2$  dissolved in two litres of distilled seawater (23.08 %  $\text{MgCl}_2$ ). In both cases 170 ml of this stock solution was added to each litre of seawater in the bath.

A clear perspex bath, covered externally with black waterproof material and an opaque grey lid was used to reduce any external stimuli and help settle the animal. Individual cuttlefish were transferred from the holding aquaria to the  $\text{MgCl}_2$  bath using a soft hand-held net and placed in a covered basin of seawater for transport. Periodic observations were then made to assess the degree of immobilisation and the surgical tagging procedure commenced once sufficient immobilisation was attained. Sufficient immobilisation (as summarised in Table 5.1) was judged to have occurred once the individual had floated to the water’s surface, changed to a consistent white (pallid) colouration (indicating a relaxation of the chromatophores), and all medial fin undulations had ceased (Wearmouth et al. 2012). Once medial fin undulations had ceased, the back fin was gently pinched and the reaction observed, if there was no movement of the fin in response to this stimulus the procedure was commenced. However, if a curling of the fin was observed the individual was left for one or two minutes longer and then the response re-checked.

Table 5.1: Steps of visible sedation in cuttlefish using  $\text{MgCl}_2$ ; [ \*ventilation rate (King and Adamo 2006)]

Stage	Description	Visible signs
<i>Anaesthetic</i>		
A1	Reduction in activity	Slowing of movement activity, positioned on bottom or central water column
A2	Change in body position	Floating on water's surface; randomised chromatophore action
A3	Change in body colour	Floating on surface, consistent body colour pale/white
A4	Movement cessation	Floating on surface, medial fin undulations ceased; white colouration
<i>Recovery</i>		
R1	Recovery of body position	Positioned level on bottom; shallow, slow ventilation rate; pale body colouration
R2	Recovery of body colour	Resumption of randomised chromatophore action; slow but deeper ventilation activity
R3	Recovery of movement activity	Consistent colouration; recovery of fin undulations; increasing ventilation rate
R4	Recovery of regular ventilation	Strong, regular ventilation rate ( $35 \pm 9.3 \text{ breathes min}^{-1}$ )*; normal movement patterns

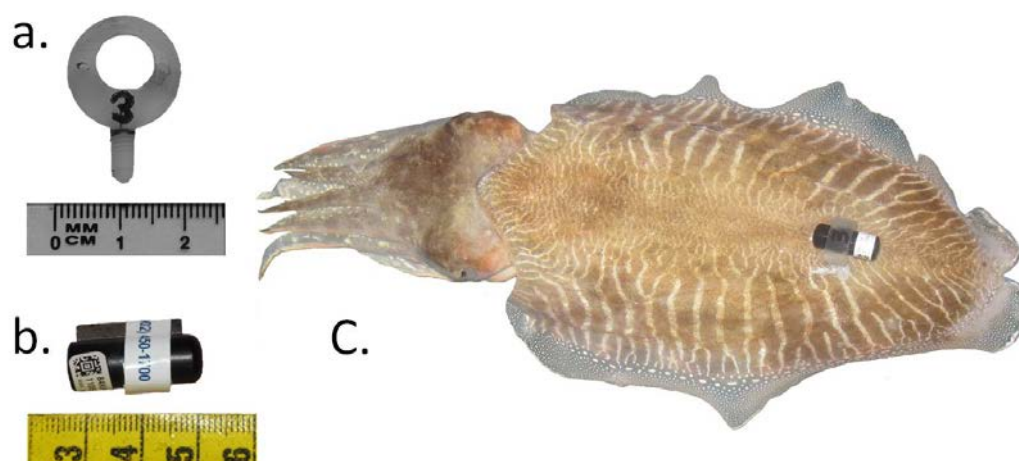
#### 5.3.5 Tagging procedure

Following immobilisation, a support frame was slowly submerged into the anaesthetic bath and the individual manoeuvred onto it, to maintain the animal at the surface of the bath and allow a laboratory assistant to hold the cuttlefish firmly by the lateral processes of the dorsal shield of the cuttlebone. This ensures that the animal remains still and that no pressure is exerted on the internal cavity of the cuttlefish which contains the internal organs (Wearmouth et al. 2012).

All individuals were tagged using a method whereby the tag harness is secured to the internal cuttlebone using a surgical procedure as first proposed by Wearmouth et al. (2012). A small incision is made at approximately 50 mm from the distal end of the cuttlebone (to enable attachment at the thickest part of the cuttlebone), both in the skin overlying the mantle and the subcutaneous membrane surrounding the cuttlebone. The skin was then held taut to expose the dorsal surface of the cuttlebone using a set of forceps. The cuttlebone has a strong external covering on the dorsal side which needs to be penetrated in order to create a hole for the transmitter harness to be attached. A battery powered, hand-held drill was used to create a preliminary starter hole in the bone (3.1 mm width). Extreme care was taken to ensure that once the external hard layer had been penetrated, the pressure on the drill was immediately released to ensure that the hole did not penetrate the remaining soft part of the cuttlebone. Once the external surface had been penetrated the hole was then widened using a second battery powered drill (3.8 mm width). The screw end of the tag harness was then tapped into the hole in the dorsal surface of the cuttlebone by hand, to a depth of approximately 5 mm, and fixed firmly in place with a small amount of quick drying cyanoacrylate glue (Figure 5.4). The surgical procedure (including weighing and measuring) took less than three minutes per individual. The weights of the transmitters in water (2.2 and 2.9 g) were considered minimal, as the tag to body weight ratio was less than 2 %, and no additional flotation was fitted to the harnesses.

Following tag attachment, each individual was weighed (g) and measured (mm DML) before being transferred to a recovery aquarium, containing clean seawater and fitted with a small water (or air) pump to ensure adequate aeration for recovery. Individuals were continuously monitored for a minimum period of 10 minutes to ensure that recovery was evident. This in-

cluded observation to ensure that the siphon was active, normal colour returned to the gills and mantle and normal body movement recommenced (Table 5.1). For sub-adult cuttlefish that were sedated at a higher concentration (see Chapter 6 for further details), following transfer to the recovery aquarium, clean seawater was pumped through the mantle cavity and across the gills for several minutes to help recover normal gill movements. Following the initial period of recovery, periodic observations were then made over the course of the following 30-60 minutes to ensure that no adverse effects, or behaviours were observed from the surgical and sedation procedures prior to being re-released back at the site of capture. Adult cuttlefish were re-released on the same day as tagging, whilst sub-adult cuttlefish were retained in holding aquaria at the MBA for 48 hours prior to being released at Whitsand Bay.



*Figure 5.4:* Attachment of acoustic transmitters (V9, VEMCO) to adult cuttlefish in Torbay. (a.) Purpose built acrylic harness, (b.) V9 (Vemco) acoustic transmitter, (c.) Tag set in purpose-built acrylic harnesses and secured to the cuttlefish using a screw and acrylic superglue (following the method in Wearmouth et al. (2012)).

#### 5.3.6 Vemco Radio Acoustic Positioning Array

The movements of adult cuttlefish fitted with acoustic transmitters were monitored in real-time using a Vemco Radio Acoustic Positioning (VRAP) array (Vemco, Nova Scotia). The array consisted of three buoys deployed in a triangular array (Figure 5.5) with each buoy fitted with a hydrophone and an acoustic transmitter with a VHF radio link which allowed the buoys to communicate with each other, as well as with the computer linked base station that is maintained

on land, by way of a line of sight two way radio connection. This enables the buoys to be controlled from the base station on land and for information collected by the buoys on the transmitter signals to be passed back to the base station for analysis and calculation by the VRAP algorithm, allowing the position of a transmitter to be determined (in real time) from the arrival times of the pulse signals to each of the three buoys.

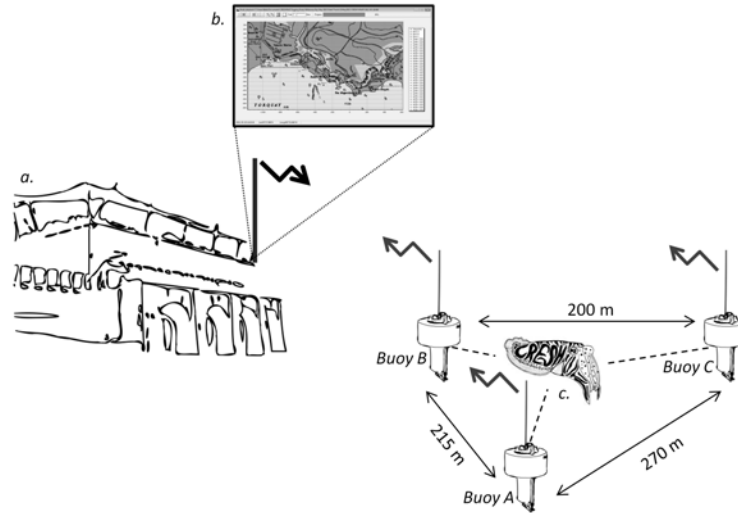


Figure 5.5: Schematic diagram illustrating the set up of the radio acoustic positioning system (VRAP) which provides continuous triangulated positions and data using the three radio-linked buoys. (a.) Living Coasts where the base station was housed, (b.) base station connected to VRAP software, (c.) VRAP buoy setup, illustrating the locations of the three buoys.

#### 5.3.6.1 Moorings

The moorings for the array were initially deployed on Friday 13th May with placement in an approximate equilateral triangle with 200 to 300 m between each (Table 5.2 and Figure 5.5). The moorings were left *in situ* for two days with small marker buoys attached in order to indicate their location. During this period the placement of the moorings was monitored using a hand-held global positioning system (GPS) unit (GPSMAP 76, Garmin (Europe) Ltd., Southampton, UK) to ensure that their position was maintained. Each of the three moorings was composed of a flat anchor (7 kg for inshore and 9 kg & 14 kg for offshore), shackled to a 2 m long section of light anchor chain by a swivel joint, to allow free rotation of the chain. A second shackle was

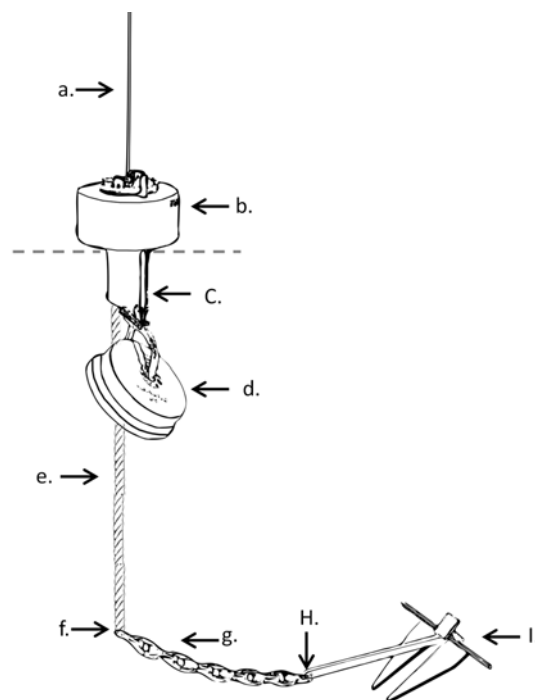


### 5.3. METHODS

---

used to attach the anchor chain to a long section of rope (Figure 5.6).

On Monday 16th May 2011 the marker buoys were replaced with the VRAP buoys to complete the set up of the array. Prior to deployment the buoys were fitted with new batteries charged at between 12.5-12.7 volts and the buoys were then tested in air before being pressure tested to 15 psi (pounds per square inch). A set of counter weights (16 kg) attached to help maintain its correct position in the water column. To prevent fraying of the rope from any movement of the weights, a section of plastic pipe was placed around the attachment rope.



*Figure 5.6:* A diagram of the moorings used for the VRAP array. (a.) antennae, (b.) VRAP buoy, (c.) hydrophone, (d.) counter weights, (e.) rope, (f.) swivel joint, (g.) light anchor chain, (h.) swivel joint, (i.) flat anchor. Grey dotted line indicates sea surface.

Table 5.2: Locations and details for the moorings for the VRAP array

Moorings	Label	Serial n.	Latitude	Longitude	Depth (m)	Dist. to A (m)	Dist. to B (m)	Dist. to C (m)
Inshore	B	2562	50° 27.367'N	03° 31.342'W	5.9 - 6.0	215	-	200
Offshore easterly	C	2563	50° 27.264'N	03° 31.309'W	9.6	270	200	-
Offshore westerly	A	2564	50° 27.345'N	03° 31.499'W	9.2	-	215	270

#### 5.3.6.2 Base station

A laptop computer was used to run the VRAP software (VRAP 5 Version 5.1.4, Vemco, Nova Scotia). A cropped bitmap image of the admiralty chart (resolution 500 DPI [dots per inch]) for the area was uploaded to the software and georeferenced using two calibration points (Calibration point A = 50° 27'N, 03° 32'W; Calibration point B = 50° 27.9'N, 03° 30'W), this enabled the base station to plot the resolved positions for each transmitter in real-time onto the admiralty chart. The base station was connected to a weather-proofed aerial antenna that was erected on the exterior of the Living Coasts building to enable a direct line of sight between the antenna and the VRAP buoys (A poster display was created and displayed at Living Coasts to inform visitors about the project; see Appendix C.3). The following settings were then used to set up the tracking regime within the VRAP 5 software. For the acoustic tags ('pingers'), the scan time was set to 12 s, the scan delay was set to 0 s and the upload interval to 12 s, the speed of sound was left at the default setting of 1500 m<sup>-1</sup> and the position average algorithm used to calculate the positions. For the buoys, the scan delay was set to 0 s, the calibration interval to 180 minutes and five positions selected to show.

#### 5.3.6.3 Passive monitoring

Passive telemetry tracking systems rely on the set of stationary receivers which are able to monitor the movements of acoustic transmitters within a predefined study area. The VRAP 5 software allows the user to program a tracking schedule for the array, the base station then instructs each of the three VRAP buoys to listen for a pre-set duration (e.g. 20 seconds) for pulses of the signal from a specified transmitter. The arrival times of these sound pulses are then transmitted via a radio signal to the base station receiver onshore (at Living Coasts). The computer software uses this information to triangulate the position of the tag as a result of the differences in arrival times of the sound pulse to each of the three buoys, allowing the position of the tagged cuttlefish to be plotted on a georeferenced map. The base station instructs the buoys to repeat this process for each of the active transmitters in turn (as defined in the tracking schedule), before repeating the process cyclically, enabling each of four active transmitters to be searched for by the array approximately every 90 seconds. In order to resolve the position

of a transmitter the pulse signal must be received by all three of the buoys. If a transmitter is positioned directly behind a buoy, then the position cannot be calculated. During this study the software program was set up to calibrate the position of the three buoys every hour in order to maximise the accuracy of their positions, and in turn, the positions of the transmitters.

**5.3.6.3.1 Positional accuracy** The positional accuracy of the VRAP system has been tested by several authors (e.g. Klimley et al. 2001; Filer 2009) which suggest that within the detection range the accuracy of the resolved transmitter positions is not uniform. The accuracy being greatest within the centre of the triangular array (approximately 2 to 3 m) and decreasing rapidly with distance outside of the array (VEMCO 2003). In addition, around each of the three buoys is a shadow zone, where the accuracy of position calculation is also reduced to approximately 20 m.

The accuracy of the VRAP system was assessed to identify the degree to which temporal error in the detection of pulses from a transmitter affected the accuracy of positional determinations by the VRAP array. This was undertaken using the VRAP Positional Simulator program (Vemco Ltd., VRAP PosSim, V. 3.01 Beta) (Figure 5.7). For the simulation, the depth of each buoy and the distances between them were set to match those recorded at the beginning of the study (Table 5.2). A temporal error for pulse detection of 0.5 ms was introduced and the simulation run for an area of approximately 1,000 by 1,500 m surrounding the VRAP buoys (Figure 5.7). The PosSim software prepares the simulation by determining the times taken for a pulse emitted, within a randomly seeded quadrat, to arrive at each of the three buoys. The arrival times are then altered by a random number (less than or equal to our pre-set temporal error of 0.5 ms) in order to simulate the variability in pulse detection by each of the three buoys. The simulated positional error occurs as the signal from the quadrant travels along slightly different pathways and at different speeds, as described by Klimley et al. (2001).

The simulation (Figure 5.7) indicates that the theoretical accuracy of positions was highest within the array (1 m accuracy) to approximately 100 m distance outside of the array (excluding the areas directly behind each buoy). The theoretical accuracy of positions was less than 20 m for a distance of approximately 400 m directly in front of the array. The theoretical accuracy

of areas behind each of the buoys was low (accuracy less than 50 m), and for areas extending outwards from the sides of the array. Whilst a large number of the data points collected during this study do fall outside of the area of highest accuracy (within the array), these ‘cleaned’ data positions still fall within the 400 m distance directly in front of the array, where the theoretical error is less than 50 m, and so are still considered sufficient to support the analysis that is presented in this chapter.

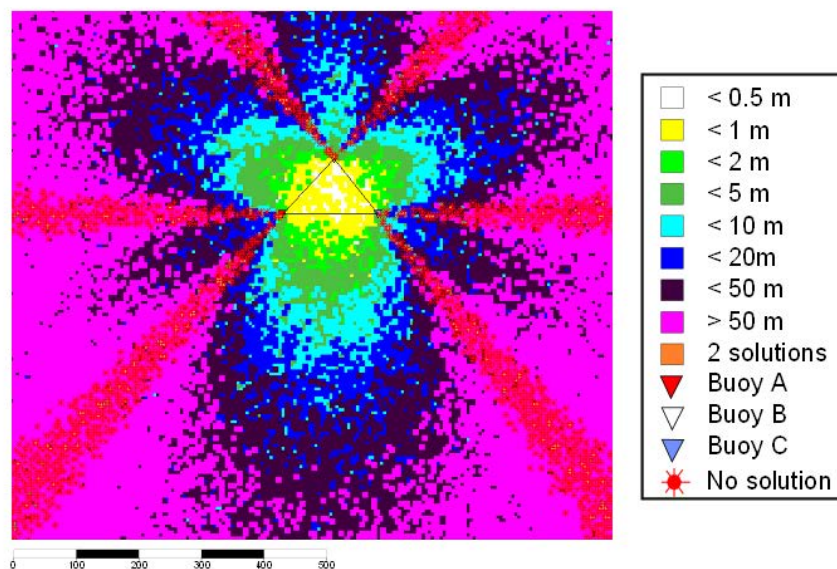


Figure 5.7: Simulation plot of the accuracy of positions determined within the detection range of the VRAP array with a receiver timing error of 0.5 ms and a transmitter depth of 10 m. The default speed of sound (1,500 ms) was also used

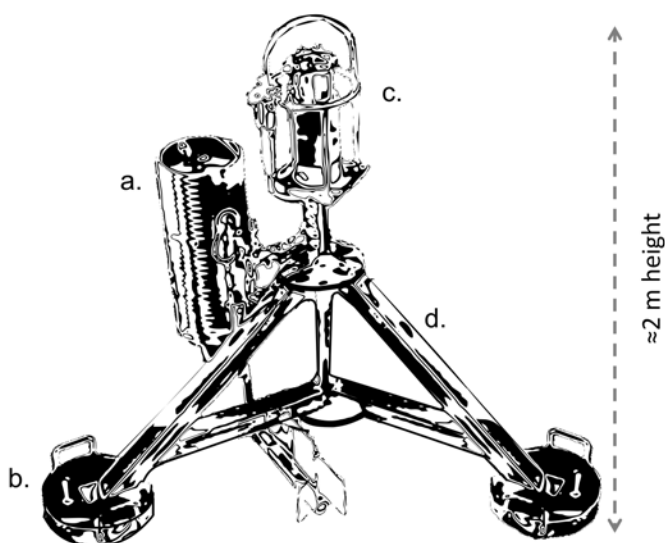
#### 5.3.6.4 Active monitoring

Active (or manual) monitoring was undertaken using a directional hydrophone and acoustic receiver (VR60 receiver, Vemco) and a hand-held GPS to locate the pulse signals emitted by the acoustic transmitters and then manually record their positions. Active monitoring was undertaken by boat on four occasions during the study (20<sup>th</sup>, 24<sup>th</sup>, 25<sup>th</sup> and 27<sup>th</sup> May 2011) and once following the removal of the acoustic array (3<sup>rd</sup> June 2011). The purpose of this monitoring was to monitor the area outside of the immediate study vicinity to confirm the presence or absence of tagged individuals, which were no longer being tracked by the VRAP system, but that might have remained in the greater Torbay area.

A typical monitoring session lasted between 1 to 2 hours, although the session undertaken at the end of the study took place over a 4 hour period, allowing a systematic search of the greater Torbay area to be undertaken. Tagged individuals were searched for, starting with their last known position recorded by the VRAP system. The directional hydrophone was set to the correct scanning frequency to detect the target transmitter and placed in the water at a depth of approximately 1 m and then rotated to 0°, 60°, 120°, 180°, 240°, 300° and 360° in order to determine the direction of the strongest signal strength. Once the direction of the target transmitter had been identified, the hydrophone was removed from the water and the boat proceeded 300 m in that direction. The process was then repeated until the position of the target tag was localised (indicated by strong detection of the signal pulse in all directions). A GPS location and a time and date stamp were then recorded. A similar process was undertaken for non-positioned tags, with systematic sampling at 300 m intervals and the hydrophone cycled through all required tag frequencies.

#### 5.3.7 Static acoustic array

Static acoustic array telemetry was conducted at Whitsand Bay, commencing on 27<sup>th</sup> October 2011 and remaining ongoing. A total of ten sub-adult *S. officinalis* were tagged using **V9-2L** coded transmitters and released back into the study site. The static acoustic array is deployed approximately 1.5 km offshore at a depth of between 14 to 25 m and covers an area of 1.5 km<sup>2</sup>. The array consists of six non-overlapping receivers (VR3-under water modem, VEMCO, Halifax, Nova Scotia) mounted 2 m above the seabed on a set of seabed landers (Figure 5.8), that are being used principally to track fish movements and space use. When a tagged cuttlefish occurs within 250 to 300 m of one of the six receivers, at the same time as their coded transmitter emits a pulse, then that specific receiver will create a log of the tagged individual's presence, recording information on the transmitter (ID, date and time).



*Figure 5.8:* A diagram illustrating the seabed landers at Whitsand Bay. (a.) Release transponder, (b.) one of the three feet used to stabilise the lander on the seabed, (c.) VR3 acoustic receiver and data logger, (d.) the main frame of the seabed lander which is painted with anti-fouling paint to prevent biofouling. The seabed landers were designed by the Marine Biological Association's behavioural ecology group and built by Underhill Engineering Ltd.

### 5.3.8 Tag recovery

For the purpose of this study, a monetary reward (£50) was offered for the return of each tagged animal together with information on its recapture date and location. In addition, posters announcing the experiment were distributed at the port and to individual fishermen involved in the local cuttlefish trap fishery.

### 5.3.9 Data processing

#### 5.3.9.1 VRAP array

**5.3.9.1.1 Track cleaning** For data collected from the VRAP array, all tag positional fixes were calculated from the VRAP 5 software (Version 5.1.4; Vemco Ltd) using the 'position-average' algorithm. Since the determination of tag locations relies on the detection of the sound pulses by all three buoys within the VRAP array and the positional accuracy of tag positions attenuates from the centre of this array, erroneous positions can be created. Such anomalies can be created as the result of the sound pulse emitted from the transmitter reaching one or more of the VRAP buoys indirectly, causing a delay in its arrival time, as a result of reflecting

off underwater features such as the seabed or rocky outcrop or from noise of water movement created during adverse weather conditions. In addition, Klimley et al. (2001) noted that the signal is more likely to travel indirectly or by multiple paths when the tagged individual swims near the seabed or the surface of the water. Such anomalies include positions calculated on land, or those separated by greater distances than physically possible for the animal to travel. In order to analyse the data correctly, the first step must include cleaning of the tracks to remove any such anomalous positions. For this purpose a data cleaning routine was developed and used to clean the data from each individual tagged cuttlefish, as follows:

1. **Positions plotted on land:** The data were extracted from the Vemco software and transferred into ArcGIS where they were plotted onto a georeferenced map of the study area (Admiralty Chart no. 26 'Harbours on the South Devon Coast'). Any positions plotted on land were then highlighted and deleted from the data file.
2. **Positions exceeding maximum range of VRAP system:** The maximum range of the VRAP system was estimated at approximately 0.5 km from the centre of the array (after which the theoretical accuracy is greater than 50 m (Figure 5.7)) and any positions outside of this range were removed.
3. **Positions exceeding maximum swim speed ( $1 \text{ ms}^{-1}$ ):** The data points were extracted from ArcGIS and transferred to a track analysis software program (Track Analysis, MBA, 2011), step lengths between successive points were then calculated and all positions with abnormally large step lengths to and from a point (using maximum swim speed filter set to  $1 \text{ ms}^{-1}$ ) were removed. The maximum plausible speed was calculated from data recorded within the VRAP coverage area ( $0.262 \text{ ms}^{-1}$ ) in addition a calculation of the maximum swim speed of cuttlefish produced by O'Dor and Webber (1991) stated that the maximum speed for cuttlefish is  $0.65 \text{ ms}^{-1}$ , therefore a conservative estimate of  $1 \text{ ms}^{-1}$  was used for initial exclusion.
4. **Positions exceeded maximum step length distance (m):** Finally using the Track Analysis software any position resulting from a movement greater than ten times the distance



between two continuous positions, in a time interval of less than 30 minutes was considered an outlier and removed from the data set.

5. **Positions averaged for every 15 minutes section of the track:** To aid with visual display of the data positions were then averaged for every 15 minute section of the track and plotted in ArcGIS. This was done for ease of viewing and did not affect the content of the data set.

#### 5.3.9.2 Static acoustic array

The data from the static acoustic array was downloaded directly from the at-sea modem, following verification of the data with the time and date stamps, it was then transferred into Microsoft Excel for analysis.

#### 5.3.10 Data analysis

##### 5.3.10.1 Presence/absence

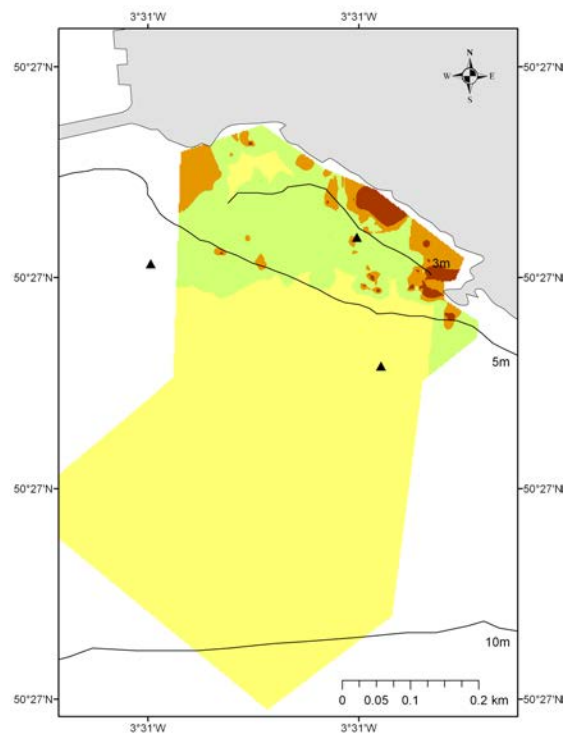
Data from both acoustic tracking systems were used to examine the patterns of presence and absence of tagged individuals. Presence of all tagged individuals was assessed on a daily basis with individuals considered present in the study area if one or more positions were detected with a single day.

##### 5.3.10.2 Maximum net displacement

Maximum net displacement of a tagged individual was calculated as the greatest straight line (at sea) distance from the point of release to any subsequently recorded position within the VRAP array (for adult individuals that remained for multiple days) or to the point of recapture (for individuals that were recovered through the fishery). Whilst this allowed the maximum net displacement to be calculated for several adult individuals, it could not be calculated for any adult individual that had left the study area immediately and was subsequently not recaptured through the fishery. Maximum net displacement was not calculated for any sub-adult individuals as only their positions at each of the six receivers were recorded and so an estimate of maximum net displacement would have been limited to within this small, static area.

### 5.3.10.3 Habitat and area use

At Millstones Bay, a habitat map was produced from video surveys during May 2012, using a drop down video camera (Colour camera used with GPS overlay, Panasonic, UK) and a hand-held GPS. The point data from these surveys were then plotted in ArcGIS and interpolated within the extent of the sampling area using inverse distance weighting to produce a continuous raster layer showing the habitats of the study area (Figure 5.9). An estimation of the total area use for this individual was undertaken using minimum convex polygons in ArcGIS (Arc Toolbox-> Data Management -> Features -> Minimum Bounding Geometry [ConvexHull, All]). This was calculated for the total duration of the tracking period as well as individually for each period of day (06:00 - 19:59) and night (20:00 - 05:59).



*Figure 5.9:* A habitat map for the Millstones Bay study area produced using drop down video surveys and interpolated from point data to a raster layer using ArcGIS. Green = seagrass, light brown = seaweed (short algal turf), dark brown = seaweed (kelp) and yellow = areas of bare sand. The black triangles show the locations of the three buoys for the VRAP acoustic array. The solid grey area denotes land. Contour lines are drawn in solid lines and labelled

At the Whitsand Bay study site, the habitat was inferred from data given on the admiralty chart for the area (Admiralty Chart 1900 Whitsand Bay to Yealm Head including Plymouth Sound), which suggests that the area is largely soft sediment with patches of gravel and broken shell and the presence of complex structures on the seabed (e.g. HMS Scylla and James Egan Layne).

**5.3.10.3.1 Diel activity patterns** Diel activity patterns were examined for only one individual (Cuttlefish 7) for which the data set covers nine consecutive days of tracking (May 19<sup>th</sup> - May 27<sup>th</sup> 2011). Data for the remaining tagged individuals was not analysed for diel activity patterns due to the restricted temporal extent of these datasets. For the period May 19<sup>th</sup> to May 27<sup>th</sup> 2011, the mean sunrise and sunset were at 04:58 (SD  $\pm$ 3 minutes) and 20:56 (SD  $\pm$ 3 minutes) respectively. By estimating the minimum convex polygons (the smallest area that contains all your data points) in ArcGIS for each 12 hr period, an analysis of the cuttlefish movement patterns during daylight hours (06:00 - 19:59) and nighttime hours (20:00 - 05:59) was possible. In addition, the dataset for Cuttlefish 7 was divided into hourly bins and the net distance moved measured and plotted by hour to observe whether movement was related to periodicity (e.g. activity occurring at regular intervals). In addition, each hour was then assigned to one of four activity patterns as previously described by Scheel and Bisson (2012) based on directional tendency and step length of the series of positions. Stationary activity was described as a scattering of positions that lacked directional tendency and that fell within a radius of the estimated error; all other sequences were termed movement. Directed movement was considered to have occurred when the sequence of positions exhibited a directional tendency and the final displacement from the start position was approximately the same as the net displacement (calculated as ratios [of net displacement and final displacement] between 1.00 - 1.50). Indirect movement occurred when the sequence of positions lacked direction and central tendency and where final displacement was less than the net displacement (calculated as ratios of 1.50 or greater). In contrast central tendency movements (or loops) occurred when net displacement occurred, but the final displacement was approximately equal to zero (calculated as 5 m or less) (Figure 5.10) (Scheel and Bisson 2012).

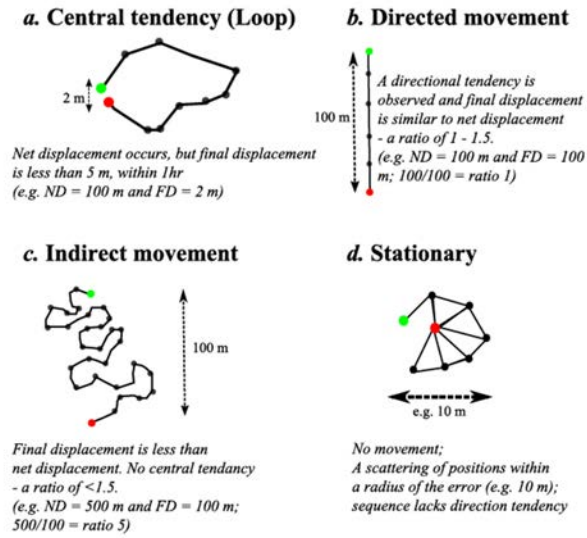


Figure 5.10: Types of movement pattern as described by Scheel and Bisson (2012). (a.) Central tendency (or loop), (b.) directed movement, (c.) indirect movement, (d.) stationary. Green dots = start position of sequence, Red dot = end position of sequence, Black dot = intermediary positions

**5.3.10.3.2 Swimming speeds** Maximum swimming speed was calculated for each adult tagged cuttlefish using portions of the tracks where directed travel had occurred and that contained a minimum of 10 consecutive points. Swimming speed was calculated by dividing the distance travelled by the time taken to travel it and was expressed as meters per second ( $\text{ms}^{-1}$ ). It was not possible to calculate maximum swimming speed from sub-adult cuttlefish due to the nature of the datasets recorded.

## 5.4 Results

### 5.4.1 Sedation

For adult cuttlefish, movement was observed to cease 12 minutes following introduction to the  $\text{MgCl}_2$  sedative bath (range = 9 - 19 min, mean = 12.1 min,  $sd = 3.25$ ,  $n = 9$ ) and individuals remained immobile throughout the tagging procedure. Recovery commenced immediately following placement into a recovery aquarium. For sub-adult cuttlefish, using a higher concentration  $\text{MgCl}_2$  sedative bath, movement was observed to cease after approximately six minutes (range = 4 - 8 min, mean = 5.9 min,  $sd = 1.2$ ,  $n = 10$ ). For adult cuttlefish recovery commenced

immediately following placement into a recovery aquarium, whilst for sub-adult individuals sedated at a higher concentration, recovery times were longer and aided by pumping clean seawater across the gills.

##### **5.4.2 Tag retention and recovery**

Tag retention was considered good for both studies, three adult individual (37.5 %) were recovered through the fisheries between two and six weeks after their release. Only the individual recaptured six weeks after release was returned to the laboratory for analysis, however, tag retention in all three individuals was considered normal as the tag was firmly attached to the cuttlebone and the area surrounding the tag was observed to be healthy. In the case of the third individual which was returned to the laboratory, somatic growth was evident around the tag harness, which resulted in a thickening of the tissue (Figure 5.11), this is considered to be a normal process as the animal's skin tissue attempts to heal itself around the shaft of the harness (Wearmouth et al. 2012). This thickened area of tissue appeared healthy and there was no sign of infection or damage to the tissue.



*Figure 5.11:* Somatic growth at surgical tag attachment sites (Cuttlefish 5): (a) Recovered cuttlefish with tag still attached following six weeks at liberty (cuttlefish recovered in Exmouth by a local cuttlefish trap fisherman); (b) A front view of the tag attachment site with tissue growth; (c) A top view of the tag attachment site with tissue growth

In addition, three of the ten sub-adult individuals were tracked intermittently by the static acoustic array over an extended period (up to 73 days) following release (Table 5.3), indicating that the tag retention in these individuals was also good. A fourth tag, still attached to the cuttlebone, was recovered on 18<sup>th</sup> January 2012 after having been washed up on Rustington beach, Sussex, some 200 miles from its original release site in Cornwall. The cuttlebone was subsequently returned to the laboratory; whilst we have no indication how long this individual lived following the tag attachment, or where in the English Channel it had died, what is evident is that the tag and harness were still well attached (suggesting tag retention is good) and that the recovery from a beach indicates that the bone was still buoyant with the tag attached, supporting the idea of a ‘life-time tag’ (see Chapter 6, Section 6.1 for further details) and a novel method for their

#### 5.4. RESULTS

recovery independent of the fisheries.

*Table 5.3:* Summary details of sub-adult cuttlefish (CF-SA) receiving V9-2L coded tags. DML: Dorsal mantle length. \* Indicates individual was recovered. All individuals were captured and released at Whitsand Bay.

Cuttlefish DML (mm) Weight(g) Released Last recorded No. pings					
CF-SA1	180	457	27/10/11	27/10/11	19
CF-SA2	145	310	27/10/11	07/12/11	30
CF-SA3*	147	279	27/10/11	27/10/11	51
CF-SA4	134	228	27/10/11	10/01/12	40
CF-SA5	160	417	27/10/11	27/10/11	1
CF-SA6	138	282	27/10/11	31/10/11	97
CF-SA7	145	322	27/10/11	27/10/11	1
CF-SA8	132	173	27/10/11	18/12/11	177
CF-SA9	145	279	27/10/11	28/10/11	80
CF-SA10	150	354	27/10/11	-	-

##### 5.4.3 Presence/absence

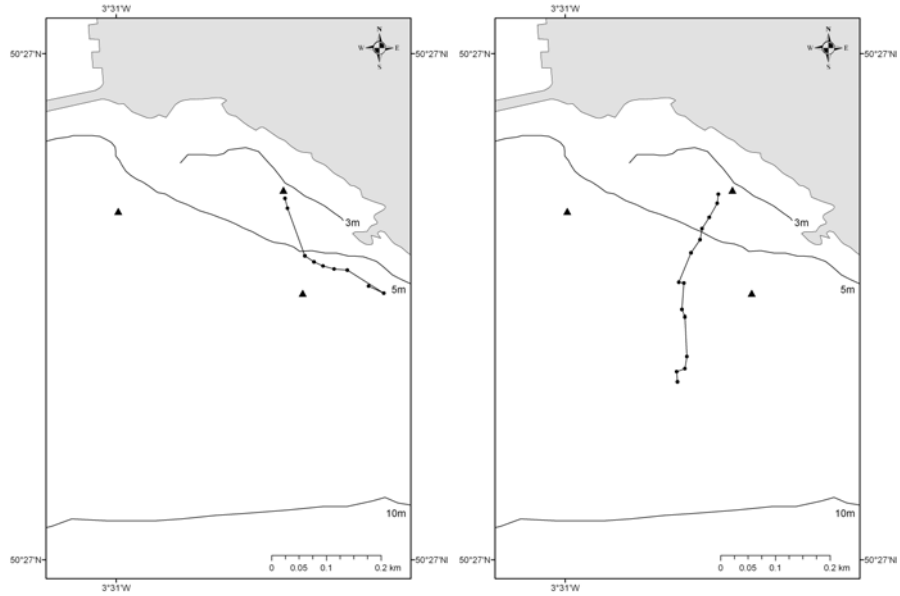
A total of six individuals left the vicinity of the array either immediately (< 45 min) or within 9 hrs of release (Cuttlefish 1, 2, 3, 5, 6 and 8). The remaining two individuals (Cuttlefish 4 and 7) stayed within the vicinity of the array for two and nine days respectively (Table 5.4 and Figures 5.12 and 5.13). Some individuals were present within the study site for continuous periods of 1-9 days, most were only present for short periods. No cuttlefish were found to return to the study area after leaving, in addition, the combination of passive and active monitoring also suggests that once an individual left the immediate vicinity of Millstones Bay it also left the greater area of Torbay as well, with at least three individuals moving along the coastline in an easterly direction.

*Table 5.4:* Summary details of adult cuttlefish receiving Vemco V9 continuous tags. DML: Dorsal mantle length (Note: weight not recorded), \* denotes that two tagged individuals were additionally recovered but their transmitter identity was not confirmed

Cuttlefish no.	Tag no.	Tag freq.	Length (mm DML)	Date released	Time monitored	Recovered*
1	A05699	66	170	19/05/11	8 hrs 30 mins	-
2	A05698	63	180	18/05/11	9 hrs	-
3	A05705	84	190	24/05/11	41 mins	-
4	A05704	81	190	19/05/11	25 hrs 45 mins	-
5	A05707	72	195	19/05/11	1 hr 15 mins	Yes
6	A05703	78	200	19/05/11	1 hr 15 mins	-
7	A05702	75	200	19/05/11	185 hrs 30 mins	-
8	A05700	69	205	18/05/11	2 hrs 45 mins	-

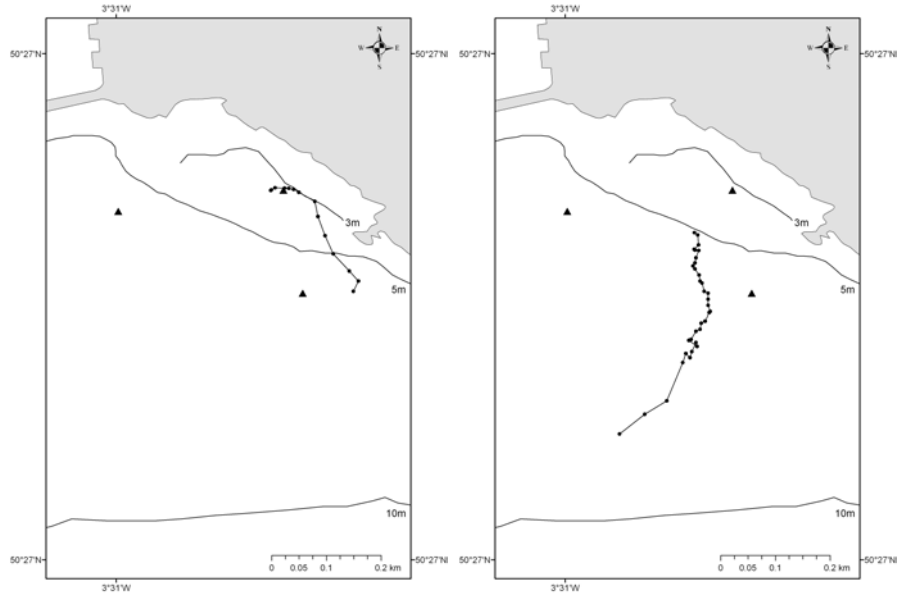


Of the ten sub-adult cuttlefish tagged and released with the study area at Whitsand Bay (Table 5.3), only nine transmitters were subsequently detected by the array. Of the nine individuals detected by the array, four (Cuttlefish SA-1, SA-3, SA-5 and SA-7) were only recorded as present in the vicinity of the array on the day of release. A further two individuals (Cuttlefish SA-9 and SA-6) were last detected by the array one and five days respectively following release. None of these six individuals have to date subsequently been detected in the vicinity of the array, indicating that they have left the area and not returned. The remaining three individuals (Cuttlefish SA-2, SA-8 and SA-4) which were detected by the array up to three days following initial release, were then subsequently re-detected within the vicinity of the array again on 7<sup>th</sup> December 2011, 18<sup>th</sup> December 2011 and 10<sup>th</sup> January 2012, respectively (Figure 5.14). Data collection from the array is ongoing and the next data upload is due to take place in September 2012.



(a) Telemetry data points and tracks (Cuttlefish 3). Tracked for 41 minutes

(b) Telemetry data points and tracks (Cuttlefish 5). Tracked for 1 hr 15 minutes



(c) Telemetry data points and tracks (Cuttlefish 6). Tracked for 1 hr 15 minutes

(d) Telemetry data points and tracks (Cuttlefish 8). Tracked for 2 hrs 45 minutes

Figure 5.12: Telemetry data points and tracks for: (a.) Cuttlefish 3, (b.) Cuttlefish 5, (c.) Cuttlefish 6, (d.) Cuttlefish 8. The solid grey area represents land. Depth contours (3 m, 5 m and 10 m) for the site are labelled and black triangles denote the locations of the three VRAP buoys.

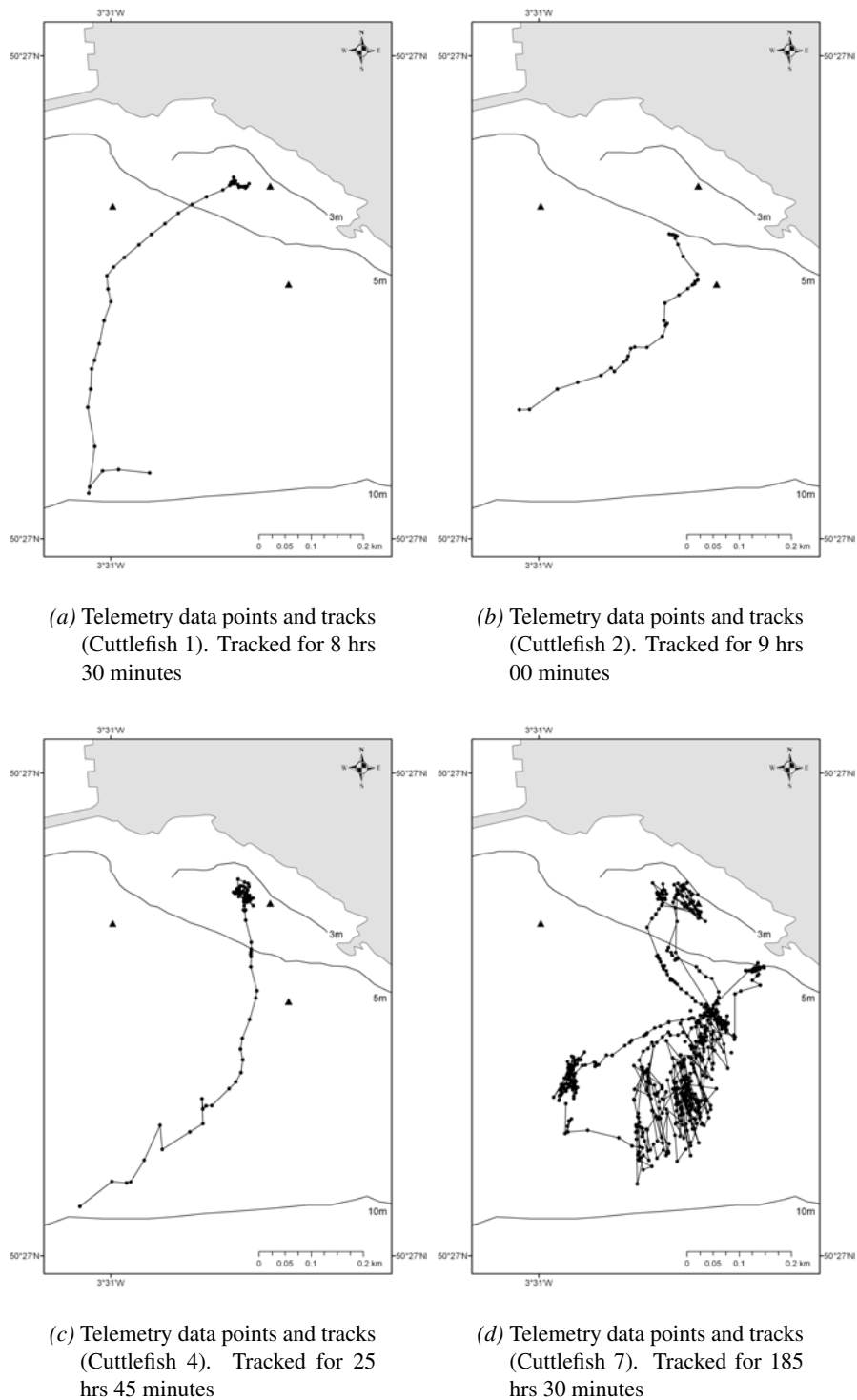


Figure 5.13: Telemetry data points and tracks for: (a.) Cuttlefish 1, (b.) Cuttlefish 2, (c.) Cuttlefish 4, (d.) Cuttlefish 7. The solid grey area represents land. Depth contours (3 m, 5 m and 10 m) for the site are labelled and black triangles denote the locations of the three VRAP buoys.

## 5.4. RESULTS

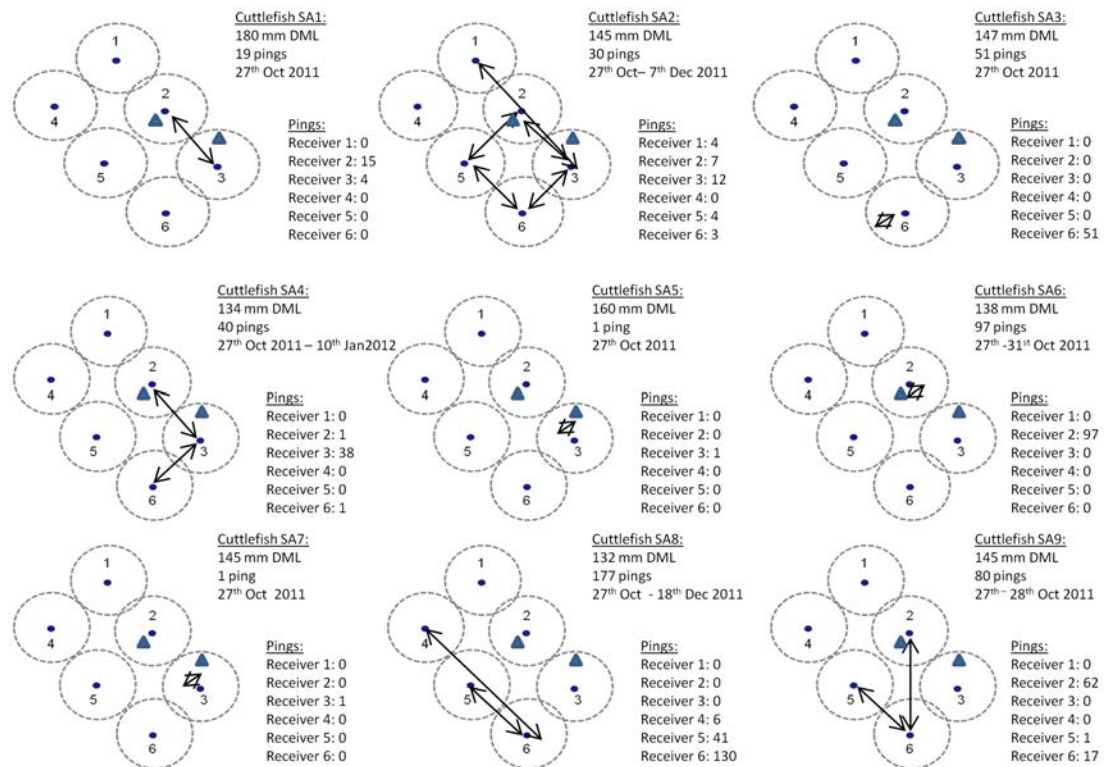


Figure 5.14: Data from tagged sub-adults within the Whitsand Bay static acoustic array. Ten sub-adult cuttlefish were tracked within the array from October 2011 - January 2012. Only nine transmitters were recorded by the array the data from these individuals are presented. Blue triangles represent the locations of complex habitats (submerged wrecks), whilst the arrows indicate transference patterns of the transmitters between receivers, and not actual directed movements. Information on the dorsal mantle lengths (DML mm) of each individual together with tracking dates and the numbers of detected pings by each receiver are also provided for each transmitter

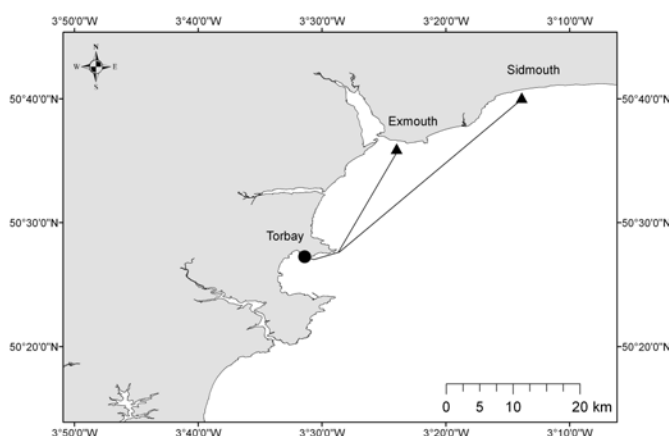
### 5.4.3.1 Active monitoring

No individuals were detected during the active monitoring sessions outside of the VRAP array, although the precise locations of those individuals being tracked by the array were ground-truthed during these monitoring sessions.

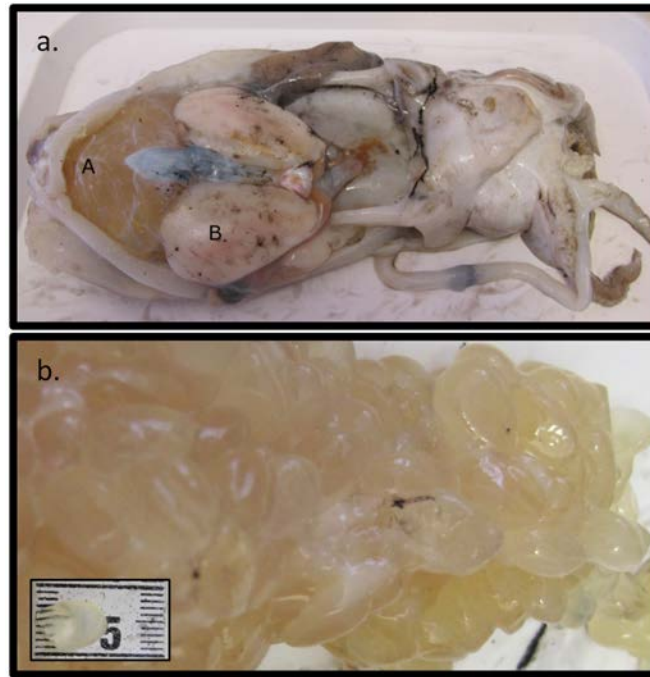
### 5.4.4 Maximum net displacement

Three adult cuttlefish were recaptured through the fishery allowing their maximum net displacement to be calculated, along with a fourth animal (Cuttlefish 7) that remained within the study site for  $\geq 9$  days (this individual was still in the study area at the end of the study period). Data

from Cuttlefish 5 that was recaptured in a commercial cuttlefish pot by the fishing vessel *Becci of Ladram* (E508) off the coast of Exmouth on 30<sup>th</sup> June 2012 (50° 35.661'N, 03° 24.129'W) had a maximum net displacement of approximately 25 km (Figure 5.15). The maturity status of this individual was identified in accordance with the new guidelines for macroscopic maturity in cephalopods produced by the report of the International Council for Exploration of the Sea workshop on maturity stages of cephalopods (ICES WGMSCPH) (ICES 2010). Using these guidelines, this individual was assessed to be a mature spawning females (stage 3-aGSA11) with an amber-coloured gelatinous ovary with oocytes > 4 mm (Figure 5.16). The other two tagged adult individuals recovered through the fishery off the coast of Sidmouth were unfortunately sold before they could be returned to the laboratory for analysis, as such we have only an approximate capture date and location provided by the fisherman and cannot identify their transmitter ID or the sex and maturity status of these individuals. However, an estimation of the maximum net displacement of these two individuals was approximated at 35 km (Figure 5.15). For the fourth individual (Cuttlefish 7), the point of recapture was taken as the position recorded by the VRAP array that was furthest from the original point of release. The maximum net displacement of this individual was accordingly estimated at 0.57 km.



*Figure 5.15:* Maximum displacement for three tagged cuttlefish. Cuttlefish 5 was recaptured through the fishery in Exmouth, 23 km from the original release site in Torbay, after six weeks at liberty. A further two tagged individuals (ID unknown) were recaptured through the fishery at Sidmouth, 34 km from the original release site, after two weeks at liberty. Black circle indicates release site in Torbay, black triangles indicate recapture sites in Exmouth and Sidmouth.

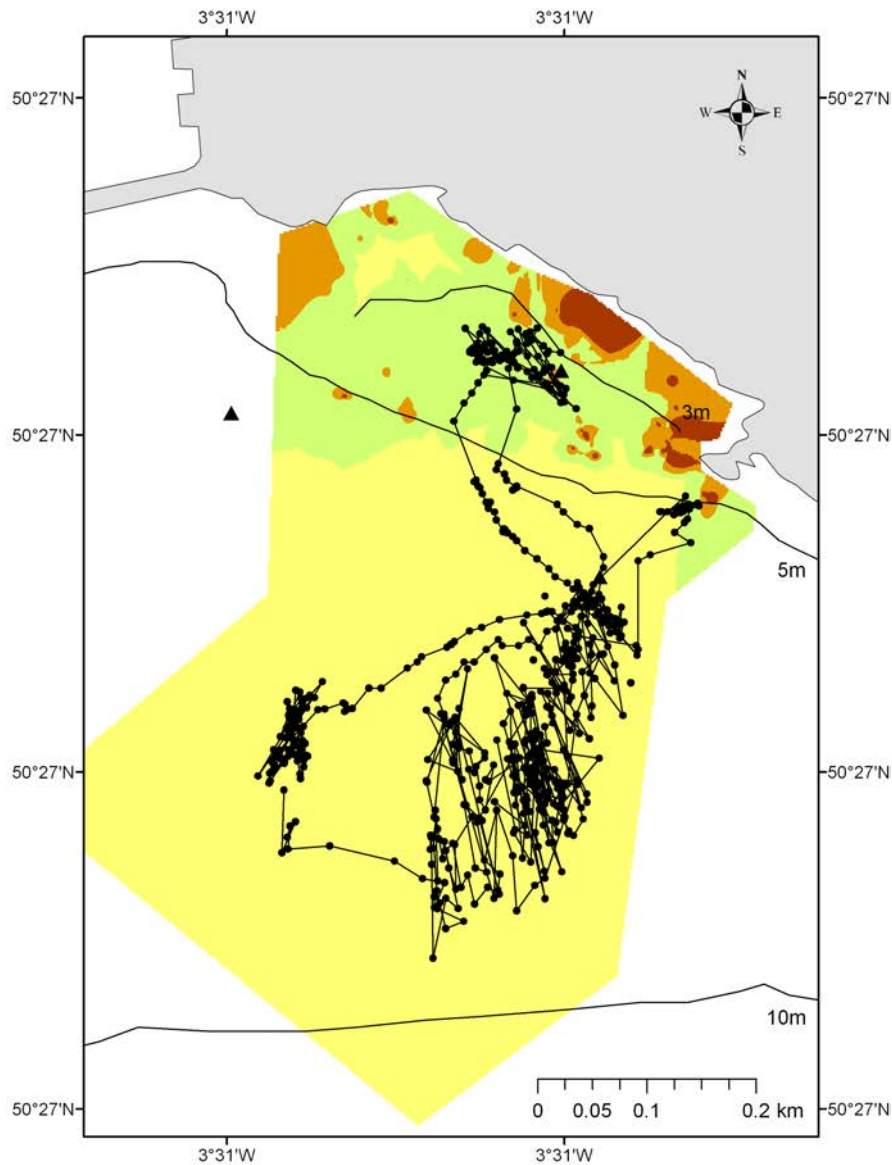


*Figure 5.16:* Dissection and macroscopic maturation analysis of Cuttlefish 5: (a) A- shows the amber coloured and gelatinous ovary containing oocytes. B- shows the enlarged nidamental and ovary glands; (b) shows part of the mass of oocytes removed from the ovary; (c) shows an example oocyte measuring 7 mm.

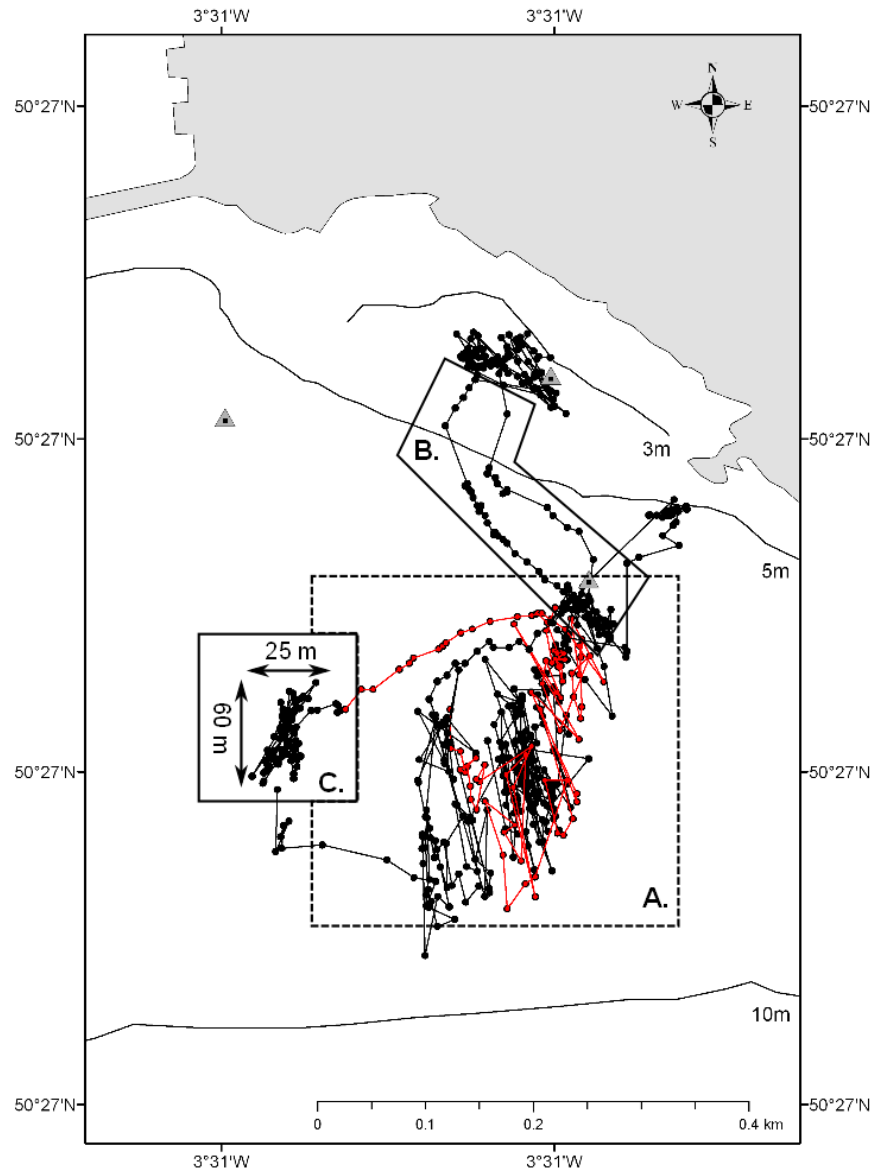
##### 5.4.5 Habitat and area use

In Figure 5.17, the data for nine days of continuous tracking for adult Cuttlefish 7 was overlaid onto the habitat maps for the study site. The results indicate that the individual actually spent the majority of the time it was tracked (83 %) in the bare sand habitat adjacent to the seagrass bed, with gradual exploratory movements around the area over a period of several days, before returning and re-passing over the same area (Figure 5.18 A.). The movement patterns during this time could suggest periods of resting on the seabed (when no movement was recorded) or periods of foraging or searching for potential mates (when movement was recorded). In addition, as well as the time spent in the seagrass bed immediately following release (approx 20 hrs) the individual had two additional, discrete trips into the seagrass bed, each lasting around 6 hrs and taking place in the early morning (02:00 - 09:00) where the individual could have been engaged in spawning activities (egg laying or copulation). This equates to the tracked individual spending only 17 % of the total time that it was tracked in the seagrass bed. This individual did

not use any of the adjacent seaweed (kelp and short-algal turf) habitat.



*Figure 5.17:* The movement tracks for Cuttlefish 7 over a period of nine consecutive days (19th - 27th May 2011) overlaid on to a habitat map of the study area. Yellow = areas of bare sand, green = seagrass, light brown = seaweeds (short algal turf) and dark brown = seaweed (kelp). Depth contours (3 m, 5 m and 10 m) for the site are labelled and the black triangles denote the locations of the three VRAP buoys. Cuttlefish 7 spent approximately 83 % of the total time in sand habitat and only 17 % of the total time tracked within the seagrass bed. Three discrete trips were made into the seagrass bed in the early morning (02:00-09:00). This individual did not use any of the adjacent seaweed habitat.



*Figure 5.18:* Example movement patterns exhibited by Cuttlefish 7. (A.) A discrete looping excursion over a 24 hr period (here small red dots are used in addition to black dots, here the black dots illustrate the first movement path [original points] and the red dots illustrate the second movement path [loop points]) (B.) Directed movement, with net displacement equal to 215 m and final displacement to 205 m over a 3 hour period (ratio = 1.05) (C.) Stationary, the theoretical accuracy was estimated at approximately 50 m in this area and so it is likely that this sequence of points which occurred over a 24 hr period represent the tagged individual sitting stationary on the seabed. The solid grey area represents land. The small black dots denotes the locations of Cuttlefish 7 over the nine day tracking period and the lines joining them represent movement paths between points. Depth contours (3 m, 5 m and 10 m) for the site are labelled.



For Cuttlefish 7 the total area use for the nine days of tracking was estimated using a minimum convex polygon at 89,250 m<sup>2</sup> (or approximately 9 hectares) (Figure 5.19). However, ultimately this figure must be much larger for some individuals within the population as at least six individuals left the study area. The patterns of cuttlefish movement, whilst hard to distinguish at the finer-scale due to the decline of accuracy (less than 50 m) outside of the array, general movement patterns at the larger scale can still be identified. In Figure 5.18 examples of three movement types are highlighted. In box (A.) an example of a looping excursion is highlighted whereby the individual makes an initial pass over the area (black points) over a period of 2 to 3 days, before leaving the area to box (C.) where it spends a period of approximately 1 day stationary on the seabed, before returning back and passing over the original area again (red points). An example of directed movement is also highlighted in box (B.) where the track on the left hand side of the box illustrated the movement of the tagged individual from the corner of box (A.) to the far end of box (B.) over a final displacement of 205 m and a net displacement of 215 m giving a ratio of 1.05.

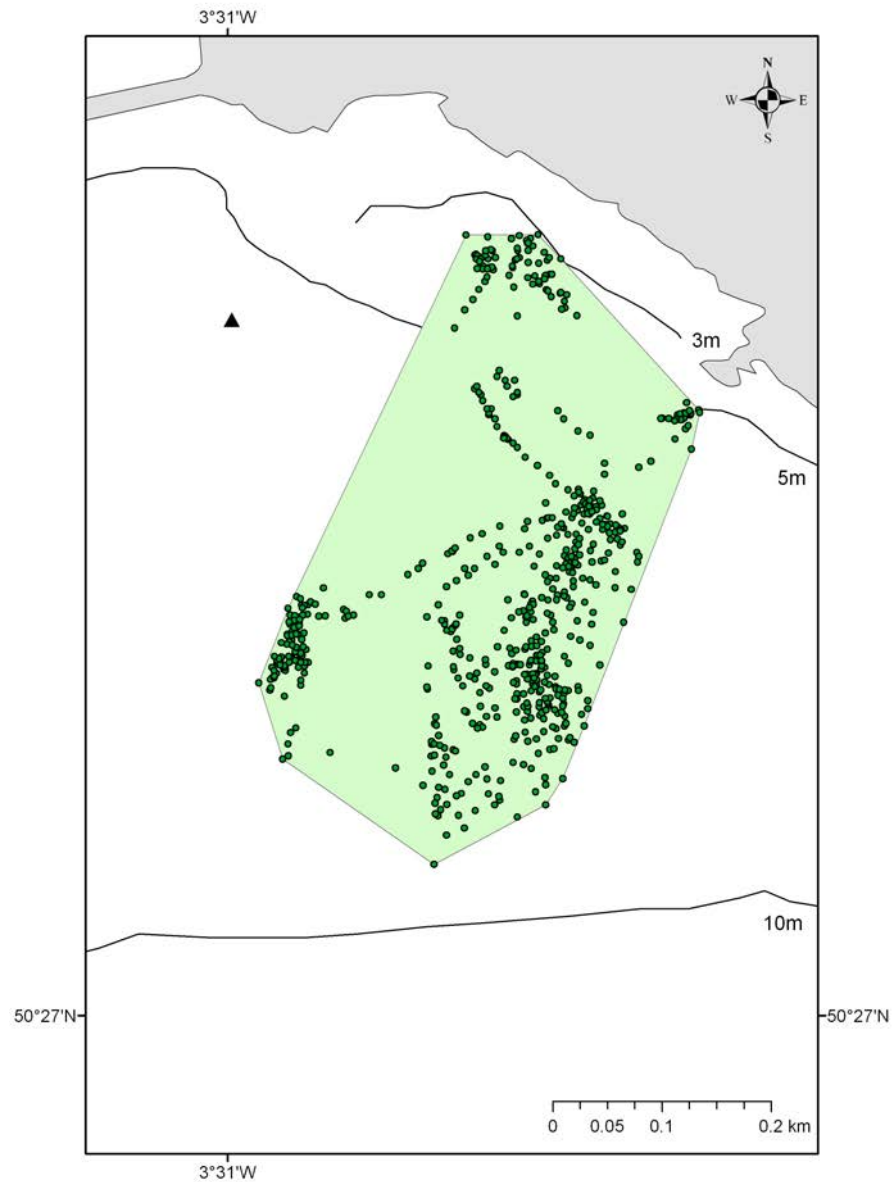


Figure 5.19: A minimum convex polygon to estimate area use (or home range) for Cuttlefish 7 over nine days of tracking. A total area of 9 ha was used by the animal over the nine day tracking period. This is the first estimation of the home range for spawning *S. officinalis*. The minimum convex polygon is outlined in green. The solid grey area represents land. The small black dots denotes the locations of Cuttlefish 7 over the nine day tracking period. Depth contours (3 m, 5 m and 10 m) for the site are labelled.

Of the tagged sub-adult cuttlefish, six out of the nine individuals spent the majority of the time they were tracked by Receiver 2 and/ or Receiver 3 (Table 5.5 and Figure 5.14) which are also

#### 5.4. RESULTS

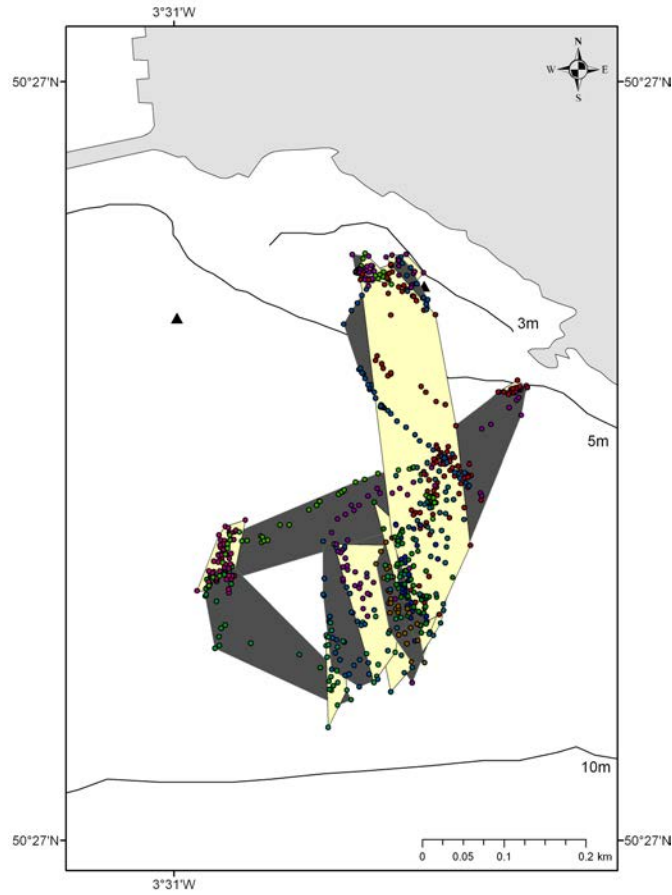
the two receivers which have complex structures on the seabed (two ship wrecks: HMS Scylla and James Egan Lane) within their detection range.

*Table 5.5: The percentage (%) of time each of the nine sub-adult cuttlefish (CF-SA) were tracked by each Receiver*

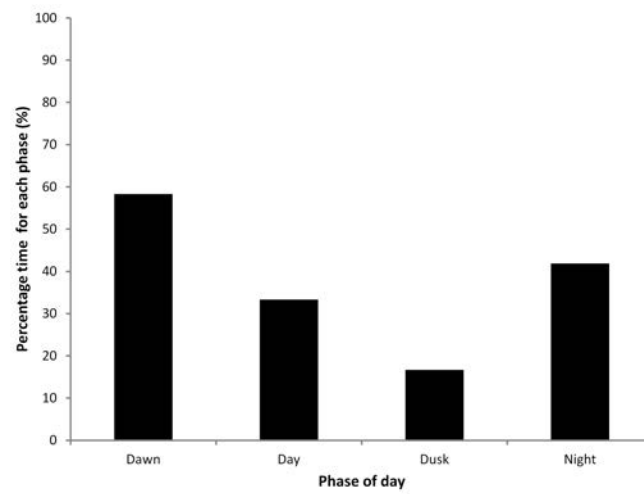
Receiver No.	CF-SA 1	CF-SA2	CF-SA3	CF-SA4	CF-SA5	CF-SA6	CF-SA7	CF-SA8	CF-SA9
Receiver 1	0.0	32.1	0.0	0.0	0.0	0.0	0.0	0.0	0.00
Receiver 2	97.1	17.2	0.0	0.4	0.0	100.0	0.0	0.0	79.16
Receiver 3	2.9	27.3	0.0	99.2	100.0	0.0	100.0	0.0	0.00
Receiver 4	0.0	0.0	0.0	0.0	0.0	0.00	0.0	1.8	0.0
Receiver 5	0.0	15.6	0.0	0.0	0.0	0.0	0.0	31.1	0.2
Receiver 6	0.0	7.8	100.0	0.4	0.0	0.0	0.0	67.1	20.7

##### 5.4.6 Diel activity patterns

The mean area use estimated for Cuttlefish 7 using minimum convex polygons was 5934 m<sup>2</sup> (SE  $\pm$  2235 m<sup>2</sup>) for daytime hours and 8356 m<sup>2</sup> (SE  $\pm$  2479 m<sup>2</sup>) for nighttime use, suggesting a greater degree of movement at night. On a daily basis movement was greatest at nighttime on four out of the eight days compared with only two for daytime movement, for the remaining two days movement was considered equal during both the day and night) (Figure 5.20). In addition, in terms of directed movement patterns, Figure 5.21 indicates that it was greatest during the dawn (58.3 %) and nighttime (41.8 %), compared with daytime (33.3%) and dusk (41.8%). Finally, whilst Figure 5.22 did not indicate any overall patterns in terms of diel activity, the use of seagrass beds during the early morning, dawn period was highlighted.



*Figure 5.20:* Daily minimum convex polygons for daytime (06:00 - 19:59) marked and night-time (20:00 - 05:59) marked for Cuttlefish 7. The positions for Cuttlefish 7 are also plotted for reference. The solid grey area represents land. The small black dots denotes the locations of Cuttlefish 7 over the nine day tracking period and the lines joining them represent movement paths between points. Depth contours (3 m, 5 m and 10 m) for the site are labelled. Day polygons are filled in yellow and night polygons are filled in dark grey. The total minimum area estimated by the minimum convex polygons was 5934 m<sup>2</sup> for daytime and 8356 m<sup>2</sup> for nighttime, suggesting a greater degree of movement at night.



*Figure 5.21:* Directed movement patterns for Cuttlefish 7 indicating the percentage of hours for directed movement during each phase of the day (Dawn: 04:00-05:00; Day: 06:00-19:00; Dusk:20:00-21:00; Night: 22:00-03:00).

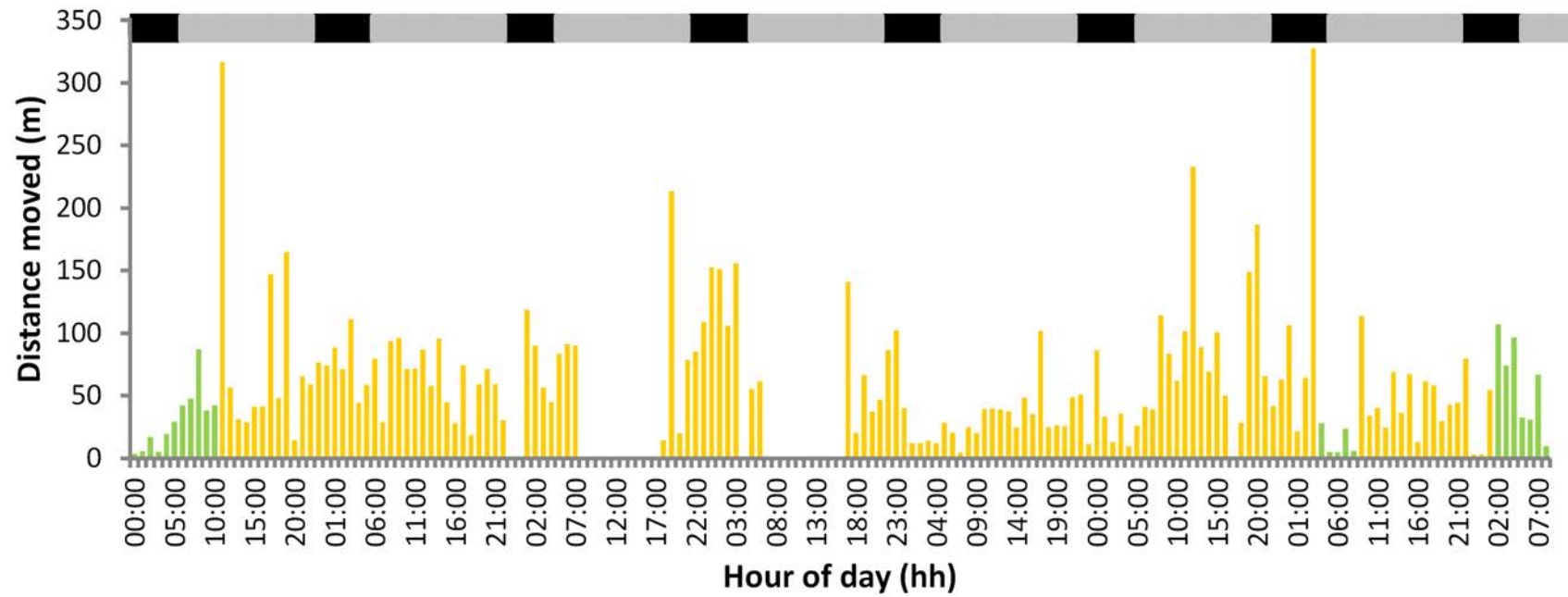


Figure 5.22: Graph of net distance moved by hour of the day for Cuttlefish 7 indicating dominant habitat type. The bar along the top denotes daytime periods in grey (calculated from the mean time for sunrise for the period 19<sup>th</sup> -27<sup>th</sup> May 2011) and nighttime periods in black (calculated from the mean time for sunset for the period 19<sup>th</sup> -27<sup>th</sup> May 2011), green bars = seagrass and yellow bars = bare sand.

### 5.4.7 Speed and distance analysis

The data from each tagged adult individual indicates that the maximum sustained average speeds (taken over 10 points or more during directed travel) recorded for these adult cuttlefish ranged between  $0.058 \text{ ms}^{-1}$  to  $0.262 \text{ ms}^{-1}$  (Table 5.6).

Table 5.6: Maximum swimming speeds of tagged cuttlefish within Millstones Bay

Animal	Date	Time start	Duration (mins)	Distance (m)	Tide state	Swim speed ( $\text{ms}^{-1}$ )
Cuttlefish 1	19 <sup>th</sup>	21:29	31	487	High	0.262
Cuttlefish 2	18 <sup>th</sup>	13:38	37	87	Low	0.058
Cuttlefish 3	24 <sup>th</sup>	13:10	16	206	Mid (ebb)	0.215
Cuttlefish 4	20 <sup>th</sup>	12:43	16	90	Mid (ebb)	0.094
Cuttlefish 5	19 <sup>th</sup>	15:55	29	319	Mid (flood)	0.183
Cuttlefish 6	19 <sup>th</sup>	16:19	25	225	Mid (flood)	0.150
Cuttlefish 7	20 <sup>th</sup>	10:58	26	260	Mid (ebb)	0.167
Cuttlefish 8	18 <sup>th</sup>	13:32	18	216	Low	0.200

## 5.5 Discussion

This is the first reported study to track the movements of the common cuttlefish *S. officinalis* in their natural habitats using acoustic telemetry. The results of this study have enabled the first insights into the subsurface movements, behaviours and activity patterns for this species (adults and sub-adults) within their natural environments. Whilst the initial results indicate that individuals (both spawning adults and non-spawning sub-adults) can exhibit a degree of seasonal (short-term) site fidelity at a small geographic scale ( $< 1.5 \text{ km}^2$ ), over a medium temporal scale (e.g. weeks or months). They also indicate that individual spawning adults can exhibit a more complex pattern of movement along the coastline over a period of one to two months. A number of (mutually exclusive or interactive) theories may explain such patterns. Firstly site fidelity may occur in these individuals, but at a larger geographic scale, for example at the extent of a geographic region (e.g. Lyme Bay or Western English Channel) rather than at a single spawning

site (e.g. Millstones Bay). Secondly, spawning adults may utilise multiple spawning sites (or habitats) during the spawning season to spread the risk of recruitment (or spawning) failure. Finally, uniseasonal-iteroparous spawning may occur within natural populations of this species, to some extent, alongside other strategies such as semelparous spawning. Further electronic tagging studies are required to validate the movement patterns and behaviours of *S. officinalis* in these inshore habitats.

### 5.5.1 Evaluation of tagging methodology for field use

One of the objectives of this field study was to assess the feasibility of using electronic tags to track the movements and behaviours of *S. officinalis* within their natural environment. Whilst previous studies have already used electronic tags to study this species in the laboratory (Wearmouth et al. 2012), this study provides the first results of their use in the wild for periods of up to six weeks for sexually mature adults and intermittently over a period of up to 11 weeks in sub-adults.

#### 5.5.1.1 Tag retention

Tag retention in these studies was considered to be good (up to 11 weeks to date). Although somatic growth was evident around the surgical site in the tagged adult individual recaptured after six weeks as liberty (Figure 5.11), the skin appeared healthy, with the tag harness remaining firmly attached to the cuttlebone with no evidence of movement. In a study by Watanuki and Iwashita (1993) on tagging in *Sepia esculenta*, the authors reported that the tissue surrounding a surgical tag attachment site generates epithelial cells for protection, and should be considered as part of the natural healing process and not an adverse reaction to the tagging procedure. This type of tissue growth was also evident in animals tagged and studied under laboratory conditions (Wearmouth et al. 2012).

The novel tag attachment technique used in these studies enabled the tag to be attached to the internal cuttlebone, this was an important feature for studies using acoustic telemetry to ensure that the transmitter signal was not blocked or reflected by the internal cuttlebone itself, as may have been the case if the transmitter had been attached to the inside of the mantle cavity or to



the ventral side of the animal, which would position the transmitter below the cuttlebone.

### 5.5.1.2 Tag recovery

Tag recovery was also considered good, with a total of four out of the eighteen tags released having been recaptured and/or returned to the laboratory. Of these four tags, three were recaptured through the fisheries and one located by a member of the general public after having been found washed up on a beach in Sussex. Tag return rates of 37.5 % for tagged adults and 10 % for tagged sub-adults, compared well with tag-recapture rates reported in other studies of *S. officinalis*, where on the Gulf of Tunis, a recovery rate of 8.96 % was reported (Ezzedine-Najai et al. 1997) and in the Gulf of Morbihan where a recovery rate of 32 % was reported Le Goff and Daguzan (1991). Several factors are known to influence the rates of tag recovery, including the interest of fishermen, the level of rewards (e.g. Taylor et al. 2006) and the ease of reporting or returning tags.

### 5.5.1.3 Tag presence

The validity of field data is dependent on the effect that the tagging process has on the individuals. The results of previous laboratory work found no changes in the detailed behaviour and/or movements between tagged and control animals, following investigation of feeding rates, buoyancy control and movement patterns (Wearmouth et al. 2012). Also within this study, general observations confirmed normal patterns of behaviour and activity in tagged individuals proceeding tagging and prior to their release. On the basis of these laboratory results, the assumption was made that the tagging process did not alter the behaviour, movement or activity pattern of these individuals in the field. In addition, the post-mortem examination of one of the tagged individuals following six weeks at liberty, allowed the individual to be assessed as being in good health at the time of capture.

One possible effect of the tagging on individuals, which has not yet been assessed in detail, is the potential for reduced survivorship or increased predation risk, especially for smaller sub-adult individuals. For example, the degree to which the cryptic ability of these tagged individuals (e.g. camouflage and sand burying), which are, among other things essential for predator avoidance

and ambush feeding, are compromised by the presence of the tag on the dorsal mantle, needs to be assessed in more detail (see Chapter 6, Section 6.5.4 for further discussion of this topic).

Another concern that has yet to be addressed is an assessment of the effects that the presence of these tags may have on the social interactions of tagged individuals to ensure that the physical presence of the transmitter on tagged individuals does not negatively influence social interactions, for example in sexually mature adults, the number of copulations received. A study by Sauer et al. (2000) which tagged the Chokka squid (*Loligo vulgaris reynaudii*) off the coast of South Africa reported that tagged male individuals were observed to engage in normal courtship behaviour, despite the presence of highly visible, external tags. Further research is still required to understand the effects (if any) of tag presence on social interactions in *S. officinalis*, both in the laboratory and in the field.

### 5.5.2 Presence/absence

Of the eight adult cuttlefish tagged and released within the study area at Torbay, only two were recorded as present within the array for longer than 24 hours, the remaining six individuals were all present for less than 24 hours, three of which could not be relocated, whilst the remaining three were subsequently recaptured through the inshore cuttlefish trap fishery. There are several possible (mutually exclusive or interacting) explanations as to why these differences in presence patterns may have occurred. One explanation, to do with site fidelity will be discussed in Section 5.5.4.1. Other potential explanations include the sex of the cuttlefish, with male and females individuals potentially exhibiting different patterns of movements and behaviours within the spawning season. The spawning state of the individuals on capture and tagging was unknown and it is likely that it varied between individuals, in some cases female individuals may have spawned prior to capture or even during the duration of the period spend in the cuttlefish traps prior to tagging. These individuals, if adopting a semelparous strategy may then be at the end of their lifecycle, and entering senescence. It is not known whether cuttlefish remain at their spawning locations during senescence or if they disperse to other areas, in addition individuals that have already spawned at the site but that have not entered senescence may have left the site in search of additional mates or spawning locations. Unfortunately, this information was

difficult to assess prior or during the tagging procedure without having to significantly prolong the procedure and sedation of the individual potentially causing stress to the individual.

Of the ten sub-adult cuttlefish tagged and released within the study area at Whitsand Bay, only nine transmitters were subsequently detected by the array. It is possible that the remaining transmitter (Cuttlefish 10) failed or that the animal had simply left the area before any data was recorded and logged by the receivers. The movement patterns of sub-adult individuals was of interest in terms of migration, with three of the nine recorded individuals remaining in the inshore coastal waters of the study area until December and January (Figure 5.14). The traditional view of the life cycle within the English Channel has been based on the premise that offshore migration occurs during the autumn period (22<sup>nd</sup> September - 20<sup>th</sup> December), indicating that at least two of these tagged individuals did not migrate offshore until the very end of autumn (7<sup>th</sup> and 18<sup>th</sup> December 2011), whilst the remaining individual was detected within the array as late as 10<sup>th</sup> January 2012, suggesting that it had still not migrated offshore. An assessment of local sea surface temperature (2005-2010) for these months indicates that the average water temperature within the vicinity of the array generally ranges between 8.0 - 11.1 °C (mean 10.08 °C SE  $\pm 0.8$ ) in December and from 7.5 - 11.4 °C (mean 9.2 °C SE  $\pm 0.09$ ) in January (satellite data AVHRR for the static array, averaged between 2005-2010). The actual SST measured at the station 'L4', which is 8 km away from the static acoustic array and measured on a monthly basis by the Marine Biological Association during the standard haul, was recorded as slightly above the monthly average at 11.6 °C in December 2011 and at the upper end of the monthly average at 10.7 °C in January 2012, suggesting that the temperature at this time could have still been warm enough for an individual to remain active and survive (10 °C and 7 °C respectively Richard 1971). This data indicates that a greater degree of plasticity in the timing of migration may occur within natural populations than has previously been considered. The possibility that variation in the timing of migration for this species might be linked to larger scale climate mediated regulation (e.g. North Atlantic Oscillation), as has been previously demonstrated in the English Channel for the squid species *Loligo forbesi* (Sims et al. 2001), could be an interesting area for future research.

### 5.5.3 Maximum net displacement

Within the inshore areas of the English Channel, spawning individuals were found to move relatively large distances during the spawning season, reaching as far as 35 km in 14 days (approx 2.5 km/d) and 25 km in 42 days (approx 0.6 km/d) (Figure 5.15). The movements of all three relocated individuals was in an easterly direction along the coastline and could imply a general population-level movement in that direction, although further tagging research would be required to verify this. Whilst these distances are likely to be underestimated given that they are based on the most direct, straight line, 'at sea' distance between the point of release and capture, these figures are in line with those found by Ezzedine-Najai et al. (1997) in the Gulf of Tunis where maximal and minimal individual distances of 4 km in 2 days (approx 2 km/d) and 25 km in 21 days (approx 1.2 km/d) were reported.

### 5.5.4 Habitat and area use

For the tagged adult Cuttlefish 7, the degree of time spent outside of the seagrass bed itself (83 %), indicates the importance of adjacent habitats which may provide suitable conditions for sand burying, resting and feeding, as well as the seagrass beds themselves which provide the structures for spawning, when considering the potential management options such as closed areas for the protection of spawning in this commercial species. The area use estimated for Cuttlefish 7 was estimated at approximately 89,262 m<sup>2</sup> and is the first estimate of habitat use for this species in their natural environments. It is likely that this area is an underestimate in terms of some individuals within the population as the remaining seven tagged individuals all left the study area. An estimate of cuttlefish use area has been reported by Aitken et al. (2005) for *S. apama* to be up to 23,700 m<sup>2</sup> which is much smaller than that quoted here. However, there are several important differences between the life cycles of these two species. For example, *S. officinalis* is generally quite solitary, forming loose aggregations to mate and spawn, in inshore areas along both sides of the English Channel coastline, and within a variety of different habitats during the spring and summer. In contrast, in the Spencer Gulf, Australia, the *S. apama* population forms the only known dense spawning aggregation (105 individuals per 100 m<sup>2</sup>) of cuttlefish in the world (Hall and Hanlon 2002) making use of a limited area of suitable spawning

substrate that exists at the site. In terms of movement patterns of these two species, it would seem likely that the area use of *S. officinalis* may be larger in comparison to allow individuals that do not form part of a dense aggregation to find suitable mates and copulation opportunities, as well as enabling female cuttlefish, which will spawn on a wide variety of substrates to locate a suitable spawning site in which to lay her eggs.

In addition, examples of general movement patterns for Cuttlefish 7 that were highlighted in Figure 5.18 could indicate the potential of *S. officinalis* to use ‘pilotage’, a navigational ability based on either simple orientation by means of familiar landmarks (visual or otherwise) (Griffin 1955) to move around a locality. In Figure 5.18 box (A.) illustrates a looping excursion where the individual spends multiple days in one area before leaving that area to make a discrete trip elsewhere box (C.) before returning and recovering the same area again. Such movement patterns with the individual returning to the same area could suggest at least a basic form of navigation by visual pilotage. Although there remains limited knowledge on the behaviour of cuttlefish in their natural environments, it is likely that their survival may depend on the ability to find and relocate specific sites such as spawning and feeding areas, through some form of navigation. In the laboratory, *S. officinalis* has already been demonstrated to use various spatial learning strategies including both response and place (visual cue) learning (Alves et al. 2006), the use of acoustic telemetry to study spatial learning and navigation of this species in their natural habitats will provide an interesting area of research for the future.

For tagged sub-adult cuttlefish, six out of the nine individuals spent the majority of the time they were tracked within Receiver 2 and/ or Receiver 3 (Table 5.5 and Figure 5.14) which are also the two receiver ranges within which the two ship wrecks are present (HMS Scylla and James Egan Lane). In a study by Watanuki and Hirayama (2000), the authors suggest that the main motivation for the Golden cuttlefish (*Sepia esculenta*), entry into basket traps is a habit of physically occupying the internal space of a three dimensional structure. One theory to explain the predominance of tagged cuttlefish within the area of these two receivers, could be that within the study area, which is predominately ‘soft ground’ (e.g. sand and mud), the added complexity of these wrecks to the habitat is of ‘preference’ to these individuals, as it is possible for them to

occupy the internal space that these structures provide.

### 5.5.4.1 Site fidelity

A principle objectives of this study was to investigate whether individuals, both spawning adults and non-spawning sub-adults, remained associated with localised areas for any appreciable length of time, thereby indicating a degree of seasonal (short-term) site fidelity. In the present study, the results were varied with a range of different strategies evidenced among tagged individuals. For example, of the tagged adults, only two remained in the study area (which was structured around a seagrass bed, where spawning is known to occur) for greater than 24 hrs, and only one of these two individuals remaining in the area for the entire duration of the study (9 days) (Figure 5.13) and for some time afterwards, indicating a possible degree of site fidelity to the spawning ground (or spawning habitat) at a small geographic scale ( $< 1$  km). In contrast at least three of the remaining six tagged adults, were known to have travelled at least 25 to 35 km along the coastline in an easterly direction, over a period of two to six weeks (Figure 5.15), suggesting limited site fidelity, at least at the smallest geographic scale (e.g. individual seagrass bed) described above, although site fidelity at a larger geographic scale (e.g. a geographic region such as Lyme Bay) remains a possibility. In addition the location and movements of the final three tagged individuals remains unknown following their departure from the study site, it is possible that these individuals had already spawned and so were entering senescence or that they also travelled along the coast, but were simply not recaptured through the fishery, their presence within the greater Torbay area was not detected however, following extensive active monitoring surveys with a mobile hydrophone. A similarly varied pattern was exhibited by tagged sub-adult individuals with only three of the nine tagged cuttlefish recorded by the array remaining within (or at least returning to) the study area or its near vicinity, intermittently over a period of up to 73 days. The remaining six individuals dispersed away from the immediate vicinity of the study site within five days of release.

These results suggest that if site fidelity does occur in this species it may be apparent at a range of geographic spatial scales within the population, with some individuals showing seasonal site fidelity to small geographic areas or habitats (e.g.  $< 1$  km) and others potentially to much larger

geographic areas (e.g.  $\leq 50$  km). This difference in potential ‘choice’ affects the distances moved by individuals and can have direct costs in terms of energetics, growth and susceptibility to predation and mortality (including fishery mortality) (Steingrímsson and Grant 2003). However, for spawning adults the benefits of moving longer distances to utilise multiple habitats or locations within a site can also include helping to spread the risk of recruitment failure by distributing the risk of encountering poor conditions for spawning and offspring hatching (Sauer et al. 2000). The movement of sub-adult *S. officinalis* along the coastline may indicate that these individuals are searching out preferred habitats or better food availability.

### 5.5.4.2 Reproductive strategies

Traditionally, *S. officinalis* has been considered as a semelparous spawner. A definition of semelparous females was provided by Fritz et al. (1982) as those that lay a single clutch/batch of eggs within their life time and deposit it in one place. Semelparous spawning females essentially commit their entire reproductive effort into one oviposition event, at one location and die shortly afterwards (Fritz et al. 1982). The spatial clustering of eggs (both spatial and temporal) that is provided by semelparous spawning can be of particular importance for species which provide parental care, but *S. officinalis* does not. The results of this study indicate that individual sexually mature cuttlefish can show a degree of seasonal site fidelity over a period of weeks that could be indicative of a semelparous spawning strategy.

In captivity, sexually mature female cuttlefish are known to exhibit a high degree of flexibility in their spawning patterns (Boletzky 1986b), including semelparous spawning (Boletzky 1986b) and intermittent spawning, with multiple repeated spawning events over a period of several months (Boletzky 1983, 1987a, 1988, 1989). Similarly, in this study a second movement pattern was also observed with individuals moving relatively large distances (25-35 km) along the coastline during the spawning season, over a period of up to six weeks. This type of movement pattern better reflects a second, alternative spawning strategy that has been labelled by Rocha et al. (2001) as intermittent terminal spawning to define females that lay multiple egg clusters/batches, within a single breeding season, potentially at multiple spawning sites. Intermittent terminal spawners distribute their reproductive effort within a single breeding season,

over multiple oviposition events, which can be distributed within both time and space, in order to spread the risk of encountering unsuitable conditions for embryonic development and hatching. For many species, one of the major advantages in distributing reproductive effort (temporally) is to enable a greater overall investment in offspring as an individual has more time available to accrue the necessary resources required for these costly reproductive processes (Kirkendall and Stenseth 1985).

Uniseasonal-iteroparous spawning is not a new concept in terms of captive female spawners of this species (e.g. Boletzky 1983, 1987a, 1988, 1989), and whilst it has generally been acknowledged that *S. officinalis* has the capacity to exhibit extended spawning, as long as the individual can remain alive for long enough to accomplish this, whether such a strategy is exploited in natural populations is still under debate. Several authors have suggested that it does occur, with a review of cephalopod reproductive strategies by Rocha et al. (2001) indicating that *S. officinalis* should be labelled as ‘intermittent terminal spawners’ in their review of cephalopod reproductive strategies and a study by Laptikhovsky et al. (2003) comparing the potential fecundity of pre-spawning and spawning females in the Aegean Sea, also indicating that intermittent spawning is a process that is likely to occur within natural populations. This study also provides an indication that the *in situ* movements and behaviours of individual sexually mature adults could reflect the occurrence of a uniseasonal-iteroparous spawning strategy within natural populations and environments; further supporting the idea that within natural populations the potential exists for a similar degree of flexibility in spawning strategies to that already described for captive individuals.

### 5.5.5 Diel activity patterns

*S. officinalis* is generally considered to be a nocturnal species, with Denton and Gilpin-Brown (1961) demonstrating the effects on buoyancy control of the internal shell according to changes in the light conditions, attributable to day and night, whilst Castro and Guerra (1990) demonstrated nocturnal activity patterns from an analysis of stomach contents. A study by Mark et al. (2007) has also indicated that some of the physiological processes of this species operate under diurnal cycles, with the authors finding that measurements of both activity (video analysis)



and oxygen consumption suggested a strong diurnal pattern, with maximum physical activity occurring shortly after midnight and a relatively constant minimum value during the daytime. A more recent study by Frank et al. (2012) provides support for the concept of nocturnality in this species, for juvenile cuttlefish, which showed clear diurnal organisation in rest and activity, with an increase in activity during the night. However, this study also indicated that for adult/senescent animals such a division was not detectable (Frank et al. 2012). In this present study, the analysis of tracks from Cuttlefish 7 over a period of nine days indicated that although activity did appear to be slightly more pronounced during the nighttime (increased area use and directed movement), that activity was also still apparent during the daylight hours, suggesting a lack of clear diurnal pattern for this spawning adult.

### 5.5.6 Swimming speeds

In a study by O'Dor (2002) the speeds for *S. apama* on the breeding grounds at Whyalla, South Australia were calculated from the track of a mature female with an average speed of  $0.038 \text{ ms}^{-1}$  over a 2 hr period. The speeds calculated in this study for *S. officinalis* which is a smaller species than *S. apama* are generally quicker and range from between  $0.06$  to  $0.26 \text{ ms}^{-1}$ . Given a general inshore migration distance during the spring of around 100-200 km a speed of  $0.06$  to  $0.26 \text{ ms}^{-1}$  would equate to a migration period of between 1 to 6 weeks depending on the speed and distance travelled.

The primary mode of locomotion in *Sepia* is considered to be undulatory swimming with a skirt fin, while jet propulsion is a secondary mode of locomotion primarily used as an escape response (O'Dor and Webber 1991). The maximum speed that is capable via this primary mode of fin-wave locomotion has been estimated at around  $0.15 \text{ ms}^{-1}$  (O'Dor and Webber 1991). The potential to combine fin and jet locomotion would enable cuttlefish to travel above this maximal speed for fin-wave locomotion alone (e.g.  $0.26 \text{ ms}^{-1}$ ).

### 5.5.7 Conclusions

In conclusion, this field study has proved the validity of using electronic tags (acoustic telemetry) in natural environments to study the movement and behaviour patterns of *S. officinalis*.

Demonstrating the potential power of acoustic telemetry as a tool for obtaining useful information on the ecology of this species but also essential information on key life stages that are required for sustainable management of this commercial species in the future. Tag retention and recovery using the novel tag attachment technique were shown to be good in the field, which also shows the potential of these methods for longer term studies such as archival (data storage tag) tagging studies. The results from this study indicate that within inshore spawning grounds, cuttlefish can be relatively mobile, over a relatively long spawning phase (up to six weeks), and exhibiting a high degree of plasticity in their reproductive behaviour and general movement patterns. Further electronic tagging research is required in order to gain a proper understanding of whether the results here represent persistent, large scale phenomena, and if so, to what extent seasonal site fidelity (in both adults and sub-adults) occurs and to what degree unisexual-iteroparous spawning may occur within natural populations and under what conditions. This research has begun to explore the potential of electronic tagging methods for the study of *S. officinalis* and other inshore cephalopod species within the English Channel (and further afield) and further research using these techniques is now required to gain a thorough understanding of the complex phenomena and processes that may occur in natural populations to further both our ecological understanding and our capacity to sustainably manage this fishery.



## Chapter 6

# Long-term tagging of *S. officinalis* using data storage tags

### 6.1 Introduction

As the fishing pressure on non-quota species like *S. officinalis* continues to increase (FAO 2010), a need for accurate life history, growth and movement data becomes ever more apparent. Whilst studies using acoustic telemetry have enabled the first insights into the fine-scale subtidal movements of this species over relatively small temporal and spatial scales (see Chapter 5), in order to study larger-scale (spatial and temporal) movements (e.g. migration) as well as potential vertical movements within the water column, the implementation of alternative electronic tagging methods using data storage tags (DSTs) is required. DSTs (also known as archival) are able to continuously monitor and store information on several key environmental variables (e.g. light intensity, water pressure and water temperature), that enables the movements and behaviours of marine species to be studied. The major drawback of any data storage tagging study, is the requirement that tags be recovered in order to download the stored data (Semmens et al. 2007). In spite of these disadvantages, the data provided by DSTs can often be of great importance to understanding the movements and behaviours of natural marine populations, allowing vertical movements in the water column to be recorded, together with the inhabited depths and temperature range of the individual. In addition, once the data have been downloaded, if the tagged individual has spent sufficient time on the seabed, it is possible to compare the data collected by the tag to models of tidal cycles and water movements in order to estimate the horizontal movements of tagged individuals (e.g. Neuenfeldt et al. 2004).

To date, only a few studies have been published that have used DSTs to study cephalopod species in the wild (Replinger and Wood 2007; O’Dor et al. 2002; Gilly et al. 2006). Of these studies, O’Dor et al. (2002) used hybrid acoustic/archival tags to study the energetics of *S. apama*, although only one tag was recovered. Jackson et al. (2005) also used hybrid acoustic/archival tags to study the movements of *S. apama*, allowing the location and retrieval of tagged individuals. Using the technique, the authors were able to track two individual cuttlefish for over one week, one of which was subsequently recovered by divers using a hand-held acoustic receiver, enabling the environmental data collected by the tag to be retrieved and downloaded (Jackson et al. 2005). A third study by Gilly et al. (2006) used 96 DSTs and 10 PAT (Pop-up Archival Transmitting) tags to study the migrations of the jumbo squid (*Dosidicus gigas*) in the Gulf of California. The authors only managed to recover a single DST, however seven of the ten PAT tags successfully up-loaded, providing data on temperature, pressure (water depth) and light intensity (horizontal movements) allowing the tracks of these tagged animals to be reconstructed and their movements inferred. The fourth study undertaken by Replinger and Wood (2007) used DSTs to study the growth rates of Caribbean reef squid (*Sepioteuthis sepioidea*) in the wild.

Whilst the general migration patterns of *S. officinalis* are well accepted (see Chapter 2, Section 2.2.2.3), the fine-scale detail of these movements remains lacking, including the exact locations and routes that these migrations follow, and the factors that regulate and direct these movements. The daily activity patterns of this species, including the presence/absence of diel vertical migrations and growth rates within wild populations remain unknowns. The potential for DST studies to answer these knowledge gaps is a new and promising field of research, which has previously been unavailable due to the relatively large size of DSTs in comparison to individuals of this species. However, rapid advancement in electronic tagging technology has seen an increasing miniaturisation of the hardware available and combined with new tag attachment methods for this species have now enabled these methods to be used, not just for larger adults but also for smaller sub-adults.

The new tag attachment technique that has been used in all the studies within this chapter

enables the tag to be attached to the internal cuttlebone (Wearmouth et al. 2012). There are several important features of this method, firstly it provides a visible tag location (with the tag raised above the dorsal mantle) and in prominent view for fishermen. Secondly, the attachment of the tag to a hard part of the animal (e.g. cuttlebone), provides a lasting attachment that will continue even after the cuttlefish has died. The cuttlebone grows with the individual during its life time and in contrast to the soft tissue and muscle, which will decompose or be eaten once the animal has died, the hard structure of the cuttlebone remains long after the death of the animal. In fact, these bones are often washed up en masse on beaches around the English Channel following the mass mortality of adults at the end of the spawning season. Tagging of cuttlefish as small as 100 mm dorsal mantle length (DML), that are in their first year of life, is possible using this new technique, and the tag may remain in place as the cuttlefish grows. Should it die of natural causes the potential exists for the tagged cuttlebone to be recovered (e.g. washed up on a beach and located by recreational beach users).

The aims of this study were to use DSTs to investigate and describe the migration movements and patterns of sub-adult (1 year) cuttlefish *S. officinalis* in the English Channel, as well as to observe their behaviours and daily activity patterns (e.g. vertical migrations) for periods of up to one year (or the remainder of their natural lifespan). For commercially important species such as *S.officinalis* an understanding of the habitat utilisation (including the locations of juvenile feeding grounds, migratory routes and the temporal and spatial movement patterns of individuals in relation to environmental variables, will help to provide baseline knowledge for the future management and sustainability of this commercial fisheries resource.

### 6.2 Objectives

1. To trial a new tagging methodology for long-term tag attachment in sub-adult individuals (over 100 mm DML).
2. To investigate and discuss the use of different anaesthetic concentrations on the sedation rates of sub-adult cuttlefish.
3. To provide the first fisheries independent information on the movements and behaviour

of sub-adult cuttlefish over seasonal scales, including the patterns of migrations and locations of offshore wintering grounds within the English Channel (e.g. geolocation through tidal algorithms).

4. To record the patterns of vertical movements of these individuals within the water column on a daily basis (e.g. feeding migrations etc.) as previously demonstrated in laboratory studies.
5. To assess the recovery and return rates of DSTs through the fishery and through the general public (beaches) to validate the concept, and use in the field, of a 'life-time' tag attachment technique and a method of recovering tags that is fisheries independent and possible if the animal dies of natural causes or predation and to determine how recovery and return rates can be optimised.

## 6.3 Methods

### 6.3.1 Study animals

Individual sub-adult cuttlefish ranging in size from 118 - 160 mm DML were captured by short hauls of a demersal trawl (12 m otter trawl, cod-end mesh size 12 mm) in Whitsand Bay (50° 33'N, 04° 24'W), Cornwall, U.K (17 individuals) and Bigbury Bay (50° 15'N, 03° 54'W), Devon, U.K. (4 individuals), by the research vessel *RV MBA Sepia* between July and September 2011. On deck, animals were held in aquaria with a constant supply of clean seawater before being transferred to the MBA laboratory where they were held in aerated, recirculating aquaria for up to three and a half weeks prior to tagging. The difference in capture sites was a result of opportunistic sampling to obtain individuals suitable for tagging, however all individuals were subsequently released at Whitsand Bay (Figure 6.1). The first eight individuals were released on 4<sup>th</sup> August 2011 at 08:38h at the position 50° 19.538'N; 04° 15.181'W, the next twelve cuttlefish were released on 11<sup>th</sup> August 2011 at 09:38h at the position 50° 19.234'N; 04° 14.125'W. The final individual was released on 27<sup>th</sup> October 2011 at 09:05h at the position 50° 19.549'N; 04° 15.251'W (Table 6.1).

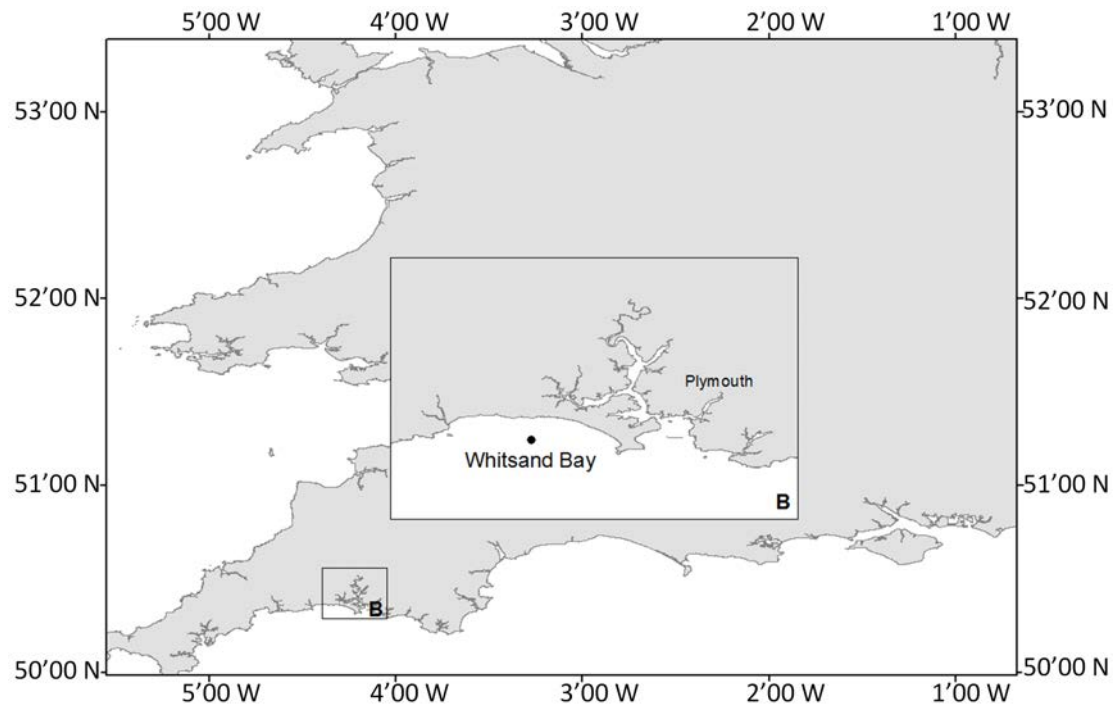


Figure 6.1: A Map of south-west England showing the location of the release site for sub-adult cuttlefish fitted with data storage tags. The study site Whitsand Bay is shown in inset B.

### 6.3.2 Tagging methodology

All 21 sub-adult cuttlefish (Year 1) were fitted with Cefas G5 DSTs (31 mm long x 8 mm diameter and weighed 1.3 g in water, Cefas G5 DSTs, Cefas Technology Limited, Lowestoft, U.K.), which were surgically attached to the internal cuttlebone as outlined previously (Chapter 5, Section 5.3.5). Each transmitter records the ambient water pressure and water temperature and has an estimated battery life of between 1 - 2 years. It should be noted that Tag A07558 (from Cuttlefish D10) was redeployed following its retrieval 3 days after initial deployment (Table 6.1).



### 6.3. METHODS

*Table 6.1:* Summary details of cuttlefish receiving Cefas G5 (20bar 2MB) DST tags. ML: Mantle length. (\*) indicates tags that have been redeployed following recovery

Cuttlefish	Tag no.	ML (mm)	Weight(g)	Capture location	Capture date	Tagging date	Release date
Cuttlefish D1	A07548	150	241	Whitsand	26/07/11	29/07/11	04/08/11
Cuttlefish D2	A07550	140	275	Whitsand	26/07/11	03/08/11	04/08/11
Cuttlefish D3	A07551	150	277	Bigbury	03/08/11	03/08/11	04/08/11
Cuttlefish D4	A07552	118	94	Bigbury	03/08/11	03/08/11	04/08/11
Cuttlefish D5	A07553	130	251	Bigbury	03/08/11	03/08/11	04/08/11
Cuttlefish D6	A07554	140	291	Whitsand	26/07/11	03/08/11	04/08/11
Cuttlefish D7	A07555	130	251	Bigbury	03/08/11	03/08/11	04/08/11
Cuttlefish D8	A07556	130	224	Whitsand	26/07/11	03/08/11	04/08/11
Cuttlefish D9	A07557	130	246	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D10	A07558	151	306	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D11	A07560	130	207	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D12	A07561	125	200	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D13	A07562	140	243	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D14	A07563	135	261	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D15	A07565	142	272	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D16	A07566	148	305	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D17	A07567	138	271	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D18	A07568	138	265	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D19	A07569	150	321	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D20	A07570	142	299	Whitsand	04/08/11	10/08/11	11/08/11
Cuttlefish D21	A07558*	160	309	Whitsand	29/09/11	24/10/11	27/10/11

Each tag has a memory of 2 megabytes which can be programmed to the user's specific data collection needs (e.g. start date and time and data collection intervals) prior to deployment. In this study, the DSTs were programmed to save data on ambient water pressure at 20 s time intervals for the first 274 days and then at 120 s intervals for the next 92 days. Whilst data on

ambient water temperature were programmed to save at 600 s time interval for 366 days or the duration of the battery life.

#### 6.3.3 Sedation procedures

Sedation procedures are as described in Chapter 5, Section 5.3.4. However, during an initial sedation session (29<sup>th</sup> July 2011 (Table 6.2) using a concentration of 13.04 %  $\text{MgCl}_2$  at 19.1 °C, the first cuttlefish placed into the anaesthetic bath was still unaffected after 40 minutes (Table 5.1). A new anaesthetic bath was prepared at the same dosage, using a fresh, unopened batch of  $\text{MgCl}_2$  to eliminate any potential issues with the quality of the original sedation bath, but again the anaesthetic bath was not effective after 18 minutes. The original dosage was considered insufficient to induce the required effect on these smaller sub-adult individuals and the concentration was increased, adding an additional 300 ml of stock solution over the following 12 minutes until the anaesthesia began to show visual signs of effect. This gave an increased concentration of 2.04 %, this individual was tagged and then placed into recovery. A third cuttlefish (140 mm DML) was then immersed into the anaesthetic bath at 11:50 hrs, after 7 minutes the effects of the anaesthetic were still not apparent and so an additional 650 ml of stock solution was added to give an increased concentration of 2.33 %, this individual was tagged, but did not recover. A fourth cuttlefish was immersed into the anaesthetic bath at 12:27 hrs and following 10 minutes it was decided that the anaesthetic was not taking adequate effect and so an additional 48.3 g of  $\text{MgCl}_2$  dissolved in 100 ml of distilled water was added to the bath to give a final concentration of 2.52 %. After a total time of twenty minutes the individual was still showing little visual sign of anaesthesia. The session was terminated and the individual placed into recovery.

Whilst working at Living Coasts in Torbay the water used for maintaining the animals and preparing the anaesthetic bath was 12 °C whilst the water used while working at the MBA was 19.1 °C. In order to evaluate whether temperature was altering the effectiveness of the anaesthetic, a second tagging session (3<sup>rd</sup> August 2011 (Table 6.2) was initiated using the same concentration bath as had been used in Torbay (13.04 %), but at a reduced water temperature of 11 °C. The first cuttlefish (130 mm DML) immersed in the anaesthetic bath was still moving

### 6.3. METHODS

---

after 15 minutes and exhibited no visible signs of anaesthesia. The reduction in the water temperature of the bath was therefore considered to have little or no effect on these smaller sub-adult animals and the concentration of the bath was again gradually increased until a visible effect of the anaesthetic was seen. All the remaining cuttlefish tagged during this session were successfully anaesthetised at this increased concentration and therefore subsequent sedation sessions (10<sup>th</sup> August and 23<sup>th</sup> October 2011 (Table 6.2) used a concentration of 3.35 %.

### 6.3. METHODS

*Table 6.2:* Summary details of cuttlefish receiving anaesthetic ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ). D: Dorsal mantle length. (\*) denotes that the individual was not immersed immediately at the dose recorded but that the dose was gradually increased over a period of time in order to obtain an effective dosage, WSB refers to Whitsand Bay, Cornwall and BBB refers to Bigbury Bay, Devon. '-' indicates that no length or weight measurements were recorded as the anaesthetic procedure was terminated and the individual placed into recovery without measurement as they were not considered suitably anaesthetised to undertake this procedure without incurring potential stress

Session	CF	DML (mm)	Weight(g)	Dose (%)	Time (min)	Temp (°C)	Location
one	1	-	-	1.9	< 40	19.1	WSB
one	2*	150	241	2.04	30	19.1	WSB
one	3*	140	255	2.33	29	19.3	WSB
one	4*	-	-	2.52	< 20	19.3	WSB
two	5*	130	312	3.12	58	11.3	WSB
two	6	150	277	3.12	07	11.3	BBB
two	7	118	94	3.12	03	11.3	BBB
two	8	130	251	3.12	05	11.3	BBB
two	9	140	291	3.12	14	11.3	WSB
two	10	130	251	3.12	07	11.3	BBB
two	11	130	224	3.12	09	11.3	WSB
two	12	140	275	3.12	13	11.3	WSB
three	13	130	246	3.35	11	15.6	WSB
three	14	170	408	3.35	17	15.6	WSB
three	15	130	207	3.35	12	15.6	WSB
three	16	141	281	3.35	14	15.6	WSB
three	17	140	243	3.35	10	15.6	WSB
three	18	135	261	3.35	10	15.6	WSB
three	19	142	272	3.35	10	15.6	WSB
three	20	148	305	3.35	11	15.6	WSB
three	21	138	271	3.35	10	15.6	WSB
three	22	138	265	3.35	05	15.6	WSB
three	23	150	321	3.35	09	15.6	WSB
three	24	142	299	3.35 251	05	15.6	WSB
three	25	125	200	3.35	11	15.6	WSB
three	26	151	306	3.35	11	15.6	WSB
four	27	160	309	3.35	04	17.5	WSB

### 6.3.4 Tagging procedure

*(All surgical tagging procedures were undertaken by a team of scientists which included Prof. D.W. Sims (MBA) who provided surgical techniques, Isobel Bloor, Dr. V.J. Wearmouth and M. McHugh.)*

Following immobilisation, all individuals were surgically fitted with CEFAS G5 DSTs with tagging following the same procedure described previously for acoustic tags (Chapter 5, Section 5.3.5). The only difference pertained to the method by which the tag was attached within the purpose-built harness. Whilst for acoustic tags, which do not store data, the tags were simply attached to the harnesses using quick drying cyanoacrylate glue (Chapter 5, Section 5.3.3), for DSTs which store data and so need to be released from the harness once the tags are recovered, the tag harnesses had two small holes inserted to each side of the tag insertion area, enabling them to be wired into place (Figure 6.2). On retrieval this wire was simply cut to remove the tag from the harness for data downloading.



*Figure 6.2: DST wired into harness prior to attachment (tag harnesses for DSTs were developed by the MBA behavioural ecology group).*

### 6.3.5 Tag recovery

In order to recover the data from the deployed DSTs it is essential that the tags are recovered and returned to the laboratory by either members of the public (e.g. recreational beach goers) who may find the tags attached to cuttlebones that have washed up on the beach, or fishermen that find the tagged cuttlefish among their catch. The DSTs are printed with contact details

and details of the reward (£50 for a cuttlefish and tag or £25 for cuttlebone and tag), it was also necessary to ensure that anyone who may come into contact with these tags was aware of the study taking place. Recovery of the whole animal (primarily through the fishery) enables a post-mortem of the individual to be undertaken, providing valuable information about health, growth rates and tag retention for the tagged individual and an accurate recovery location also enables net displacement of the animal to be calculated, therefore the reward was higher (£50). The additional value of the reward for fishermen also compensates them for the monetary loss for not selling this piece of catch.

In order to inform fishermen within the English Channel about the tagging project, a series of posters and leaflets (Appendix C.4) were produced, in French and English, and disseminated to both French and UK fishermen through the help of IFREMER and Comité régional des pêches, Basse Normandie on the French coast and the inshore fisheries and conservation authorities (IFCAs) and trawler agents on the UK coast. In addition, this information was also disseminated to a variety of recreational organisations with links to beach or coastal activities such as local conservation organisations, recreational divers and anglers, beach cleaning organisations, canoe clubs and coastal national trust properties through a series of posters (examples shown in Appendix C.4), oral presentations and press opportunities.

#### **6.3.6 Data analysis**

Data from recovered tags was downloaded using the G-series reader and G5 Host software (Version 2.2.0, Cefas technology limited, Lowestoft, U.K.) provided with the Cefas G5 tags and in Excel the tracks were separated by day and plotted graphically as depth against time (GMT). Additional analysis was also carried out using Dive Analysis software (Dive Analysis, Marine Biological Association, 2011, V6.14) allowing the average depth of the cuttlefish to be calculated by hour.

## 6.4 Results

### 6.4.1 Sedation

In sub-adult cuttlefish the optimal sedation dosage was found to differ considerably from that used for adult cuttlefish in previous studies with a dosage of 1.9 % found to have little or no effect. At an increased dose of 3.12 % movement in sub-adult cuttlefish was found to cease in approximately 8 minutes following introduction to the  $\text{MgCl}_2$  sedative bath (range = 3 - 14 mins, mean = 8.3 mins, sd = 4.03,  $n = 7$ ) and individuals remained immobile throughout the tagging procedure. At a dose of 3.35 % movement in sub-adult cuttlefish was found to cease in approximately 10 minutes following introduction to the  $\text{MgCl}_2$  sedative bath (range = 4 - 17 mins, mean = 10 mins, sd = 3.4,  $n = 15$ ) and individuals remained immobile throughout the tagging procedure (Table 6.2). For sub-adults sedated at this higher concentrations, anaesthesia-free, clean seawater was directed over the gills to enhance the recovery phase.

### 6.4.2 Tag retention and recovery

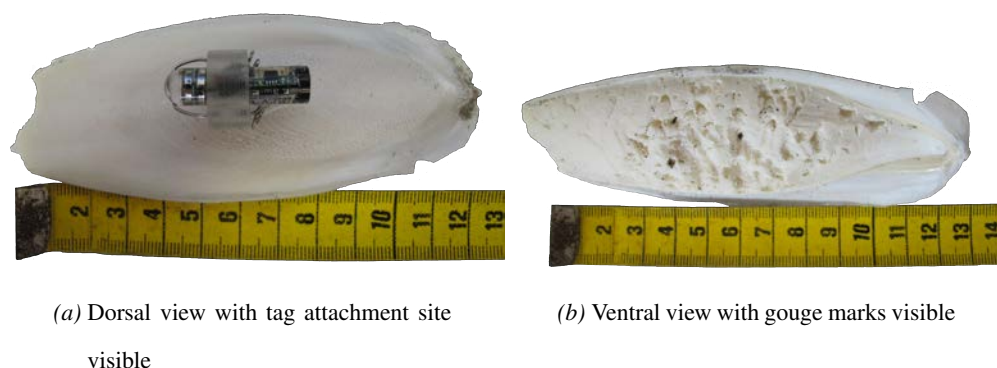
The long-term retention of DSTs deployed in the field cannot be directly assessed from this study as there has to date been only one tag returned, which occurred only two days following release. However, as part of this series of tagging studies, following post-mortem, individuals that had either not recovered from the anaesthetic procedure or that were returned to us through the fisheries, were placed in a tank with shore crabs to monitor how the tag attachment to the bone might be affected during the scavenging and removal of the surrounding flesh (Figure 6.3), the results of these tests indicated that in all cases, the tag and harness remained firmly attached to the cuttlebone, with no sign of movement.



*Figure 6.3:* Photographs showing crabs eating the flesh off the dead cuttlefish. (a.) crab tank (b.) cuttlefish 1 day on (c.) cuttlefish after 2 days (d.) cuttlefish floating on surface with tag attached after being cleaned by crabs

Tag recovery for this study was low (5 %), with only one (Cuttlefish D10) of the original 21 DSTs that were released, recovered and returned to the laboratory (Figure 6.4). The tag from Cuttlefish D10 was recovered on the beach at Whitsand Bay on 13<sup>th</sup> August 2011 by a local lifeguard. The tag had been released on 11<sup>th</sup> August 2011 at 09:38 hrs and so was at liberty for only two days before it was recovered after being washed ashore. On return to the laboratory the tag was still firmly attached to the cuttlebone with no signs of movement, whilst the underside of the cuttlebone was punctuated with a series of gouge marks (Figure 6.4).





*Figure 6.4:* Cuttlebone with DST attached from recovered Cuttlefish D10, located on the beach at Whitsand Bay on 13<sup>th</sup> August 2011

### 6.4.3 Data analysis

From the tracks (Figure 6.5) it is possible to identify periods when the individual is resting on the seabed (indicated by I and II). The point of release has been marked with an arrow at the beginning of the track on Figure 6.5a, from this point we see a steady increase in depth down to approximately 23 m as the cuttlefish swims into deeper water. For the next five hours the individual seems to have moved up into the water column at a much shallower depth of 5 m, potentially indicating a period of feeding activity. Following this, the individual then returned back to the seabed (around 23 m depth) and remained on the bottom (denoted by I) for the next two hours, before returning to a depth of approximately 5 m for the next four and a half hours during dusk/night (18:00 - 22:30 GMT), this could indicate a vertical migration in the water column to feed. After this time, the individual returns to slightly deeper water at around 10 m depth, before returning again to shallower water (< 5 m). At this point, a second period of resting on the seabed is observed between 04:00-05:30 GMT (denoted by II).

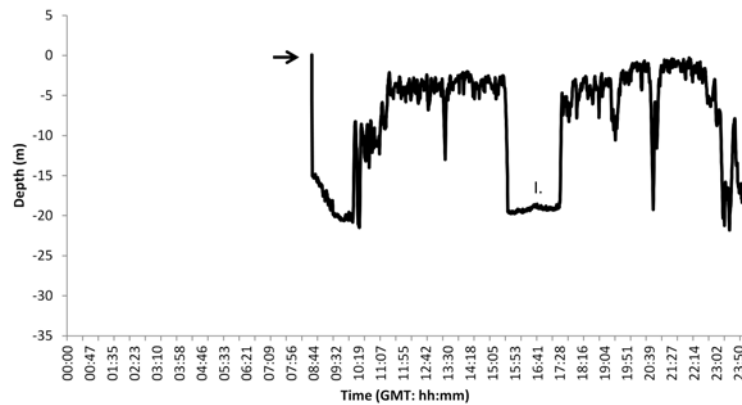
A second arrow has been marked on the track on Figure 6.5b where the individual sank to the bottom of the seabed, which is the time of presumed death (19:00 GMT). As the individual was near the surface at this point (around 1 - 2 m depth) it is possible that it had been attacked by a seabird or other predator. Sub-adult individuals of this size (151 mm DML) are prone to predation and the effect of the tag on the visibility of the individual may have increased its

#### 6.4. RESULTS

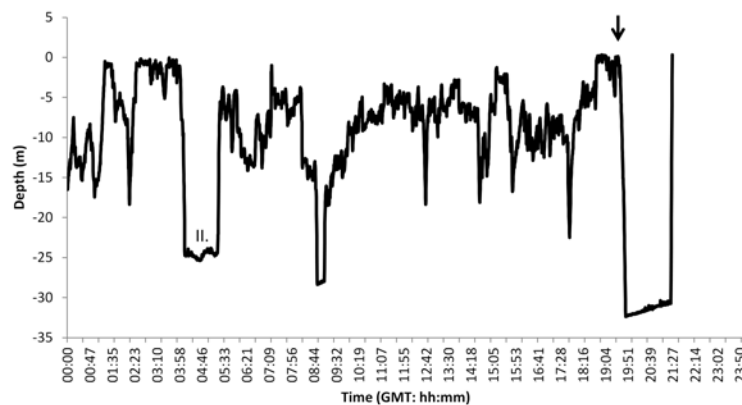
chances of a fatal predation encounter. The track then shows that movement in the individual suddenly ceases and it sinks to the seabed (around 33 m depth). At around 21:30 GMT the transmitter then re-emerges at the water's surface.

In the dive analysis software, the average depth for each hour of the day was calculated for the duration of the recorded data (Figure 6.6). These results indicate that the individual was higher in the water column at regular intervals throughout the day and not just during the night-time (01:00-03:00 [GMT], 06:00-07:00 [GMT], 11:00-15:00 [GMT], 18:00-19:00 [GMT] and 21:00-23:00 [GMT]).

The ambient water temperature recorded by the tag varied between 15.2 - 16.3 °C.



(a) Cuttlefish DST Track August 11th



(b) Cuttlefish DST Track August 11th

*Figure 6.5:* The retrieved data storage tag track from Cuttlefish D10 that was recovered from the beach in Whitsand Bay on 13<sup>th</sup> August 2011, 2 days post release. All times are specified in GMT and so 1 hr needs to be added to convert to BST

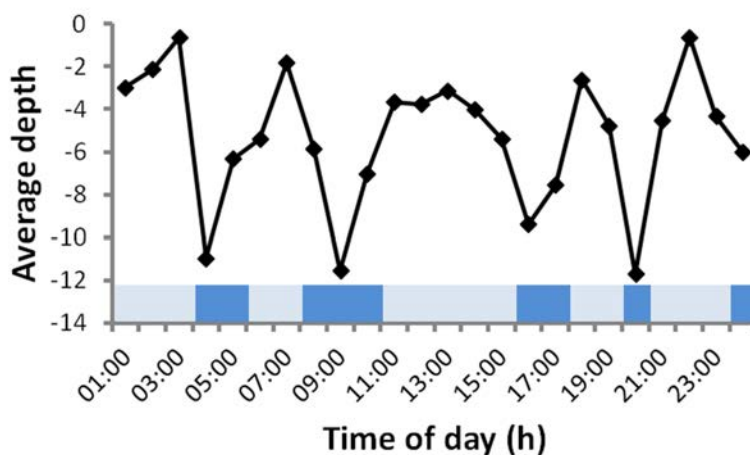


Figure 6.6: The average depth over time calculated for the retrieved data storage tag track from Cuttlefish D10 that was recovered from the beach in Whitsand Bay on 13<sup>th</sup> August 2011, 2 days post release

## 6.5 Discussion

### 6.5.1 Sedative procedures

The issues surrounding anaesthesia and analgesia are of specific importance as all cephalopods become protected under the Animals (Scientific Procedures) Act (ASPA) from 1<sup>st</sup> January 2013 onwards. Currently a wide range of methods and techniques is used throughout the research community, varying from species to species as well as among different laboratories and research groups. To date, a guidance on the best method for these procedures has not been produced by the Home Office.

The results of these studies indicate that the issue of anaesthesia and analgesia is a complex one, with a set method proposed for use in one size of animal (e.g. adult) not suitable for animals of all sizes (e.g. juveniles or sub-adults). In order to better understand the effects and processes involved in cephalopod anaesthesia and analgesia, a short summary of research on this topic that relates to *S. officinalis* is provided (additional information in Table 6.3). *Anaesthesia* can be defined as a loss of sensation that is often accompanied by a loss of consciousness and is generally considered to be formed of three components: *narcosis*, which is a state of unconsciousness; *analgesia* which is a loss of sensitivity to painful stimulation and *muscle relaxation*.

Common anaesthetic agents for cephalopods have previously included ethanol, urethane, magnesium chloride and cold seawater, however the use of many of these agents has been discontinued due to adverse effects. For example urethane was found to be carcinogenic (Gunkel 2008), whilst ethanol and urethane have both been found to cause traumatic reactions such as inking and jetting (Andrews and Tansey 1981).  $\text{MgCl}_2$  has generally been considered to be a suitable agent for use in cephalopods, as it produces rapid sedation and fast recovery, whilst being cheap and easy to use (Messenger et al. 1985). The site of action of  $\text{MgCl}_2$  in cephalopods is thought to be the central nervous system (Scimeca 2006). There remains some debate about whether  $\text{MgCl}_2$  is able to produce adequate analgesia and sedation combined by blocking nerve transmission and neurotransmitter release, or if it is solely acting as a neuromuscular blocking agent producing muscle relaxation (Lewbart and Mosley 2012). Additional anaesthetic agents have been proposed for use in cephalopods, including 2-phenoxyethanol which was used with success by Şen and Tanrikul (2009) to anaesthetise the musky octopus *Eledone moschata*. The authors of this study reported no traumatic effects (e.g. inking or escaping), and shorter induction times than those reported by Messenger et al. (1985) for  $\text{MgCl}_2$ . This agent has also been used successfully for anaesthesia in juvenile cuttlefish by Sykes et al. (2011), although whilst induction times were shorter for 2-phenoxyethanol (2 mins) compared with  $\text{MgCl}_2$  (3-7 mins), recovery times were greater for 2-phenoxyethanol (> 15 mins) compared to  $\text{MgCl}_2$  (1-2 mins) (Table 6.3).

There are several possible theories as to why sub-adult individuals may require a higher dose of anaesthetic than adults. One reason could include the fact that sub-adults, which are in their first year are stronger and fitter than their adult counterparts for whom spawning will be their final act. As such a large degree of the energy stores of these adults has either been expended during migration or converted into reproductive resources. It is also unknown whether adults in natural populations continue to feed during the spawning period; whilst sub-adults, in contrast, spend this period feeding and growing and may have a greater degree of muscle reserves. In comparison to adults, that requires a higher dosage of anaesthetic.

Table 6.3: Review of anaesthesia and analgesia in *S. officinalis*. \*indicates that the dosage quoted refers to the stock solution and not the concentration of the final solution

Drug	Dose	Size/weight	Induction (min)	Duration (min)	Recovery (min)	Reference
Chloral hydrate	0.2 %	70 - 1900 g	5	-	2-10	Abbott et al. (1985)
Urethane	0.5 %	100-150 mm DML	-	≥ 30	-	Collewijn (1970)
Ethanol	1.5 to 3.0 %	220 g	≤ 1	44	20	Harms et al. (2006)
Ethanol	10.0 mL.L <sup>-1</sup>	25-37 g	3-7	1	1-2	Sykes et al. (2011)
MgCl <sub>2</sub>	6.8 g/L	114.7 g	6-12	-	-	Gore et al. (2005)
MgCl <sub>2</sub>	7.5 % *	365-890 g	5-12	-	2-20	Messenger et al. (1985)
MgCl <sub>2</sub>	1.9 %	170-205 mm DML	9-19	3	-	Chapter 5, Section 5.3.4
MgCl <sub>2</sub>	3.12 -3.35 %	118-160 mm DML	3-14	3	-	Section 6.4.1
MS222	50.0 mL.L <sup>-1</sup>	25-37 g	3	3	11-15	Sykes et al. (2011)
Clove oil	5.0 mL.L <sup>-1</sup>	25-37 g	4-8	0	< 15 (100% mortality)	Sykes et al. (2011)
Hypothermia	4-10 °C	25-37 g	≤ 1	> 3	1 - 3	Sykes et al. (2011)
2-Phenoxyethanol	0.20 mL.L <sup>-1</sup>	25-37 g	2	0	> 15	Sykes et al. (2011)

### 6.5.2 Tag recovery

One of the major disadvantages of data storage tagging studies is that the cost of electronic tags are high and the necessity to recover the tags before the data can be download and analysed. Despite the use of a continuous communication campaign to promote data storage tagging studies, in an attempt to raise the awareness of fishermen and marine managers, recovery rates can still vary dramatically as a result of many interacting factors. These include the level of rewards, the views of fishermen towards scientific research, the species being tagged, the timing of the study, and the modes of the fishery.

In this study tag recovery and return rates were low (5 %) with only one tag being returned to the laboratory despite the use of a reward scheme, communication strategy (e.g. leaflets, posters and press articles) together with oral presentations, and recovery details printed on individual tags and harnesses. The return of the tag from Cuttlefish D10, which was found attached to the bone after being washed up on a beach, highlights the potential for a 'life-time' tagging technique. This confirms that the tags, which were surgically attached to the internal cuttlebone, can still be recovered should the cuttlefish evade the fishery and die of natural mortality. Under laboratory conditions, the tags and harnesses from dead cuttlefish were found to remain firmly attached to the cuttlebone after the flesh had been removed by crabs and the tagged bone floated on the water surface, creating an opportunity (in the natural marine environment) for the tagged cuttlebone to be washed ashore as a result of the prevailing winds and currents and recovered by recreational beach users.

The hard internal shell of the cuttlebone is a rich source of calcium. The gouge marks on the underside of the cuttlebone could have potentially been made by crabs picking at the bone using their chela, and causing deep groves to be scored on the surface. Cuttlebones are often used by aquaculture hobbyists in the rearing of hermit crabs for this purpose, with cuttlebones added to the tank to provide an additional source of calcium, calcium supplementation may additionally be of particular importance for some crab species during the moulting period. No gouge marks were observed in the cuttlebones that had been placed in laboratory crab tanks. However, as the bones were observed to float the right side up, with the hard surface upright, in would have been

difficult for the crabs in these tanks to access the submerged softer underside of the bone. It may be more pertinent to suggest that these gouge marks were made after the bone was washed onto the beach, possibly by hermit or shore crabs.

Tag recovery rates in this study (5 %) were low compared to those for acoustic telemetry tags in Torbay (37.5 %). There may be many reasons for this disparity, including the timings of the study and the biphasic seasonal nature of the fisheries for this species. For example, in order to record the fine-scale movements and behaviours of adults on spawning grounds the Torbay study (Chapter 5) targeted spawning adults (2nd year) and was conducted in May, which is considered to be a peak spawning month. In comparison, the aim of this study was to record the movements (and migration patterns) of immature, sub-adult (1st year) cuttlefish as they made their second autumn migration offshore, and their final inshore migration the following spring. This study was therefore conducted towards the end of the spawning season (August-October), so that the majority of spawning adults would have already spawned and died, enabling individuals in their 1st year, undertaking these migrations, to be selected. The coastal trap fishery which specifically targets spawning adults generally operates between March to July, although the exact duration is dependent on the timing of the inshore migration, which may vary interannually by up to two or three weeks. The trap fishery is a small sector of the English Channel cuttlefish fishery and is generally undertaken by <10 m boats which are often operated single handed. The traps are set either in strings or individually and raised every 2-3 days. The small-scale nature of this aspect of the fishery, where landings are often sorted by hand on the boat, to allow females to be extracted and used for re-baiting pots, allows the tags to be easily spotted among the catch. In contrast, the offshore and inshore trawlers which operate almost year round, with a dip in activity in June, July and August when the cuttlefish are too far inshore to fish, operates at a much larger scale, with long tows and large catches. The quantity of ink and the lack of requirement for sorting of the catch (in most cases) mean that even if caught the tags may be easily passed over. In addition, the physical nature of the trawl, can be high impact on the catch suggesting that tags and harnesses could become detached during the trawl, before the catch is hauled onboard the deck. These vast differences in the modes of the fishery suggest that differences in return rates through these two fisheries would not be unexpected. A more recent

study that has evolved from the work of this pilot study, undertaken at the Marine Biological Association (MBA) and funded by the Department for Environment, Food and Rural Affairs (DEFRA) began in summer 2012 and has 100 additional Cefas G5 DSTs. Already higher return rates, relative to those from this pilot study, have been recorded. From the first release batch of nine tags, that were deployed prior to August, three have already been returned, at least one of these was through the coastal trap fishery (Pers comms V.J. Wearmouth and D.W. Sims), indicating that the timing of release, in relation to the modes of fisheries in operation, may have an impact on the number of tags returned. In fact, to date, from all four of the tagging studies mentioned, no tags have been returned from the offshore fishery, despite its dominance at the largest métier within the English Channel cuttlefish fishery. It should also be noted that the preliminary research and methodological development, alongside the sustained publicity campaign that was undertaken within this project and included regional and national radio and news coverage has provided the groundwork for tag recovery that is now providing high levels of returns within the subsequent DEFRA funded DST study and enabling valuable data on cuttlefish behaviour and movement to be obtained using DSTs.

### 6.5.3 Data analysis

The returned tag (Cuttlefish D10), provided a total of < 2 days data showing that the cuttlefish inhabited depths of between 2 and 33 m, spending periods resting on the seabed, but also making frequent vertical movements to shallower depths. A study by Jackson et al. (2005) that used hybrid tags (acoustic and archival) to record movements of *S. apama* on their spawning grounds at Whyalla, South Australia showed a ‘regular pattern of diurnal vertical migration, with the animal moving deeper during the night’. The data from this study suggests that rather than being a discrete diurnal division in the pattern of vertical migration, this sub-adult individual exhibited regular vertical movements between the shallower and deeper zones of the water column throughout the day. However, we are limited to only a short data coverage, for a single tag and so in order to understand whether such patterns are indicative of the population or simply of this individual will require additional tags to be returned.

It is not possible to interpret from the track exactly how this individual died. However, the



individual had been monitored along with the other tagged individuals prior to release for up to 24 hrs and no unusual behaviours or movements had been recorded during this time to suggest that any negative effects from the tagging procedure had occurred. The estimated time of death (19:00 GMT) as indicated by an arrow on Figure 6.5b, shows that the individual was near the water's surface at this time (between 0 - 3 m depth). At this depth below the surface of the water, it is possible for the individual to have suffered a fatal attack by a seabird or from a marine predator. The pattern of the track, with movement appearing to cease suddenly, and the cuttlefish subsequently sinking vertically to the seabed, could be indicative of a sudden predator attack. Had the individual death been related to the tagging process, the movement in the individual may have ceased more gradually with a slow loss of buoyancy and a gradual decline in movement and increase of depth.

After sinking to the seabed the transmitter remained there over the next few hours (19:50 - 21:30 GMT), at which point it is likely that the flesh from around the bone was scavenged by benthic organisms (e.g. crabs; see Figure 6.3). As this happens, the natural buoyancy of the bone dominates and the bone with the tag attached floated to the surface, where it arrived at approximately 21:30 GMT. This scenario, if valid, highlights the rapidity with which the natural processes are at operation within the marine environment, from its estimated time of death (19:00 GMT) it took under 24hrs for the bone to be completely stripped of its flesh, washed ashore and recovered.

### 6.5.4 Predation rates of juvenile cuttlefish

Cuttlefish are susceptible to predation (Table 6.4) at almost all stages of their life cycle from hatching to spawning. The majority of cephalopod species have soft unarmoured bodies which means they have little structural defence and instead rely heavily on behavioural responses to avoid predation (Hanlon and Messenger 1996; Messenger 2001; Poirier et al. 2004). For example, primary defences such as crypsis serve to reduce the risk of detection by potential predators, whilst secondary defences such as inking, whereby an individual will release a cloud of ink to either screen itself or to act as a decoy, distracting the predator whilst it escapes (Boyle and Rodhouse 2005) are used only once the individual has been detected (Messenger 2001;

Hanlon and Messenger 1996; Poirier et al. 2004). Cuttlefish are able to adapt their colouring and texture to reduce their visibility in a wide variety of substrates and habitats. However, in the natural environment it remains unknown whether the presence of these tags affects the ability of individuals to camouflage themselves from predation within some substrates and habitats. In natural populations the potential predation rate for tagged sub-adult cuttlefish may be higher than for tagged adult cuttlefish, as both the smaller size of these individuals, combined with their presence in both shallow and deeper habitats over a larger period of time, is likely to expose them to a greater range of potential predators (e.g. Table 6.4). The issue of how these tags affect the cryptic defences of these smaller sub-adult individuals may be of particular importance to study.

Table 6.4: Review of predators of *S. officinalis*, describing species which have had cuttlefish found in their stomach contents analysis

Common name	Scientific name	Location	Reference
Blue sharks	<i>Prionace glauca</i> L.	English Channel	Clarke and Stevens (1974)
Risso's dolphin	<i>Grampus griseus</i>	English Channel	Clarke and Pascoe (1985)
Whiting	<i>Merlangius merlangus</i>	North Sea	Pinnegar and Platts (2011) [2006]
European hake	<i>Merluccius merluccius</i>	Celtic Sea	Pinnegar and Platts (2011) [1991]
Megrim	<i>Lepidorhombus whiffiagonis</i>	Celtic Sea	Pinnegar and Platts (2011) [1993]
Lesser spotted dogfish	<i>Scyliorhinus canicula</i>	North Sea	Pinnegar and Platts (2011) [1991]
Anglerfish (Monk)	<i>Lophius piscatorius</i>	Celtic Sea	Pinnegar and Platts (2011) [1991]
Cod	<i>Gadus morhua</i>	North Sea	Pinnegar and Platts (2011) [1990]
Conger eel	<i>Conger conger</i>	Morbihan Bay	Blanc and Daguzan (1999)
Grey triggerfish	<i>Balistes carolinensis</i>	Morbihan Bay	Blanc and Daguzan (1999)
Ballan wrasse	<i>Labrus bergylta</i>	Morbihan Bay	Blanc and Daguzan (1999)
Common cuttlefish	<i>S. officinalis</i>	Ria de Vigo	Castro and Guerra (1990)

#### **6.5.5 Conclusions**

With only two days of accumulated data for only one tagged individual it was not possible to make any strong assertions about the movements and behaviours of sub-adult cuttlefish from this data. However, this study has demonstrated the successful use of electronic tagging methods for field studies using smaller sub-adult individuals, whilst highlighting the potential for a ‘life-time’ tag. The potential for the tag to be retained in the cuttlebone for the remainder of the individual’s lifespan, enabling recovery either through the fisheries (e.g. fishery mortality) or from the beach (e.g. natural mortality) has also been demonstrated. Further work, with a focus on the timing and location of deployment of these tags is now required.



## Chapter 7

### General discussion

In order to provide sustainable fisheries management for *S. officinalis* populations it is essential that we first have a thorough understanding of the ecology and life history of this species, in particular the factors affecting spawning, early life stage survival and recruitment variability. This thesis aimed to address critical gaps in the knowledge of these areas and this final chapter provides a discussion and assimilation of the key findings of this research, whilst additionally suggesting directions for potential future research in this area.

#### 7.1 Summary of new contributions of this thesis

1. The potential distribution of spawning habitat for *S. officinalis* was mapped for the first time within the English Channel. A presence-only modelling technique (MaxEnt) was utilised that enabled information from pre-existing records of egg cluster presence (a true measure of spawning), collated from a range of open-source datasets, to be exploited and modelled against a set of pre-determined environmental predictor variables.
2. The first in depth qualitative study of spawning structures used by female *S. officinalis* within the English Channel was presented. Surveys conducted on both the French and English coasts of the Channel contributed to this work. Whilst overall, a wide variety of spawning structures were observed, at certain sites (e.g. Torbay and Poole Bay) a dominance of a single spawning structure (e.g. *Z. marina*) was recorded, highlighting the degree of plasticity in spawning behaviour that occurs at a spatial scale and highlighting the potential for ‘selection’ or ‘preference’ processes to exist.
3. A temporal analysis of spawning patterns within seagrass beds was undertaken using *in*

*situ* observations. The results demonstrated the impact of interannual variability in the spatial dynamics of spawning structures (e.g. as a result of natural or anthropogenic disturbance) on the patterns and intensity of spawning by *S. officinalis*. This highlighted both the direct and indirect effects that interannual fluctuations in environmental conditions can have on the patterns and intensity of spawning in this species.

4. Electronic tagging methods were used for the first time to study the free-ranging movements and behaviours of *S. officinalis* (adult and subadult) within their natural environments. The results highlighted the degree of plasticity that was evident in the movement patterns and behaviours of spawning adults. Whilst two tagged adults were observed to exhibit a degree of seasonal site fidelity to the study site, others adopted a more complex movement pattern, travelling relatively large distances (up to 35 km) along the coastline in an easterly direction over a period of up to 6 weeks. These different movement patterns could potentially mirror patterns of both semelparous and 'intermittent terminal' spawning strategies.
5. Monitoring of sub-adult individuals in a static acoustic array showed similar patterns of plasticity with three individuals repeatedly monitored over a period of up to 73 days. That sub-adult individuals were recorded in inshore waters as late as December and January (with water temperatures above 10 °C) could demonstrate a large degree of plasticity in the timing of this migration.
6. The proof of concept of a life-time tagging technique for use in the field was also demonstrated as part of this research. Tags attached to the internal cuttlebone were washed ashore by prevailing winds following the natural mortality of the animals. These tagged cuttlebones were then subsequently returned to the laboratory after being located by members of the general public, thereby validating this novel method of tag recovery for cuttlefish.

## 7.2 Discussion

There is a range of general difficulties associated with the collection of direct field observations from subtidal marine species, which has led to a paucity of data for several aspects of the ecology of these species. For example for *S. officinalis* details of free-ranging movements, behaviours and habitats use, information that is essential for good fisheries management and conservation, have to date been limited. This thesis has adopted a combination of methodological approaches to try and address these significant gaps in the knowledge of this species within its natural environments.

### 7.2.1 Spawning location and distribution

In order to model the spawning distribution of *S. officinalis* within the English Channel, archival and opportunistic datasets were utilised. Despite the issue of unknown bias that is associated with these datasets, the techniques currently available enable the extraction of valuable information on the distribution of marine species, without the requirement for new and original datasets, which can be both difficult and costly to obtain. The results of Chapter 3 highlighted differences in the distribution of spawning effort within the English Channel based on environmental conditions with a predominance of suitable habitat in the Eastern English Channel. In this study data from a wide variety of sources on the known presence of *S. officinalis* were collated for all life stages. The data on the presence of cuttlefish egg clusters (a true measure of spawning) was then extracted for use in construction of a spawning distribution model, prepared using a maximum entropy (MaxEnt) modelling approach.

SDMs have the potential to address many applications for ecological, conservation and fisheries management. They provide accurate and cost effective tools, especially for areas or species for which limited data exist, by allowing the spatial distribution patterns of a target species to be identified, and analysed in terms of the environmental and physical drivers behind these observed distributions. In this way, such models are able to provide essential information on where and why a species may occur at a particular location, enabling suitable areas for habitat management or protection to be highlighted, which is especially important for key life stages or phases of a species life cycle such as spawning which is predicted in this study. Models of



cuttlefish spawning distribution within the English Channel could also be used to highlight important spawning areas that could be targeted for periodic closure to fishing during the spawning season. However, one problem with model interpretation or its use in fisheries management to assess the impact of area closures, is that the exact nature of spawning in these wild populations is still unknown. For example, whether spawning site fidelity occurs and the extent to which intermittent spawning exists in these natural populations are areas of research that need to be addressed before such models could realistically be for utilised in such management contexts. There has been a focus in recent years on the use of SDMs to predict the effects of climate change scenarios on species distribution. Whilst this can be a useful exercise, especially in terms of a management strategy to predict the effects of future environmental change on species distribution, this type of modelling requires extrapolation beyond the range of the current dataset and brings with it a whole range of additional limitations and issues.

The model and outputs from Chapter 3 contributed to the baseline data for spawning habitats and location for *S. officinalis* which were previously limited at the fine-scale resolution and provided a distribution map of potential spawning habitat for *S. officinalis* around the English Channel to focus further study within this thesis. In addition the outputs provided in Chapter 3 have provided a potential source of information for fisheries managers (e.g. IFCA's). For example, both the geo-database, which contains records of cuttlefish presence, and maps of potential spawning distribution can help identify areas within the jurisdiction of individual IFCA's where potential spawning habitats occur to highlight areas that may be sensitive to commercial fishing activities or other disturbance during the spawning season.

### 7.2.2 Characteristics of spawning habitat

A clear definition of spawning habitat was lacking for *S. officinalis* within the English Channel. This thesis provided the first detailed study, within the English Channel, to describe the structures and habitats used by *S. officinalis* for spawning. Extensive surveys incorporated sites from both the Eastern and Western basins of the Channel, and from both the North (UK) and South (French) coasts. Although the wide variety of hydrodynamic conditions among sites required the use of multiple survey techniques, for a shared fishery resource like *S. officinalis*,

information collected from across the distributional range of the population (allowing incorporation of the full range of plasticity observed in the behaviour of spawning individuals) will be vital to provide accurate management advice for this fishery. In addition, Chapter 4 showed that whilst a wide range of structures can be used by female cuttlefish for spawning (including sessile animals (e.g. *S. pavonina*), sponges, seaweeds (e.g. *H. siliquosa*) and angiosperms (e.g. *Z. marina*)), at several study sites (e.g. UK: Torbay and Poole Bay) a single structure dominated (e.g. *Z. marina*), potentially indicating a localised ‘preference’ which requires further investigation. The temporal analysis of seagrass beds undertaken at Torbay additionally suggested that factors (e.g. natural and anthropogenic disturbance) affecting the spatial dynamics and subsequent suitability of a spawning habitat can also indirectly affect the patterns and intensity of *S. officinalis* spawning observed. The seagrass beds surveyed in Chapter 4 were found to vary in their spatial structure between years (in 2012 seagrass was significantly more fragmented than in 2011) and the associated spawning effort varied in relation (in 2012 there were significantly fewer eggs and egg clusters laid than in 2011), linked to changes in the bed heterogeneity at this local level. This indicates that in addition to the direct effect of environmental conditions on the spawning and recruitment success of *S. officinalis*, environmental conditions may also indirectly effect these important population parameters, through changes in the quality of available spawning habitat.

The results of this chapter also demonstrated that cuttlefish are able to utilise a wide range of natural structures which vary in physical dimensions. For example, structures with very small diameter were used by grouping multiple leaves or thalli together in order to attain a suitable diameter for egg attachment. Such behaviour was observed both on the French coast for *C. filum* and on the UK coast for *Z. marina*. Spawning patterns were also affected by the distribution of spawning structures at a site, such that at sites where *Z. marina* was present, a seagrass species that forms large beds or meadows, there was a greater availability of spawning structures and a higher density of spawning. In contrast at sites where suitable structures were sparsely distributed, lower densities of spawning were observed across a wider range of structures. The size and architecture of a structure also affected the number of eggs laid, whilst multiple, small (mean size 10 eggs) clusters are located in seagrass beds, fewer but larger egg clusters can be

observed on more rigid structures such as *H. siliquosa*, which were capable of supporting large numbers of eggs without subsiding under the pressure or weight of the clusters. In much the same way, artificial structures such as commercial cuttlefish traps exhibit the same structural rigidity and are also capable of supporting large numbers of eggs. Preliminary estimates of egg coverage on these structures ranged from 150 to 3700 eggs on a single trap. That the rigidity of these structures may aid spawning, especially in areas where swells or currents would otherwise affect the movement of the structures to which females were attaching eggs, is a potential hypothesis that requires additional exploration. This research has demonstrated the wide variety in factors that can affect the pattern and density of spawning within a single location or across the entire extent of the Channel. The English Channel fishery for the common cuttlefish (*S. officinalis*) is known, like many cephalopod species, for its intrinsic variability in recruitment levels. The availability of suitable spawning habitat, which can vary interannually with environmental conditions, may be a contributing factor, at least at a local level, to this variability in annual recruitment.

It is expected that the results of Chapter 4 will contribute to the elucidation of key spawning grounds and habitats within the English Channel. This information will help inform management strategies of this population in the future, enabling fisheries managers to select specific habitats that may help protect cuttlefish spawning stocks through the use of closed fishing areas during years of or following poor recruitment. This information could also identify habitats that should be included in marine protected areas to help maintain spawning or areas where habitat maintenance or enhancement may be of benefit to the conservation of local cuttlefish stocks (e.g. seagrass beds in Torbay). In addition, data relating to the spawning density of cuttlefish at different sites or within different habitats can provide useful information for fisheries and conservation managers in estimating the ecosystems value of an area.

### 7.2.3 Spawning behaviour

In addition to spawning patterns, the behaviours and movements of spawning adults were assessed using acoustic tagging, providing the first reported use of acoustic tags for the study of *S. officinalis* within the field. Such novel tools will help develop our understanding of the

spatial dynamics and spawning behaviour of this species enabling an assessment of the level of recruitment from spawning areas, which will be a critical factor for the effective management of this species within the English Channel. The use of acoustic telemetry to assess the movements and habitat use of adults within a seagrass bed that is a known spawning ground for this species provided evidence for extended periods of spawning activity and a larger degree of movement within the spawning season than had originally been thought, suggesting a connectivity between multiple spawning habitats and coastal locations that would require tagging studies to be undertaken across a larger spatial scale.

The successful development of a long-term tagging technique for *S. officinalis* to allow the use of DSTs represented the first small step towards better understanding patterns of migration, site fidelity and the processes behind navigation (e.g. natal homing). In terms of DSTs the results provided within this study in terms of collected data are limited. However, as with any study that is undertaken with a species for the first time, the value is in the lessons learned from undertaking it. To optimise tag return rates the timing of tag deployment should coincide with the inshore commercial trap fishery where possible. It is apparent that in order to collect data from these tags, that is so vital for our increased knowledge, the quantity of tags that is deployed needs to be significantly increased from the twenty originally completed within this study in order that some individuals can evade the offshore fishery and return to inshore grounds the following spring to enable tags to be picked up through the trap fishery or should the animal die of natural causes for it to be washed up on a beach and located. In addition, in order to obtain the best chance of relocating tagged cuttlefish cooperation from offshore trawlers (both French and UK) is required. Another potential recovery avenue could be from the processing factories where the cuttlefish is taken following sale at the fish market for processing before it is exported abroad. A study into this operation and whether the cuttlefish is processed in the UK or abroad and to assess if any of the processes within the operation may be manually overseen, providing an additional opportunity for the retrieval of these tags from these processing factories. Essentially any way of increasing the rate of tag recovery will drastically improve the degree of information obtained from this exciting and novel method of research.

The data collected and methods used in Chapters 5 and 6 can help provide advice for fisheries management by examining the scale at which management measures would be best applied within the English Channel population. For example, the degree of movement exhibited by spawning individuals within the inshore waters of the Channel would suggest that localised management of spawning stocks may not be effective in isolation. In addition, data on the migration patterns of *S. officinalis* will help fisheries scientists and managers to better understand the spatial patterns of and factors driving navigation in this species (e.g. natal homing) that will be critical in the management of this species.

## 7.3 Conclusions

Throughout this thesis a combination of methodologies was used to address critical gaps in the understanding of the distribution, movement patterns and spawning behaviour of *S. officinalis* within the inshore waters of the English Channel. This research has highlighted the use of complementary research methods (traditional and novel) to provide fundamental insights into the ecology of this commercially important fishery species both at the individual and population level, building on the knowledge and baseline data that is required for good fisheries management and conservation of *S. officinalis* both in the English Channel and further afield.

## 7.4 Future work

Within the Western English Channel, the MaxEnt model produced within this thesis predicted only limited areas of suitable spawning habitat. In addition from the Plymouth area and west towards Penzance the presence of commercial cuttlefish trap fisheries is reported as limited. The model output of mapped potential spawning distribution for *S. officinalis* provided a basis from which exploration and investigation of areas within the Western English Channel, that could potentially support important spawning locations, can be systematically sampled based on the predicted logistic probability of presence calculated for each area. By surveying the areas for the presence of spawning and also identifying the habitat available in these areas, an assessment of the usage of these location by cuttlefish as spawning grounds can be made. By undertaking these surveys using a remotely operated vehicle it would be possible to accurately record the

location and habitat of the areas surveyed overlaid with GPS. In addition, such techniques would allow the exploration of habitats at depths greater than are feasible using SCUBA.

Following on from the study of natural structures that was undertaken within this thesis, an investigation of spawning on artificial structures (e.g. cuttlefish traps) would now be of interest. Large quantities of eggs are thought to be laid on commercial cuttlefish traps during the fishing season by spawning females. The eggs laid on these traps can be lost from the system, when the traps are removed at the end of the season and the eggs cleaned off using pressure hoses before being placed in storage over the autumn and winter. In order to provide a proper assessment of the potential issues associated with this aspect of the inshore trap fishery, an in depth study to quantify the proportion of eggs laid on trap needs to be undertaken to quantify the extent spawning effort that is distributed on these commercial fishing traps such data will provide a valuable comparison to egg densities that have now been calculated for natural substrates and will also allow an assessment of the true extent of the problem. In addition, complementary studies using ‘baited video surveys’ in which the traps themselves essentially provide the ‘bait’ could be undertaken both within the laboratory and within the field to observe and assess the behaviours of cuttlefish in relation to these structures. By understanding how cuttlefish interact with these structures both individually and in groups, including their approach, exploration, entry and use as a spawning device will help provide an understanding of their utilisation form which mitigation practices can be better suggested. In addition the undertaking of these ‘baited video surveys’ in natural environments could help to provide information on how many cuttlefish use a single trap for spawning and the level of predation on eggs on these traps that occurs within natural environments.

This study explored the powerful techniques and potentials of electronic tagging for monitoring and recording the movements and behaviours of *S. officinalis* within their natural environments. The work here has only begun to highlight the potential uses and data that can be obtained with these methods and it is hoped that in the next few years studies using these techniques will help expand our current knowledge of the behaviour and ecology of this species. For example, the use of DSTs to monitor growth rates of *S. officinalis* within natural populations has yet to be

explored. Using DSTs there is the potential for research to elucidate natural growth rates to be undertaken. To date, the majority of information which provides the basis of our understanding of temperature regulated growth in cephalopods has been determined by laboratory based studies. Such studies are often undertaken under set (fixed) temperature regimes and without the interactions of other environmental factors. Whilst they have provided valuable information on the effects of temperature on early life history parameters, they may not accurately reflect the situation in natural populations when temperature regimes are dynamic (seasonal changes in water temperature) and complicated or compounded by multiple additional environmental factors. DSTs record information on ambient water temperatures and the profiles extracted from these tags can then be used to analyse the growth rate on a tagged individual following recapture. Understanding how growth rates of ELS *S. officinalis* are determined is critical to research on stock assessments and sustainable exploitation of the fishery and would provide an interesting area for future research using these novel electronic tagging techniques.

## Appendix A

### Chapter 4: Raw data tables

#### A.1 Results

##### A.1.1 Comparison of spawning strata (structures)

*Table A.1:* Torbay 2010 subtidal survey results for May

Date	Site	Stratum	No. egg clusters recorded		
			Rep 1	Rep 2	Rep 3
May	Meadfoot	Mixed	0	0	0
May	Roundham Head	Mixed	0	0	0
May	Salturn Cove	Mixed	0	0	0
May	The Ridge	Mixed	0	0	0
May	Millstones Bay	Seagrass	5	19	93
May	Corbyn's Head	Seagrass	0	2	0
May	Fishcombe Cove	Seagrass	0	1	8
May	Torre Abbey Sands	Seagrass	4	15	0



## A.1. RESULTS

Table A.2: Torbay 2010 subtidal survey results for July. '\*' indicates that these replicates were aborted due to adverse weather conditions

Date	Site	Stratum	No. egg clusters recorded			
			Rep 1	Rep 2	Rep 3	Rep 4
July	Silver Cove	Mixed	0	0	0	0
July	North Corbyn	Mixed	0	0	0	0
July	North Meadfoot	Mixed	0	0	0	0
July	Hope's Nose	Mixed	0	0	0	0
July	London Bridge	Mixed	0	0	0	0
July	Elberry Cove	Seagrass	0	2	0	1
July	Hollicombe Head	Seagrass	0	0	1	0
July	Millstones Bay	Seagrass	0	15	1	6
July	Fishcombe Cove	Seagrass	0	7	0	0
July	Torre Abbey Sands	Seagrass	1	11	*	*

*Table A.3:* Torbay 2011 subtidal survey results for June. \* denotes insufficient space for the full number of transects to be undertaken within the specified habitat stratum

Date	Site	Stratum	No. egg clusters recorded							
			Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
June	Outer Millstones	Mixed	0	0	0	0	0	1	0	0
June	Long Quarry	Mixed	0	0	0	0	0	0	0	0
June	Babbacombe Bay	Mixed	0	0	12	14	16	12	0	0
June	Meadfoot	Mixed	0	0	0	0	*	*	*	*
June	Hollicombe Head	Seagrass	18	12	0	2	*	*	*	*
June	Torre Abbey Sands	Seagrass	0	20	0	0	8	24	15	0
June	Millstones Bay	Seagrass	14	0	0	68	50	0	32	3
June	Hope Cove	Seagrass	0	0	0	0	0	0	8	0

Table A.4: Poole Bay 2011 subtidal survey results for June. \* denotes that the full number of transects was not undertaken at a site due to adverse diving conditions or spatial restrictions

Date	Site	Stratum	No. egg clusters recorded							
			Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
June	Branksome Reef	Mixed	0	0	0	0	*	*	*	*
June	Ball Cliff	Mixed	0	0	0	0	0	0	0	0
June	Handfast Point	Mixed	1	0	0	0	*	*	*	*
June	Ballard Pinnacle	Mixed	0	0	0	0	*	*	*	*
June	Training bank	Seagrass	0	0	0	0	0	0	0	0
June	Handfast bay	Seagrass	23	2	5	18	31	14	0	4
June	Middle beach	Seagrass	0	0	0	0	*	*	*	*
June	Studland Bay	Seagrass	1	0	0	0	0	0	0	1

Table A.5: Summary details of subtidal survey transect data for Agon-Coutainville in June 2011

Transect	Stratum	Area covered (m)	Structures	Egg clusters	Eggs	Egg density (Eggs per m <sup>2</sup> )
1	0-5	1018	-	0	0	0
2	0-5	723	-	0	0	0
3	0-5	856	-	0	0	0
4	0-5	1982	<i>D. ligulata</i>	1	10	0.005
5	0-5	1443	<i>S. pavonina</i>	21	2300	1.594
1	5-10	1487	-	0	0	0
2	5-10	626	-	6	350	0.559
3	5-10	1769	<i>S. pavonina</i>	11	780	0.441
4	5-10	832	-	0	0	0
5	5-10	1342	-	0	0	0
1	10-15	1451	<i>Nemertesia</i> sp. and <i>D. ligulata</i>	9	700	0.482
2	10-15	1787	<i>Nemertesia</i> sp.	6	550	0.308
3	10-15	1936	-	0	0	0
4	10-15	1204	<i>Nemertesia</i> sp.	1	50	0.0415
5	10-15	956	<i>Nemertesia</i> sp.	1	100	0.105

### A.1.2 Comparison of spawning strata (depth)

Table A.6: Summary details of subtidal survey transect data for Langrune in June 2011

Transect	Stratum	Area covered (m)	Structures	Egg clusters	Eggs	Egg density (Eggs per m <sup>2</sup> )
1	0-5	156	<i>Nemertesia</i> sp.	1	150	0.9615
2	0-5	90	-	1	60	0.6667
3	0-5	410	-	0	0	0
4	0-5	260	-	0	0	0
5	0-5	116	-	0	0	0
6	0-5	114	-	0	0	0
7	0-5	1196	-	2	40	0.0334
8	0-5	478	-	0	0	0
9	0-5	992	-	0	0	0
1	5-10	122	-	0	0	0
2	5-10	888	-	0	0	0
3	5-10	88	-	0	0	0
1	10-15	1230	-	3	150	0.1220
1	10-15	1972	-	0	0	0
3	10-15	152	-	0	0	0
4	10-15	366	<i>Porifera</i> sp.	9	450	1.2295
5	10-15	424	-	1	20	0.0472
6	10-15	146	-	0	0	0

## A.1. RESULTS

Table A.8: Fractal dimension slopes for seagrass transects at Torre Abbey Sands 2011 and 2012

Transect	May		June		July	
	2011	2012	2011	2012	2011	2012
1	*	0.1437	0.0492	*	0.0153	0.1437
2	*	0.1396	0.0153	0.5885	6E-16	0.068
3	*	0.1106	0.0492	0.0616	0.0261	0.1152
4	0.1653	*	0.0153	0.0492	6E-16	0.3617
5	0.0553	0.093	6E-16	0.1621	0.0317	0.1266
6	0.0746	0.0553	0.005	0.2081	0.005	0.1667
7	6E-16	0.2317	6E-16	0.2023	6E-16	0.1409
8	0.068	0.4069	0.0153	0.3537	0.0616	*

### A.1.3 Fractal dimension

Table A.7: Fractal dimension slopes for seagrass transects at Millstones Bay 2011 and 2012

Transect	May		June		July	
	2011	2012	2011	2012	2011	2012
1	0.1231	0.3989	0.0492	0.3064	0.1106	0.0432
2	*	0.1409	0.0101	0.1243	0.0884	0.1204
3	*	0.3419	0.2398	0.1396	0.0363	0.2351
4	0.0207	0.2093	0.0793	0.2161	0.0432	0.2981
5	0.068	*	6E-16	0.3909	*	0.1679
6	0.0153	0.2457	0.0599	0.2658	*	0.0492
7	0.3419	0.2754	6E-16	0.1865	*	0.3235
8	0.1437	0.2457	0.0553	0.1621	*	0.3155



## Appendix B

### Laboratory Observations 2011.

#### B.1 Introduction.

Whilst one of the better studied cephalopod species, a full understanding of the behaviour and ecology of *S. officinalis* in the wild, and in particular direct observations of ELS (pre-recruit stages) remain limited. Within the English Channel, large interannual variations in recruitment, and subsequently landings of cuttlefish are known to occur (Royer et al. 2006). In order to produce enough eggs to allow average recruitment each year, a sufficient number of spawners are required, but beyond this stock size is likely to have little effect on recruitment strength as mature adults generally exhibit mass mortality following spawning (Caddy 1983). Instead ELS survival rates are considered to be of particular importance to recruitment. During these ELS, mortality rates are presumed to be at their highest (Caddy 1996) and individuals are considered more sensitive to environmental conditions (e.g. Forsythe 1993; Boyle and Boletzky 1996; Rodhouse 2001).

Conditions encountered within pre-recruit environments (e.g. temperature, oxygen saturation, light, predation and food availability) can account for a significant proportion of the variation in annual recruitment rates both temporally (e.g. between years) and spatially (e.g. between spawning sites). Variation in the conditions encountered by ELS is initially generated through the reproductive dynamics of spawning adults, through the timing and location of spawning, oviposition site selection and variable egg production or quality. These variations will then be amplified or dampened through the embryonic or ELS. It is therefore important that research to investigate the effects of the heterogeneous conditions in ELS habitats on the behaviour and survival and growth rates of ELS, and which thus directly or indirectly affect recruitment



success, is also undertaken. Such information will be crucial to the successful and sustainable management of this species both within the English Channel fishery and further afield.

In order to better understand annual recruitment success, this research aims to examine the degree to which the physical composition of ELS habitats may impact growth and survival rates of ELS. For example whether changes in feeding patterns, movement patterns and defence strategies occur in different habitat types (i.e. with or without structure or sediment) will be examined. In addition the ability of ELS cuttlefish to adapt their behaviour sufficiently among different habitat types will be assessed to determine whether survival rates among habitats can be optimised by adapting behavioural responses or if a particular habitat type may provide an advantage to ELS in terms of optimal feeding and shelter from predation indicating the potential for nursery habitats to exist. For this purpose an investigation of the behaviours and movements exhibited by ELS cuttlefish across a range of habitat complexities, and in the presence and absence of predators and prey are of interest. Previous research has demonstrated the viability of a range of predator cues for use in studies of cephalopods. Langridge et al. (2007) demonstrated the viability of crabs (*Necora puber*), juvenile dogfish (*Squalus acanthias*) and juvenile sea bass (*Dicentrarchus labrax*) for direct use as predators of ELS cuttlefish in the laboratory. Adamo et al. (2006) demonstrated the viability of using inanimate objects as visual predator stimuli, such that the threat of an aerial bird predator was stimulated through use of a model suspended above the tank. Boal and Golden (1999) demonstrated the ability of cuttlefish to detect small changes in odour within an aquarium, suggesting a potential for chemical predator cues. Whilst Pronk et al. (2010) demonstrated the use of high definition video playback as a means of providing visual stimuli to cephalopods.

### **B.1.1 Aims and objectives**

The aim of this experimental research was to analyse the hunting sequence, visual defence displays (e.g. changes in body pattern) and movement patterns of ELS cuttlefish across a range of habitat complexities when exposed to prey items or stimuli from predators (visual or chemical). Enabling an investigation into whether the behaviours or defence displays invoked are modulated by the presence or absence of different habitat complexity components (e.g. struc-

ture and/or sediment). Such information may help us to understand the ‘choices’ or ‘decisions’ associated with spawning habitat ‘selection’, with the intention of better understanding survival and recruitment rates from different ELS habitats and the potential for the existence of nursery habitats. The specific objectives of this research were:

- Develop, construct and test a video arena to study the effects of habitat complexity on ELS movement and behaviour.
- Acquire video footage of trials across a range of habitat complexities for ELS cuttlefish in response to the presence or absence of prey and predator stimuli.
- Analyse data from the video footage regarding predation, defence and movement patterns of ELS cuttlefish across a range of habitat complexities to assess whether the response is modulated by habitat type.

## B.2 Methodology

*(Prior to the commencement of experimental work approval was attained, via the standard application procedure, from the ethical committee of the Marine Biological Association of the United Kingdom.)*

### B.2.1 Study animals

For the preliminary stages of these trials, *S. officinalis* were initially sourced from wild eggs that were collected from both Selsey (646 eggs) and Torbay (148 eggs) on 12<sup>th</sup> July 2011. Hatchlings emerged from these eggs from 20<sup>th</sup> July 2011 onwards.

A second source of study animals, wild-caught individuals from the western English Channel, were obtained using short hauls of a demersal trawl (12 m otter trawl, cod-end mesh size 12 mm) in Whitsand Bay (50° 33’N, 04° 24’W), Cornwall, U.K. and Bigbury Bay (50° 15’N, 03° 54’W), Devon, U.K. Following capture individuals were subsequently maintained at the Marine Biological Association of the United Kingdom. A total of four animals (DML 30 - 50 mm) were tested on each of the treatments in a random sequence order. Prior to the start of the trials all cuttlefish were kept in holding tanks and fed *ad libitum*. However, food was withheld

for cuttlefish for 24 hrs prior to the commencement of a trial.

### B.2.2 Prey

The brown shrimp (*Crangon crangon*) was used as prey for the purposes of experimental trials and also for feeding of the cuttlefish during general maintenance and rearing.

### B.2.3 Predators

For preliminary trials individual shore crabs (*Carcinus maenas*) were used to simulate visual predator cues. Prior to and following experimental trials all shore crabs were housed as per the requirements of Marine Biological Association and each crab used only once in any trial to ensure minimal stress. The predators in this experiment simply represent stimuli to evoke cuttlefish defensive behaviours; as such there was no direct physical contact between predators and cuttlefish during any of the trial scenarios. The tank was divided with a clear Perspex screen (with small holes to allow water flow) to ensure that the predators could be seen and/or chemically detected and that they could approach or recede from the cuttlefish, but that no direct physical contact between the cuttlefish and predators was possible. If the cuttlefish demonstrated any signs of abnormal behaviour or stress during the trials, the experiment was terminated immediately, with an opaque screen placed between the two animals to terminate visual contact and both individuals removed from the trial tank and returned to their holding tanks. After each trial the aquarium was fully drained and wiped clean to ensure that all predator stimuli are removed. If the cuttlefish inked before the start of the trial, that trial was terminated and the aquarium again drained and wiped clean prior to the commencement of the next trial.

### B.2.4 Water quality

The water quality of the experimental and holding tanks was recorded every five days. During these tests temperature, salinity, pH, ammonia, nitrite and nitrate were all sampled to ensure consistency.

### **B.2.5 Experimental design**

Experimental trials were conducted across a range of habitat complexities with three levels of habitat complexity and one control represented in this study:

1. Sediment no structure (Level 1)
2. Structure no sediment (Level 2)
3. Structure and sediment (Level 3)
4. No sediment and no structure (Control)

Artificial seagrass and natural sediment were used to simulate the basic components of physical complexity. The level of structural complexity reflected that found in nature, with the density of shoots and the number of leaves per shoot taken from data on seagrass bed structure in Torbay, presented in (Attrill et al. 2000). Shoot density per m<sup>2</sup> was taken to be medium (120 shoots) and the average number of leaves for seagrass beds of 120 shoots was taken to be 5 leaves per shoot (Attrill et al. 2000). This was scaled down for the area covered by the artificial substrates in each tank so that a total of 12 shoots each with 5 leaves were evenly distributed within the experimental tank. Artificial seagrass structure was created from green polypropylene ribbon cut into 0.5 cm width strips of 15 cm length. The leaves were attached to a small (1 cm diameter) clear circular aquarium suckers in groups of 5 to create shoots. The artificial shoots were attached to the base of the experimental tank. Natural sediment (sand, pebbles and small shells) was collected from an offshore area just outside Plymouth.

The experimental arena was constructed from a sea water tank (122 cm long and 61 cm wide with a depth of 20 cm) with the sides of the tank covered with waterproof white material to prevent disturbance or stress by unwanted visual stimulation. The experimental arena was illuminated from above by standard fluorescent ceiling lights. A Sony high definition camcorder was fixed directly above the experimental arena to enable video recording of all trials.

Experimental trials were conducted in February 2012 using four juvenile cuttlefish within the size range 30 - 50 mm (DML). A series of eight trials (Table B.1) were conducted on each test

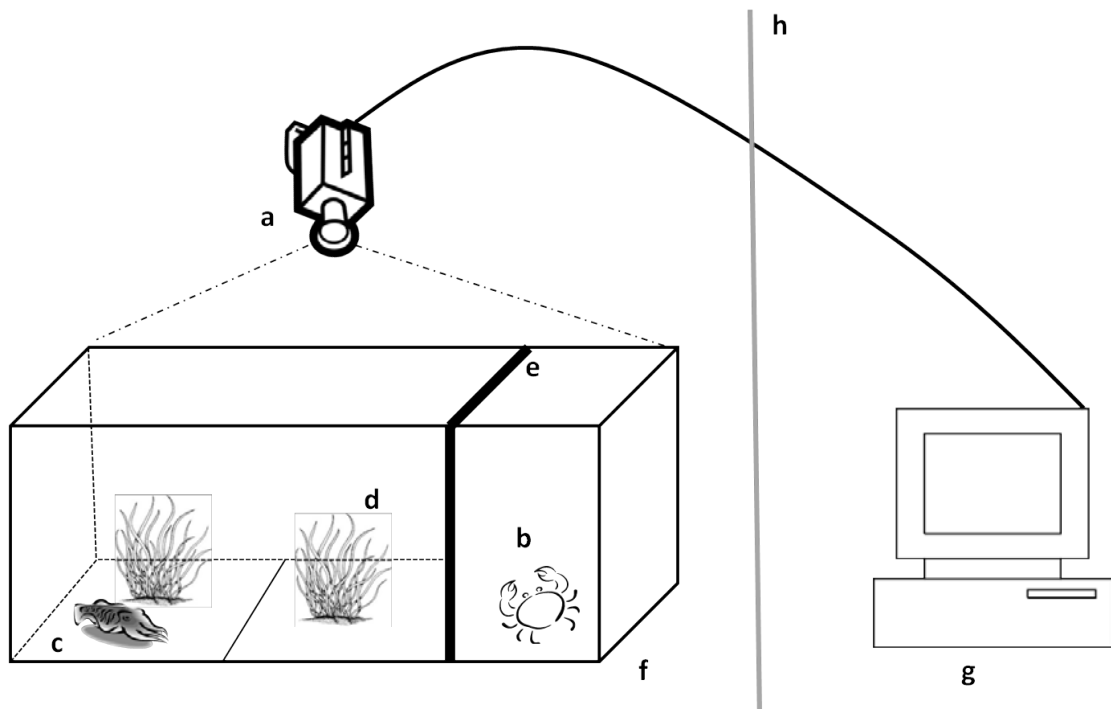
individual. For each test, individual trials were randomised, using a random number generator in Excel. Prior to the commencement of each trial, a cuttlefish was introduced into the trial arena with one of the four habitat complexities (Level 1, Level 2, Level 3 or Control) and allowed a period of acclimatisation (around 20 minutes). At the start of a trial a predator (1 shore crab) or prey (5 brown shrimp) stimuli was introduced to the trial arena and the behaviour and movement of the trial subject monitored and recorded by video. Each trial lasted no longer than 30 minutes. Each cuttlefish was used in only one trial per day and only once for any given stimulus. No more than four trials took place on any one day. At no point did the predators and cuttlefish come into direct contact. The use of naturalistic habitats, with hiding places (e.g. artificial seagrass or sediment for burying) and relatively low light levels was considered to reduce any potential stress associated with laboratory observations. If either species (cuttlefish or predator) showed signs of abnormal behaviour or stress, the trial would have been immediately terminated and the individuals immediately returned to their holding tanks.

*Table B.1:* a description of the 12 different trials used

Trial #	Habitat complexity	Cuttlefish	Prey	Predator
1	Level 1	Yes	Yes	-
2	Level 2	Yes	Yes	-
3	Level 3	Yes	Yes	-
4	Control	Yes	Yes	-
5	Level 1	Yes	-	Yes
6	Level 2	Yes	-	Yes
7	Level 3	Yes	-	Yes
8	Control	Yes	-	Yes

## B.2.6 Data collection

**B.2.6.0.1 Computer-aided video monitoring** Movement of the juvenile cuttlefish was recorded from above during all trials. The video camera was mounted directly above the video arena and the video recorded was situated in the next room, with cables exchanged between the wall to link the two systems (Figure B.1). Video trials were recorded and saved to DVD to allow subsequent analysis of the hunting sequences, defence mechanisms and movement patterns of individuals.



*Figure B.1:* Tank setup showing experimental arena and video monitoring system: (a) Ceiling mounted video camera, (b) Prey, (c) Test subject, (d) Habitat complexity components (e) Tank divider, (f) Tank, (g) Video monitoring and recording system (h) Wall separating experimental arena and video monitoring system

## **B.2.7 Data analysis**

### **B.2.7.1 Predation**

In trials where prey was used the number of attacks (successful and unsuccessful) was recorded and observations on the initiation and sequence of attacks described. This data was then compared between habitats to examine whether cuttlefish exhibit ‘habitat specific hunting’ patterns.

### **B.2.7.2 Defence**

In trials where a predator was used, observations on the defence behaviours and patterns were described and the data compared between habitats to examine what differences were exhibited by ELS among different habitat complexities.

### **B.2.7.3 Movement**

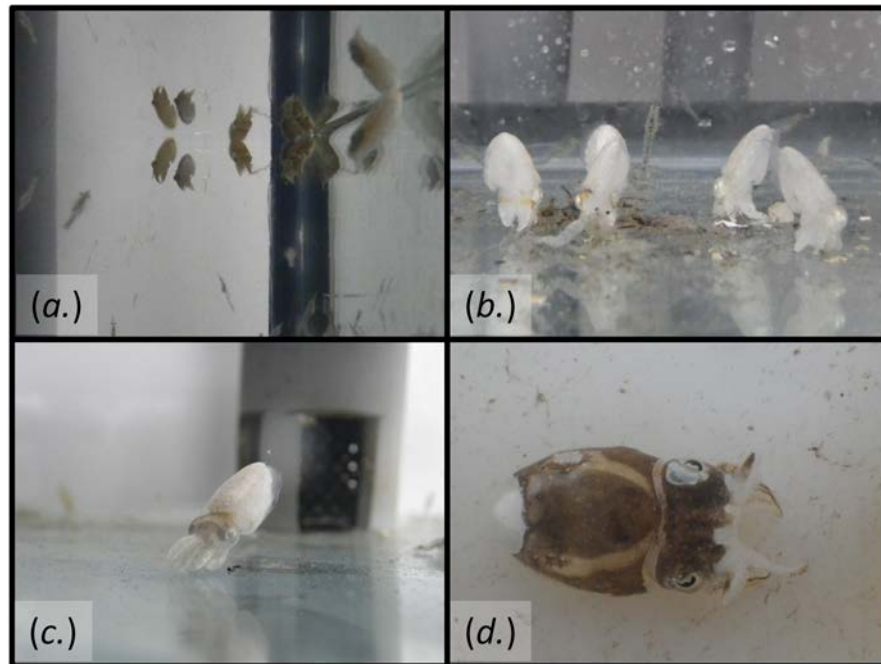
Data on the area covered and time spent moving or still was also recorded from the trials and the data compared between habitats to examine differences in movement patterns of ELS among different habitat complexities.

## **B.3 Results**

*(The presence of a bacterial infection (Vibrio sp.) within the sea water reservoir resulted in large scale death of cuttlefish hatchlings in both 2011 and 2012. As a result, trials were undertaken on only four individuals and thus the data collected within this study were considered too limited in scope to provide any robust analysis or discussion and so are not included below)*

### **B.3.1 Study animals**

Eggs collected from Torbay and Selsey began hatching on 20<sup>th</sup> July 2012. On 15<sup>th</sup> August 2011 the cuttlefish hatchlings began to die en masse. The water from the system was tested and high levels of bacteria were found to be present. The bacterial presence on the system only affected the cuttlefish hatchlings and all other fish species (of variable life stages) and adult cuttlefish remained unaffected. The infection spread rapidly around the hatchlings (Figure B.3.1) and approximately 800 hatchlings in total were affected and the commencement of experimental trials suspended.



infection on cuttlefish hatchlings.]A demonstration of the effects of the bacterial (*Vibrio* sp.) infection on cuttlefish hatchlings. (a.) The first signs of the infection are the loss of buoyancy control in the hatchlings which float with their back ends at the water surface, and hatchlings expending large amounts of energy swimming trying to maintain their buoyancy and position on the tank floor, (b.) and (c.) Hatchlings subsequently become very pale and attach themselves to the tank floor, moving very little, (d.) within around two three days from the first symptoms, the hatchlings die, often sores are visible on the mantle with flesh missing

A second source of juvenile cuttlefish was obtained from wild-caught specimens. On arrival at the MBA the four individuals captured were maintained and monitored in holding tanks and fed *ad libitum* for up to one month prior to the start of trials to ensure that no adverse behaviours or visible symptoms of bacterial infections were observed. It was only possible to collect four individuals, which was not sufficient for a full experimental trial, but enabled the methodology to be tested, with the aim of repeating a full scale study in 2012.

#### **B.4 Discussion.**

*(The presence of a bacterial infection (Vibrio sp.) within the sea water reservoir resulted in large scale death of cuttlefish hatchlings in both 2011 and 2012, the data collected within this*



#### *B.4. DISCUSSION.*

---

*study were considered too limited in scope to provide any robust discussion and so are not included below)*

## **Appendix C**

### **Supplementary information**

#### **C.1 Species occurrence data [DAASH]**

The data sources within the DAASH archive included:

- CEFAS survey data
- Seasearch survey and observation data
- Conchological Society data

#### **C.2 ‘Cuttlewatch’ sightings scheme**

Cuttlewatch was a scheme set up by the Marine Biological Association of the United Kingdom to encourage members of the general public to report their sightings of cuttlefish. The posters produced for the scheme (Figure C.1) were distributed to local dive groups, shops and organisations and provided information on how to report these sightings.

**CUTTLEWATCH**

Images: Keith Hiscock, Paul Naylor, John Rundle, Steve Trewinella.

**Take part in this exciting project by sending us your cuttlefish sightings!**

Where and when did you see them?  
 What sort of habitat were the cuttlefish in (seagrass, rocky, sandy)?  
 What were they doing (courting, fighting, spawning)?  
 Were you lucky enough to see a juvenile cuttlefish?  
 Did you come across some cuttlefish eggs?  
 What were the eggs attached to?

Sending us this information couldn't be easier:  
**email us: [CRESH@mba.ac.uk](mailto:CRESH@mba.ac.uk)**  
**phone us (24hr hotline): 01752 255026**  
**text your sightings to: 07806 938789**

In spring cuttlefish move from deeper parts of the English Channel into coastal waters to breed. As part of the CRESH Project (Cephalopod Recruitment from English-Channel Spawning Habitats) we are gathering information on cuttlefish breeding to help our understanding of stocks.

And remember, a picture tells a thousand words so if you have any photos we would love to see them!

Logos: Cefas, Ifremer, University of Plymouth, Marine Institute, Devon Sea Fisheries Committee, Royal Holloway University of London.

Figure C.1: Poster for the cuttlefish sightings scheme 'Cuttlewatch' to enable members of the general public to report cuttlefish sightings

### C.3 Poster display on cuttlefish acoustic tagging at Living Coasts

A poster display was designed and displayed at the Living Coast aquarium where the base station for the VRAP acoustic tagging study was situated. This enabled communication with the general public about this particular study as well as on the general ecology of the common cuttlefish.

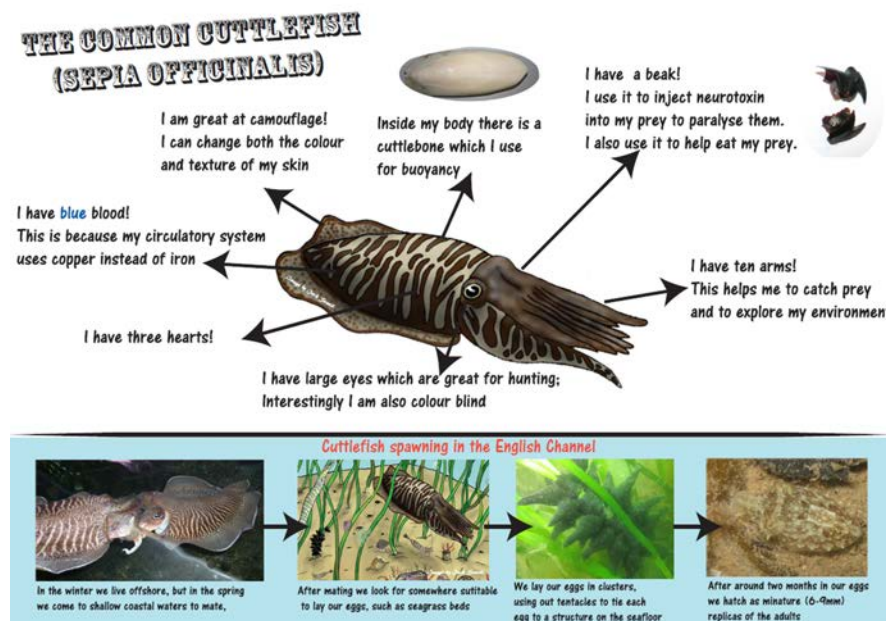


Figure C.2: Poster display (1 of 2) for Living Coasts on the general ecology of the common cuttlefish

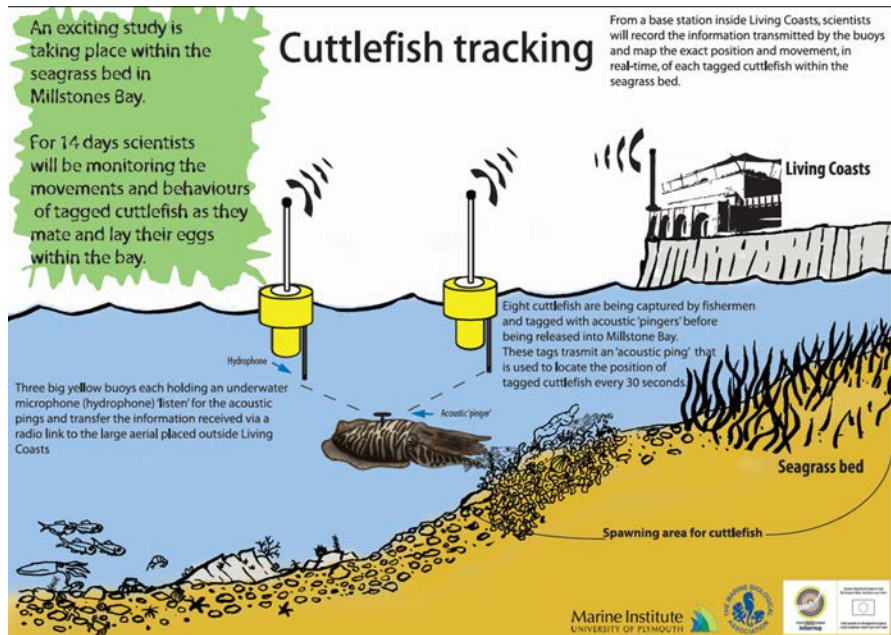


Figure C.3: Poster display (2 of 2) for Living Coasts on the VRAP acoustic tagging study conducted in Torbay

C.4 DST recovery

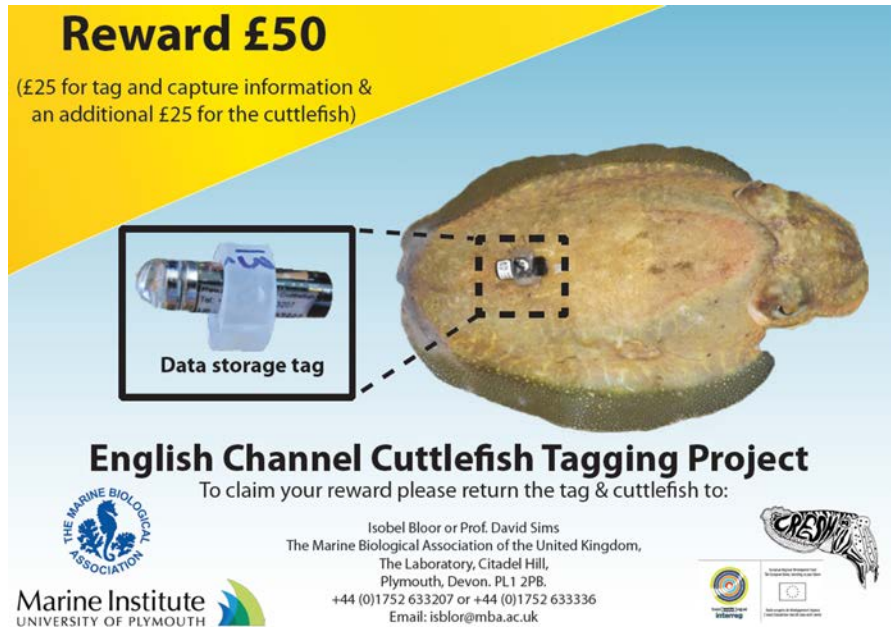


Figure C.4: Reward poster for DSTs aimed at Fishermen





Figure C.5: Reward poster for DSTs aimed at recreational beach goers



## List of references.

- Aarts, G., MacKenzie, M., McConnell, B., Fedak, M. and Matthiopoulos, J. (2008), 'Estimating space-use and habitat preference from wildlife telemetry data', *Ecography* **31**(1), 140–160.
- Abbott, N. J., Bundgaard, M. and Cserr, H. F. (1985), 'Brain vascular volume, electrolytes and blood-brain interface in the cuttlefish *Sepia officinalis* (Cephalopoda).', *The Journal of Physiology* **368**(1), 197–212.
- Adamo, S. A., Ehgoetz, K., Sangster, C. and Whitehorne, I. (2006), 'Signaling to the enemy? body pattern expression and its response to external cues during hunting in the cuttlefish *Sepia officinalis* (Cephalopoda)', *The Biological Bulletin* **210**(3), 192–200.
- Agnew, D. J., Beddington, J. R. and Hill, S. L. (2002), 'The potential use of environmental information to manage squid stocks', *Canadian Journal of Fisheries and Aquatic Sciences* **59**(12), 1851–1857.
- Agnew, D. J., Hill, S. and Beddington, J. R. (2000), 'Predicting the recruitment strength of an annual squid stock: *Loligo gahi* around the Falkland Islands', *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 2479–2487.
- Aitken, J. P., O'Dor, R. K. and Jackson, G. D. (2005), 'The secret life of the giant Australian cuttlefish *Sepia apama* (Cephalopoda): behaviour and energetics in nature revealed through radio acoustic positioning and telemetry (RAPT)', *Journal of Experimental Marine Biology and Ecology* **320**(1), 77–91.
- Alves, C., Chichery, R., Boal, J. G. and Dickel, L. (2006), 'Orientation in the cuttlefish *Sepia officinalis*: response versus place learning', *Animal Cognition* **10**(1), 29–36.
- Ambrose, R. F. (1988), 'Population dynamics of *Octopus bimaculatus*: Influence of life history patterns, synchronous reproduction and recruitment.', *Malacologia*. **29**(1), 23–39.
- Andersson, M. B. (2006), 'Condition-dependent indicators in sexual selection: development of theory and tests', *Essays in Animal Behaviour* pp. 255–269.
- Andrews, P. L. R. and Tansey, E. M. (1981), 'The effects of some anaesthetic agents in *Octopus vulgaris*', *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* **70**(2), 241–247.
- Araujo, J. N., Mackinson, S., Ellis, J. R. and Hart, P. J. B. (2005), An Ecopath model of the Western English Channel ecosystem with an exploration of its dynamic properties, *Science*



- Series technical Report Number 125, Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Lowestoft. 45pp.
- Arkhipkin, A. and Laptikhovsky, V. (1994), 'Seasonal and interannual variability in growth and maturation of winter-spawning *Illex argentinus* (Cephalopoda, Ommastrephidae) in the Southwest Atlantic', *Aquatic Living Resources* **7**(4), 221–232.
- Arkley, K., Jacklin, M., Boulter, M. and Tower, J. (1996), 'The cuttlefish (*Sepia officinalis*): a guide to its exploitation in UK waters', *Seafish Report no. SR567. SFIA, Hull* **81**.
- Attrill, M. J., Strong, J. A. and Rowden, A. A. (2000), 'Are macroinvertebrate communities influenced by seagrass structural complexity?', *Ecography* **23**(1), 114–121.
- Augustyn, C. J. (1990), 'Biological studies on the chokker squid *Loligo vulgaris reynaudii* (Cephalopoda; Myopsida) on spawning grounds off the south-east coast of South Africa', *South African Journal of Marine Science* **9**(3D1), 11–26.
- Bachmann, M. D., Carlton, R. G., Burkholder, J. A. M. and Wetzel, R. G. (1986), 'Symbiosis between salamander eggs and green algae: microelectrode measurements inside eggs demonstrate effect of photosynthesis on oxygen concentration', *Canadian Journal of Zoology* **64**(7), 1586–1588.
- Baeza-Rojano, E., García, S., Garrido, D., Guerra-García, J. M. and Domingues, P. (2010), 'Use of Amphipods as alternative prey to culture cuttlefish (*Sepia officinalis*) hatchlings', *Aquaculture* **300**(1-4), 243–246.
- Barber, I. and Arnott, S. A. (2000), 'Split-clutch IVF: a technique to examine indirect fitness consequences of mate preferences in sticklebacks', *Behaviour* **137**(8), 1129–1140.
- Bateman, A. J. (1948), 'Intra-sexual selection in *Drosophila*', *Heredity* **2**(Pt. 3), 349.
- Beck, M. W., Heck Jr, K. L., Able, K. W., Childers, D. L., Eggleston, D. B., Gillanders, B. M., Halpern, B., Hays, C. G., Hoshino, K., Minello, T. J., Orth, R. J., Sheridan, P. F. and Weinstein, M. P. (2001), 'The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates', *BioScience* **51**(8), 633–641.
- Begg, G. A., Friedland, K. A. and Pearce, J. B. (1999), 'Stock identification and its role in stock assessment and fisheries management: an overview', *Fisheries Research* **43**, 1–8.
- Bernardo, J. (1996), 'Maternal effects in animal ecology', *American Zoologist* **36**(2), 83–105.
- Blanc, A. (1998), *Recherches Bio-Ecologique Et Ecophysiologique De La Phase Juvenile De La Seiche *Sepia officinalis* Linne (Mollusque, Cephalopode, Sepiidae) Dans Le Golfe Du Morbihan (Sud Bretagne)*, PhD thesis, University of Rennes.

LIST OF REFERENCES.

---

- Blanc, A. and Daguzan, J. (1998), 'Artificial surfaces for cuttlefish eggs (*Sepia officinalis* L.) in Morbihan Bay, France', *Fisheries Research* **38**(3), 225 – 231.
- Blanc, A. and Daguzan, J. (1999), 'Young cuttlefish *Sepia officinalis* (Mollusca: Sepiidae) in the Morbihan Bay (South Brittany, France): accessory prey of predators', *Journal of the Marine Biological Association of the United Kingdom* **79**(06), 1133–1134.
- Blanc, A., Du Sel, G. P. and Daguzan, J. (1998), 'Habitat and diet of early stages of *Sepia officinalis* L.(Cephalopoda) in Morbihan Bay, France', *Journal of Molluscan Studies* **64**(3), 263–274.
- Boal, J. G. (1997), 'Female choice of males in cuttlefish (Mollusca: Cephalopoda)', *Behaviour* pp. 975–988.
- Boal, J. G. and Golden, D. K. (1999), 'Distance chemoreception in the common cuttlefish, *Sepia officinalis* (Mollusca, Cephalopoda)', *Journal of Experimental Marine Biology and Ecology* **235**(2), 307–317.
- Boavida-Portugal, J., Moreno, A., Gordo, L. and Pereira, J. (2010), 'Environmentally adjusted reproductive strategies in females of the commercially exploited common squid *Loligo vulgaris*', *Fisheries Research* **106**(2), 193–198.
- Boles, L. C. and Lohmann, K. J. (2003), 'True navigation and magnetic maps in spiny lobsters', *Nature* **421**(6918), 60–63.
- Boletzky, S. V. (1975), 'A contribution to the study of yolk absorption in the Cephalopoda', *Zoomorphology* **80**(3), 229–246.
- Boletzky, S. V. (1983), *Sepia officinalis*, in P. R. Boyle, ed., 'Cephalopod Life Cycles', Vol. I, Academic Press, pp. 31–52.
- Boletzky, S. V. (1986a), 'Encapsulation of cephalopod embryos: a search for functional correlations', *American Malacological Bulletin* **4**(2), 217–227.
- Boletzky, S. V. (1986b), 'Reproductive strategies in cephalopods: variation and flexibility of life-history patterns', *Advances in Invertebrate Reproduction* **4**.
- Boletzky, S. V. (1987a), 'Fecundity variation in relation to intermittent or chronic spawning in th cuttlefish, *Sepia officinalis* L. (Mollusca, Cephalopoda)', *Bulletin of Marine Science* **40**(2), 382–387.
- Boletzky, S. V. (1987b), Embryonic phase, in P. R. Boyle, ed., 'Cephalopod Life Cycles', Vol. II, Academic Press.

- Boletzky, S. V. (1987c), Juvenile behaviour, in P. Boyle, ed., 'Cephalopod Life Cycles', Vol. II, Academic Press.
- Boletzky, S. V. (1988), 'A new record of long-continued spawning in *Sepia officinalis* (Mollusca, Cephalopoda)', *Rapports de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranéet* **31**(3D2), 257.
- Boletzky, S. V. (1989), 'Recent studies on spawning, embryonic development, and hatching in the Cephalopoda', *Advances in Marine Biology* **25**, 85–115.
- Boletzky, S. V. (1994), 'Embryonic development of cephalopods at low temperatures', *Antarctic Science* **6**(02), 139–142.
- Boletzky, S. V., Erlwein, B. and Hofmann, D. K. (2006), 'The *Sepia* egg: a showcase of cephalopod embryology', *Vie et Milieu* **56**(2), 191–201.
- Boucaud-Camou, E. and Boismery, J. (1991), The migrations of the cuttlefish (*Sepia officinalis* L.) in the English Channel, in 'La seiche: actes du Premier Symposium international sur la seiche, Caen, 1-3 juin 1989', pp. 1–3.
- Boucaud-Camou, E., Koueta, N., Boismery, J. and Medhioub, A. (1991), The sexual cycle of *Sepia officinalis* l. from the Bay of Seine, in 'La seiche: actes du Premier Symposium international sur la seiche, Caen, 1-3 juin 1989', Institut de biochimie et de biologie appliquee, Universite de Caen, p. 141.
- Bouchaud, O. (1991a), 'Energy consumption of the cuttlefish *Sepia officinalis* l. (Mollusca: Cephalopoda) during embryonic development, preliminary results', *Bulletin of Marine Science* pp. 333–340.
- Bouchaud, O. (1991b), Recherches physiologiques sur la reproduction de la seiche, *Sepia officinalis*, PhD thesis, University of Rennes, P265.
- Bouchaud, O. and Daguzan, J. (1989), 'Etude du développement de oeuf de *Sepia officinalis* l (céphalopode, sepioidea) en conditions expérimentales', *Haliotis* **19**, 189–200.
- Bouchaud, O. and Daguzan, J. (1990), 'Etude expérimentale de l'influence de la température sur le déroulement embryonnaire de la seiche *Sepia officinalis* L. (Cephalopoda, Sepioidae). experimental study of temperature effects on the embryonic development of the cuttlefish *Sepia officinalis* L.(Cephalopoda, Sepioidae)', *Cahiers de Biologie Marine* .
- Bouchaud, O. and Galois, R. (1990), 'Utilization of egg-yolk lipids during the embryonic development of *Sepia officinalis* L. in relation to temperature of the water', *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **97**(3), 611–615.

- Boucher-Rodoni, R., Boucaud-Camou, E., Mangold, K. and Boyle, P. R. (1987), Feeding and digestion, in P. R. Boyle, ed., 'Cephalopod Life Cycles', Vol. II.
- Boyle, P. R. (1990), 'Cephalopod biology in the fisheries context', *Fisheries Research* **8**(4), 303–321.
- Boyle, P. R. and Boletzky, S. V. (1996), 'Cephalopod populations: definition and dynamics', *Philosophical Transactions: Biological Sciences* **351**(1343), 985–1002.
- Boyle, P. R. and Rodhouse, P. (2005), *Cephalopods: ecology and fisheries*, Wiley-Blackwell.
- Brambilla, M. and Ficetola, G. F. (2012), 'Species distribution models as a tool to estimate reproductive parameters: a case study with a passerine bird species', *Journal of Animal Ecology* **81**, 781.787.
- Brigden, K. (2010), A study into the cuttlefish fishery of Torbay, Devon: Investigating spawning areas from the fishermen's perspective and the potential for using artificial egg laying structures., Technical report, MSc Thesis, Plymouth University.
- Budelmann, B. U. (1994), 'Cephalopod sense organs, nerves and the brain: Adaptations for high performance and life style', *Marine Behaviour and Physiology* **25**(1), 13–33.
- Budelmann, B. U. (1996), 'Active marine predators: The sensory world of cephalopods', *Marine and Freshwater Behaviour and Physiology* **27**(2), 59–75.
- Bunker, F., Brodie, J., Maggs, C. and Bunker, A. (2010), *Seasearch Guide to Seaweeds of Britain and Ireland*, Marine Conservation Society.
- Busby, J. R. (1991), 'BIOCLIM-a bioclimate analysis and prediction system', *Plant Protection Quarterly* **6**.
- Bustamante, P., Teyssié, J. L., Danis, B., Fowler, S. W., Miramand, P., Cotret, O. and Warnau, M. (2004), 'Uptake, transfer and distribution of silver and cobalt in tissues of the common cuttlefish *Sepia officinalis* at different stages of its life cycle', *Marine Ecology Progress Series* **269**, 185–195.
- Bustamante, P., Teyssié, J. L., Fowler, S., Cotret, O., Danis, B. and Warnau, M. (2002), 'Biokinetics of cadmium and zinc accumulation and depuration at different stages in the life cycle of the cuttlefish *Sepia officinalis*', *Marine Ecology Progress Series* **231**, 167–177.
- Bustamante, P., Teyssié, J. L., Fowler, S. and Warnau, M. (2006), 'Contrasting bioaccumulation and transport behaviour of two artificial radionuclides ( $^{241}\text{Am}$  and  $^{134}\text{Cs}$ ) in cuttlefish eggshell', *Vie et Milieu* **56**(2), 153–156.

## LIST OF REFERENCES.

---

- Caddy, J. F. (1983), The cephalopods: factors relevant to their population dynamics and to the assessment and management of stocks, Fisheries technical paper, FAO.
- Caddy, J. F. (1996), 'Modelling natural mortality with age in short-lived invertebrate populations: definition of a strategy of gnomonic time division', *Aquatic Living Resources* **9**(3), 197–207.
- Calabrese, A. and Nelson, D. A. (1974), 'Inhibition of embryonic development of the hard clam *Mercenaria mercenaria*, by heavy metals', *Bulletin of Environmental Contamination and Toxicology* **11**(1), 92–97.
- Carpenter, G., Gillison, A. N. and Winter, J. (1993), 'DOMAIN: a flexible modelling procedure for mapping potential distributions of plants and animals', *Biodiversity and Conservation* **2**(6), 667–680.
- Carpentier, A., Martin, C. S. and Vaz, S. (2009), *Channel Habitat Atlas for marine Resource Management, final report*, INTERREG 3a Programme, IFREMER, Boulogne-sur-mer, France.
- Castro, B. G. and Guerra, Á. (1990), 'The diet of *Sepia officinalis* (Linnaeus, 1758) and *Sepia elegans* (D'Orbigny, 1835)(Cephalopoda, Sepioidea) from the Ría de Vigo (NW Spain)', *Scientia Marina* **54**(4), 375–388.
- Caverivière, A., Domain, F. and Diallo, A. (1999), 'Observations on the influence of temperature on the length of embryonic development in *Octopus vulgaris* (Senegal)', *Aquatic Living Resources* **12**(2), 151–154.
- Challier, L., Dunn, M. R. and Robin, J. P. (2005a), 'Trends in age-at-recruitment and juvenile growth of cuttlefish, *Sepia officinalis*, from the English Channel', *ICES Journal of Marine Science* **62**(8), 1671–1682.
- Challier, L., Pierce, G. J. and Robin, J. P. (2006), 'Spatial and temporal variation in age and growth in juvenile *Loligo forbesi* and relationships with recruitment in the English Channel and Scottish waters', *Journal of Sea Research* **55**(3), 217–229.
- Challier, L., Royer, J., Pierce, G. J., Bailey, N., Roel, B. and Robin, J. P. (2005b), 'Environmental and stock effects on recruitment variability in the English Channel squid *Loligo forbesi*', *Aquatic Living Resources* **18**(4), 353–360.
- Challier, L., Royer, J. and Robin, J. P. (2002), 'Variability in age-at-recruitment and early growth in English Channel *Sepia officinalis* described with statolith analysis', *Aquatic Living Resources* **15**(5), 303–311.

LIST OF REFERENCES.

---

- Cheung, W. W. L., Lam, V. W. Y., Sarmiento, J. L., Kearney, K., Watson, R. and Pauly, D. (2009), 'Projecting global marine biodiversity impacts under climate change scenarios', *Fish and Fisheries* **10**(3), 235–251.
- Choe, S. (1966), 'On the eggs, rearing, habits of the fry, and growth of some Cephalopoda', *Bulletin of Marine Science* **16**(2), 330–348.
- Clark, R. (2007), Preliminary investigation into the feasibility of laying artificial substrates as receptors for cuttlefish eggs, Technical report, Sussex Sea Fisheries Committee.
- Clarke, M. R. and Pascoe, P. L. (1985), 'The stomach contents of a Risso's dolphin (*Grampus griseus*) stranded at Thurlestone, South Devon.', *Journal of the Marine Biological Association of the United Kingdom* **65**(3), 663–665.
- Clarke, M. R. and Stevens, J. D. (1974), 'Cephalopods, blue sharks and migration', *Journal of the Marine Biological Association of the United Kingdom* **54**(04), 949–957.
- Cohen, C. S. and Strathmann, R. R. (1996), 'Embryos at the edge of tolerance: effects of environment and structure of egg masses on supply of oxygen to embryos', *The Biological Bulletin* **190**(1), 8.
- Collewijn, H. (1970), 'Oculomotor reactions in the cuttlefish, *Sepia officinalis*', *Journal of Experimental Biology* **52**(2), 369–384.
- Collins, K. (2007), 'Poole Bay/Harbour marine habitat surveys: Post channel deepening EIA studies 2006', *Final Report to PHC* .
- Cope, S. N. (2005), *Coastal Dynamics*, American Society of Civil Engineers, chapter Predicting Overwashing and Breaching of Coarse-Clastic Barrier Beaches and Spits - Application to Medmerry, West Sussex, pp. 1–14.
- Correia, M., Palma, J. and Andrade, J. P. (2008a), 'Effects of live prey availability on growth and survival in the early stages of cuttlefish *Sepia officinalis* (Linnaeus, 1758) life cycle', *Aquaculture Research* **39**(1), 33–40.
- Correia, M., Palma, J., Kirakowski, T. and Andrade, J. P. (2008b), 'Effects of prey nutritional quality on the growth and survival of juvenile cuttlefish, *Sepia officinalis* (Linnaeus, 1758)', *Aquaculture Research* **39**(8), 869–876.
- Cowan, J. H. Shaw, R. F. (2002), *Recruitment*, Blackwell Science, chapter Recruitment, pp. 88–111.
- Cronin, E. R. and Seymour, R. S. (2000), 'Respiration of the eggs of the giant cuttlefish *Sepia apama*', *Marine Biology* **136**(5), 863–870.

## LIST OF REFERENCES.

---

- Darmaillacq, A. S., Chichery, R. and Dickel, L. (2006b), 'Food imprinting, new evidence from the cuttlefish *Sepia officinalis*', *Biology Letters* **2**(3), 345–347.
- Darmaillacq, A. S., Chichery, R., Poirier, R. and Dickel, L. (2004), 'Effect of early feeding experience on subsequent prey preference by cuttlefish, *Sepia officinalis*', *Developmental Psychobiology* **45**(4), 239–244.
- Darmaillacq, A. S., Chichery, R., Shashar, N. and Dickel, L. (2006a), 'Early familiarization overrides innate prey preference in newly hatched *Sepia officinalis* cuttlefish', *Animal Behaviour* **71**(3), 511–514.
- Darmaillacq, A. S., Lesimple, C. and Dickel, L. (2008), 'Embryonic visual learning in the cuttlefish, *Sepia officinalis*', *Animal Behaviour* **76**(1), 131–134.
- Dauvin, J. C. (2012), 'Are the eastern and western basins of the English Channel two separate ecosystems?', *Marine Pollution Bulletin* **64**, 463–471.
- De Wachter, B., Wolf, G., Richard, A. and Declair, W. (1988), 'Regulation of respiration during juvenile development of *Sepia officinalis* (Mollusca:Cephalopoda)', *Marine Biology* **97**, 365–371.
- De'Ath, G. (2007), 'Boosted trees for ecological modeling and prediction', *Ecology* **88**(1), 243–251.
- Den Hartog, C. (1987), '"wasting disease" and other dynamic phenomena in *Zostera* beds', *Aquatic botany* **27**, 3–14.
- Denis, V., Lejeune, J. and Robin, J. P. (2002), 'Spatio-temporal analysis of commercial trawler data using General Additive Models: patterns of *Loliginid* squid abundance in the north-east Atlantic', *ICES Journal of Marine Science* **59**(3), 633–648.
- Denis, V. and Robin, J. (2001), 'Present status of the French Atlantic fishery for cuttlefish (*Sepia officinalis*)', *Fisheries Research* **52**(1-2), 11–22.
- Denis, V. and Robin, J. P. (2000), 'Spatio-temporal patterns in cephalopod resources exploited by the French Atlantic Fishery-Empirical models of abundance based on environmental parameters'.
- Denton, E. J. and Gilpin-Brown, J. B. (1961), 'The effect of light on the buoyancy of the cuttlefish', *Journal of the Marine Biological Association of the United Kingdom* **41**(02), 343–350.
- Derby, C. D., Kicklighter, C. E., Johnson, P. M. and Zhang, X. (2007a), 'Chemical composition of inks of diverse marine molluscs suggests convergent chemical defenses', *Journal of Chemical Ecology* **33**(5), 1105–1113.

## LIST OF REFERENCES.

---

- DeRusha, R. H., Forsythe, J. W., DiMarco, F. P. and Hanlon, R. T. (1989), 'Alternative diets for maintaining and rearing cephalopods in captivity.', *Laboratory Animal Science* **39**(4), 306.
- Dickel, L., Chichery, M. P. and Chichery, R. (1997), 'Postembryonic maturation of the vertical lobe complex and early development of predatory behavior in the cuttlefish (*Sepia officinalis*)', *Neurobiology of Learning and Memory* **67**(2), 150–160.
- Dictionary, C. O. (1999), *Concise Oxford Dictionary*, Oxford University Press.
- Dingle, H. (1996), *Migration: the biology of life on the move*, Oxford University Press.
- Dingle, H. and Drake, V. A. (2007), 'What is migration?', *Bioscience* **57**(2), 113–121.
- Dodson, J. J. (1997), *Behavioural Ecology of Teleost Fish*, Oxford University Press, USA, chapter 2 Fish migration: an evolutionary perspective, pp. 10–36.
- Domingues, P. M., Kingston, T., Sykes, A. and 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**(11), 923–930.
- Domingues, P. M., Sykes, A. and 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**(4), 319–331.
- Domingues, P., Poirier, R., Dickel, L., Almansa, E., Sykes, A. and Andrade, J. P. (2003), 'Effects of culture density and live prey on growth and survival of juvenile cuttlefish, *Sepia officinalis*', *Aquaculture International* **11**, 225–242.
- Domingues, P., Sykes, A., Sommerfield, A., Almansa, E., Lorenzo, A. and 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**(1-4), 239–254.
- Donohue, K. (1999), 'Seed dispersal as a maternally influenced character: mechanistic basis of maternal effects and selection on maternal characters in an annual plant', *American Naturalist* **154**(6), 674–689.
- Dudík, M., Phillips, S. J. and Roberts, E. S. (2004), Performance guarantees for regularized maximum entropy density estimation, in 'Proceedings of the 17th Annual Conference on Computational Learning Theory'.
- Dudík, M., Phillips, S. J. and Schapire, R. E. (2007), 'Maximum entropy density estimation with generalized regularization and an application to species distribution modeling', *Journal of Machine Learning Research* **8**, 1217–1260.



- Dudík, M., Schapire, R. E. and Phillips, S. J. (2005), 'Correcting sample selection bias in maximum entropy density estimation', *Advances in Neural Information Processing Systems* **17**, 323–330.
- Dunn, M. R. (1999), 'Aspects of the stock dynamics and exploitation of cuttlefish, *Sepia officinalis* (Linnaeus, 1758), in the English Channel.', *Fisheries Research* **40**, 277–293.
- Eberhard, W. G. (1985), *Sexual selection and animal genitalia*, Harvard University Press.
- Eberhard, W. G. (1996), *Female control: sexual selection by cryptic female choice*, Princeton University Press.
- Edrén, S., Wisz, M. S., Teilmann, J., Dietz, R. and Söderkvist, J. (2010), 'Modelling spatial patterns in harbour porpoise satellite telemetry data using maximum entropy', *Ecography* **33**(4), 698–708.
- Elith, J. and Graham, C. H. (2009), 'Do they? how do they? why do they differ? on finding reasons for differing performances of species distribution models', *Ecography* **32**(1), 66–77.
- Elith, J., Graham, C. H., Anderson, R. P., Dudík, M., Ferrier, S., Guisan, A., Hijmans, R. J., Huettmann, F., Leathwick, J. R., Lehmann, A., Li, J., Lohmann, L. G., Loiselle, B. A., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., Overton, J. M., Peterson, A. T., Phillips, S. J., Richardson, K. S., Scachetti-Pereira, R., Schapire, R. E., Sobero'n, J., Williams, S., Wisz, M. S. and Zimmermann, N. E. (2006), 'Novel methods improve prediction of species' distributions from occurrence data', *Ecography* **29**(2), 129–151.
- Elith, J. and Leathwick, J. (2007), 'Predicting species distributions from museum and herbarium records using multiresponse models fitted with multivariate adaptive regression splines', *Diversity and Distributions* **13**(3), 265–275.
- Elith, J. and Leathwick, J. R. (2009), 'Species distribution models: ecological explanation and prediction across space and time', *Annual Review of Ecology, Evolution, and Systematics* **40**, 677–697.
- Elith, J., Leathwick, J. R. and Hastie, T. (2008), 'A working guide to boosted regression trees', *Journal of Animal Ecology* **77**(4), 802–813.
- Elith, J., Phillips, S. J., Hastie, T., Dudík, M., Chee, Y. E. and Yates, C. J. (2011), 'A statistical explanation of MaxEnt for ecologists', *Diversity and Distributions* .
- Ezzedine-Najai, S. (1985), 'Fecundity of cuttlefish, *Sepia officinalis* L. (Mollusca: Cephalopoda) from the gulf of Tunis', *Vie et Milieu* **35**.
- Ezzedine-Najai, S., Ros, J. and Guerra, A. (1997), 'Tagging of the cuttlefish, *Sepia officinalis* L. (Cephalopoda: Decapoda), in the Gulf of Tunis', *Scientia Marina* **61**, 59–65.

#### LIST OF REFERENCES.

---

- Fabry, V. J., Seibel, B. A., Feely, R. A. and Orr, J. C. (2008), 'Impacts of ocean acidification on marine fauna and ecosystem processes', *ICES Journal of Marine Science* **65**, 414–432.
- FAO (1964), *Yearbook Fisheries Statistics. Catches and landings 1963*, Vol. 16.
- Feely, R. A., Doney, S. C. and Cooley, S. R. (2009), 'Ocean acidification: Present conditions and future changes in a high-co<sub>2</sub> world', *Oceanography* **22**, 36–47.
- Filer, J. L. (2009), Movement patterns and search behaviour of a predatory marine fish, PhD thesis, University of Leicester.
- Fisheries and Aquaculture Information and Statistics Service (FAO) (2010), Fao yearbook. fishery and aquaculture statistics. 2008, Technical report, Fishery and Aquaculture Statistics.
- Fonesca, M. S. and Bell, S. S. (1998), 'Influence of physical settings on seagrass landscapes near beaufort, north carolina, usa.', *Marine Ecology Progress Series* **171**, 109–121.
- Fonesca, M. S., Zieman, J. C., Thayer, G. W. and Fisher, J. S. (1983), 'The role of current velocity in structuring eelgrass (*Zostera marina* L.) meadows', *Estuarine, Coastal and Shelf Science* **17**, 367–380.
- Fonseca, M. S., Fisher, J. S., Zieman, J. C. and Thayer, G. W. (1982), 'Influence of the seagrass, *Zostera marina* L., on current flow', *Estuarine, Coastal and Shelf Science* **15**(4), 351–358.
- Forster, G. R. (1955), 'Underwater observations on rocks off Stoke Point and Dartmouth', *Journal of the Marine Biological Association of the United Kingdom* **34**(02), 197–199.
- Forsythe, J., Lee, P., Walsh, L. and Clark, T. (2002), 'The effects of crowding on growth of the European cuttlefish, *Sepia officinalis* Linnaeus, 1758 reared at two temperatures', *Journal of Experimental Marine Biology and Ecology* **269**(2), 173–185.
- Forsythe, J. W. (1993), 'A working hypothesis of how seasonal temperature change may impact the field growth of young cephalopods', *Recent advances in cephalopod fisheries biology*. Tokai University Press, Tokyo pp. 133–143.
- Forsythe, J. W. (2004), 'Accounting for the effect of temperature on squid growth in nature: from hypothesis to practice', *Marine and Freshwater Research* **55**(4), 331–339.
- Forsythe, J. W., DeRusha, R. H. and 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. and Hanlon, R. T. (1988), 'Effect of temperature on laboratory growth, reproduction and life span of *Octopus bimaculoides*', *Marine Biology* **98**(3), 369–379.

## LIST OF REFERENCES.

---

- Forsythe, J. W. and Hanlon, R. T. (1989), 'Growth of the eastern Atlantic squid, *Loligo forbesi* Steenstrup (Mollusca: Cephalopoda)', *Aquaculture Research* **20**(1), 1–14.
- Forsythe, J. W. and Van Heukelem, W. F. (1987), Growth, in P. R. Boyle, ed., 'Cephalopod life cycles', Vol. II, Academic Press.
- Forsythe, J. W., Walsh, L. S., Turk, P. E. and 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**(1), 103–112.
- Fox, C. W. and Mousseau, T. (1998), 'Maternal effects as adaptations for transgenerational phenotypic', *Maternal effects as adaptations* p. 159.
- Frank, M. G., Waldrop, R. H., Dumoulin, M., Aton, S. and Boal, J. G. (2012), 'A preliminary analysis of sleep-like states in the cuttlefish *Sepia officinalis*', *PloS one* **7**(6), e38125.
- Franklin, J. and Miller, J. A. (2009), *Mapping species distributions: spatial inference and prediction*, Vol. 338, Cambridge University Press Cambridge.
- Franz, M. O. and Mallot, H. A. (2000), 'Biomimetic robot navigation', *Robotics and Autonomous Systems* **30**(1-2), 133 – 153.
- Frederiksen, M., Krause-Jensen, D., Holmer, M. and Laursen, J. S. (2004), 'Spatial and temporal variation in eelgrass (*Zostera marina*) landscapes: influence of physical setting', *Aquatic botany* **78**, 147–165.
- Freeman, S. M. and Rogers, S. I. (2003), 'A new analytical approach to the characterisation of macro-epibenthic habitats: linking species to the environment', *Estuarine, Coastal and Shelf Science* **56**(3-4), 749–764.
- Friedland, K. D., Stock, C., Drinkwater, K. F., Link, J. S., Leaf, R. T., Shank, B. V., Rose, J. M., Pilskaln, C. H. and Fogarty, M. J. (2012), 'Pathways between primary production and fisheries yields of large marine ecosystems', *PloS one* **7**(1), e28945.
- Friedman, J. H. (1991), 'Multivariate adaptive regression splines', *The Annals of Statistics* pp. 1–67.
- Fritz, R. S., Stamp, N. E. and Halverson, T. G. (1982), 'Iteroparity and semelparity in insects', *The American Naturalist* **120**(2), 264–268.
- Fuiman, L. A. and Werner, R. G. (2002), *Fishery science: the unique contributions of early life stages*, Wiley-Blackwell.
- Gadsden, H., Ballesteros-Barrera, C., Hinojosa de la Garza, O., Castañeda, G., García-De la Peña, C. and Lemos-Espinal, J. A. (2012), 'Effects of land-cover transformation and climate

- change on the distribution of two endemic lizards, *Crotaphytus antiquus* and *Sceloporus cyanostictus*, of northern Mexico', *Journal of Arid Environments* **83**, 1–9.
- Garnaud, S., Lesueur, P., Lesourd, S., Poupinet, N. and Brun-Cottan, J. C. (2002), 'Caractérisation des variations lithologiques saisonnières des sédiments de la Baie de Seine orientale; impact des crues de la Seine (France)', *Comptes Rendus Geoscience* **334**(5), 323–330.
- Gauvrit, E., Goff, R. L. and Daguzan, J. (1997), 'Reproductive cycle of the cuttlefish *Sepia officinalis* (L) in the northern part of the Bay of Biscay', *Journal of Molluscan Studies* **63**(1), 19–28.
- Gilly, W. F., Markaida, U., Baxter, C. H., Block, B. A., Boustany, A., Zeidberg, L., Reisenbichler, K., Robison, B., Bazzino, G. and Salinas, C. (2006), 'Vertical and horizontal migrations by the jumbo squid *Dosidicus gigas* revealed by electronic tagging', *Marine Ecology Progress Series* **324**, 1–17.
- Ginsburg, R. N. and Lowenstam, H. A. (1958), 'The influence of marine bottom communities on the depositional environment of sediments', *The Journal of Geology* pp. 310–318.
- Gonzalez, A. F., Otero, J., Pierce, G. J. and Guerra, A. (2010), 'Age, growth, and mortality of *Loligo vulgaris* wild paralarvae: implications for understanding of the life cycle and longevity', *ICES Journal of Marine Science* **67**(6), 1119–1127.
- Gore, S. R., Harms, C. A., Kukanich, B., Forsythe, J., Lewbart, G. A. and Papich, M. G. (2005), 'Enrofloxacin pharmacokinetics in the European cuttlefish, *Sepia officinalis*, after a single IV injection and bath administration', *Journal of Veterinary Pharmacology and Therapeutics* **28**(5), 433–439.
- Gouyen, J. (2001), 'Economie et ecologie', *Samudra* pp. 37–49.
- Griffin, D. R. (1955), *Recent studies in avian biology*, University of Illinois press, chapter Bird navigation, pp. 154–197.
- Grimpe, G. (1926), 'Biologische beobachtungen an *Sepia officinalis*', *Verh dt zool Ges (Suppl Zool Anz)* **31**, 148–153.
- Grinnell, J. (1904), 'The origin and distribution of the chest-nut-backed chickadee', *The Auk* **21**(3), 364–382.
- Guerra, A. (2006), 'Ecology of *Sepia officinalis*', *Vie et Milieu* **56**(2), 97–107.
- Guerra, A. and Castro, B. G. (1988), 'On the life of *Sepia officinalis* (Cephalopoda, Sepioidea) in the Ria de Vigo (NW Spain)', *Cahiers de Biologie Marine* **29**(3).

## LIST OF REFERENCES.

---

- Guerra, A. and Castro, B. G. (1994), 'Reproductive-somatic relationships in *Loligo gahi* (Cephalopoda: Loliginidae) from the Falkland Islands', *Antarctic Science* **6**(02), 175–178.
- Guerra, A. and González, J. L. (2011), 'First record of predation by a tompot blenny on the common cuttlefish *Sepia officinalis* eggs', *Vie et Milieu* **61**(1), 45–48.
- Guerra-García, J. M. and Tierno de Figueroa, J. M. (2009), 'What do caprellids (crustacea: Amphipoda) feed on?', *Marine Biology* **156**(9), 1881–1890.
- Guisan, A., Edwards, T. C. and Hastie, T. (2002), 'Generalized linear and generalized additive models in studies of species distributions: setting the scene', *Ecological Modelling* **157**(2), 89–100.
- Guisan, A. and Thuiller, W. (2005), 'Predicting species distribution: offering more than simple habitat models', *Ecology Letters* **8**(9), 993–1009.
- Guisan, A. and Zimmermann, N. E. (2000), 'Predictive habitat distribution models in ecology', *Ecological Modelling* **135**(2), 147–186.
- Guisan, A., Zimmermann, N. E., Elith, J., Graham, C. H., Phillips, S. and Peterson, A. T. (2007), 'What matters for predicting the occurrences of trees: Techniques, data or species' characteristics?', *Ecological Monographs* **77**(4), 615–630.
- Gunkel, C. Lewbart, G. A. (2008), *Anesthesia and Analgesia in Laboratory Animals*, Academic Press, chapter 22: Anesthesia and analgesia of Invertebrates, pp. 535–543.
- Gutowska, M. A. and Melzner, F. (2009), 'Abiotic conditions in cephalopod (*Sepia officinalis*) eggs: embryonic development at low pH and high pCO<sub>2</sub>', *Marine Biology* **156**(3), 515–519.
- Gutowska, M. A., Pörtner, H. O. and Melzner, F. (2008), 'Growth and calcification in the cephalopod *Sepia officinalis* under elevated seawater pCO<sub>2</sub>', *Marine Ecology Progress Series* **373**, 303–309.
- Hall, K. C. and Hanlon, R. T. (2002), 'Principal features of the mating system of a large spawning aggregation of the giant Australian cuttlefish *Sepia apama* (Mollusca: Cephalopoda)', *Marine Biology* **140**, 533–545.
- Hanley, J. A. and McNeil, B. J. (1982), 'The meaning and use of the area under a receiver operating characteristic (ROC) curve', *Radiology* **143**, 29–36.
- Hanley, J. S., Shashar, N., Smolowitz, R., Bullis, R. A., Mebane, W. N., Gabr, H. R. and Hanlon, R. T. (1998), 'Modified laboratory culture techniques for the European cuttlefish *Sepia officinalis*', *Biological Bulletin* pp. 223–225.

- Hanlon, R. T., Ament, S. A. and Gabr, H. (1999), 'Behavioral aspects of sperm competition in cuttlefish, *Sepia officinalis* (sepioidea: Cephalopoda)', *Marine Biology* **134**, 719–728.
- Hanlon, R. T. and Messenger, J. B. (1988), 'Adaptive coloration in young cuttlefish (*Sepia officinalis* L.): the morphology and development of body patterns and their relation to behaviour', *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* pp. 437–487.
- Hanlon, R. T. and Messenger, J. B. (1996), *Cephalopod Behaviour*, Cambridge University Press.
- Harms, C. A., Lewbart, G. A., McAlarney, R., Christian, L. S., Geissler, K. and Lemons, C. (2006), 'Surgical excision of mycotic (*Cladosporium* sp.) granulomas from the mantle of a cuttlefish (*Sepia officinalis*)', *Journal of Zoo and Wildlife Medicine* **37**(4), 524–530.
- Hastie, L. C., Pierce, G. J., Wang, J., Bruno, I., Moreno, A., Piatkowski, U. and Robin, J. P. (2009), 'Cephalopods in the North-Eastern Atlantic: species, biogeography, ecology, exploitation and conservation', *Oceanography and Marine Biology* **47**, 111–190.
- Hastie, T. and Tibshirani, R. (1986), 'Generalized Additive Models', *Statistical Science* **1**(3), 297–318.
- Hatfield, E. (2000), 'Do some like it hot? temperature as a possible determinant of variability in the growth of the Patagonian squid, *Loligo gahi* (Cephalopoda: Loliginidae)', *Fisheries Research* **47**(1), 27–40.
- Hatfield, E. M. C., Hanlon, R. T., Forsythe, J. W. and Grist, E. P. M. (2001), 'Laboratory testing of a growth hypothesis for juvenile squid *Loligo pealeii* (Cephalopoda: Loliginidae)', *Canadian Journal of Fisheries and Aquatic Sciences* **58**(5), 845–857.
- Healy, S. D. (2006), 'Imprinting: Seeing food and eating it', *Current Biology* **16**(13), R501 – R502.
- Herbert, R. J. H., Southward, A. J., Sheader, M. and Hawkins, S. J. (2007), 'Influence of recruitment and temperature on distribution of intertidal barnacles in the English Channel', *Journal of the Marine Biological Association of the United Kingdom* **87**(2), 487.
- Hermosilla, C., Rocha, F. and Valavanis, V. D. (2011), 'Assessing *Octopus vulgaris* distribution using presence-only model methods', *Hydrobiologia* **670**, 35–47.
- Hernandez, P. A., Graham, C. H., Master, L. L. and Albert, D. L. (2006), 'The effect of sample size and species characteristics on performance of different species distribution modeling methods', *Ecography* **29**(5), 773–785.

## LIST OF REFERENCES.

---

- Hirzel, A. H., Hausser, J., Chessel, D. and Perrin, N. (2002), 'Ecological-niche factor analysis: how to compute habitat-suitability maps without absence data?', *Ecology* **83**(7), 2027–2036.
- Hosmer, D. W. and Stanley, L. (2000), 'Applied logistic regression', *John Wiley and Sons*.
- Ibáñez, C. M. and Keyl, F. (2010), 'Cannibalism in cephalopods', *Reviews in Fish Biology and Fisheries* **20**(1), 123–136.
- Ikeda, Y., Ito, K. and Matsumoto, G. (2004), 'Does light intensity affect embryonic development of squid (*Heterololigo bleekeri*)?', *Journal of the Marine Biological Association of the United Kingdom* **84**, 1215–1219.
- International Council for Exploration of the Sea (ICES) (2003), Report of the working group on cephalopod fisheries and life history (wgceph), 4-6 december 2002, Lisbon, Portugal, number ICES CM 2003/G:02, p. 14pp.
- International Council for Exploration of the Sea (ICES) (2010a), Report of the working group on cephalopod fisheries and life history (wgceph), 9-11 March 2010, Sukarrieta, Spain, number ICES CM 2010/SSGEF:09, p. 95pp.
- International Council for Exploration of the Sea (ICES) (2010b), Report of the workshop on sexual maturity staging of cephalopods, 8th - 11th November, Livorno, Italy, number ICES CM 2010/ACOM:49, p. 97.
- Jackson, E. L., Attrill, M. J. and Jones, M. B. (2006), 'Habitat characteristics and spatial arrangement affecting the diversity of fish and decapod assemblages of seagrass *Zostera marina* beds around the coast of Jersey (English Channel)', *Estuarine, Coastal and Shelf Science* **68**(3), 421–432.
- Jackson, E. L., Griffiths, C. A., Collins, K. and Durkin, O. (2012), An assessment of anthropogenic impact on marine angiosperm habitat, Technical report, Natural England and Marine Management Organisation, Peterborough, UK.
- Jackson, E. L., Rowden, A. A., Attrill, M. J., Bossey, S. J. and Jones, M. B. (2001), 'The importance of seagrass beds as a habitat for fishery species', *Oceanography and Marine Biology* **39**, 269–304.
- Jackson, G. D. (2004), 'Cephalopod growth: historical context and future directions', *Marine and Freshwater Research* **55**(4), 327–329.
- Jackson, G. D., Forsythe, J. W., Hixon, R. F. and Hanlon, R. T. (1997), 'Age, growth, and maturation of *Lolliguncula brevis* (Cephalopoda: Loliginidae) in the northwestern Gulf of Mexico with a comparison of length-frequency versus statolith age analysis', *Canadian Journal of Fisheries and Aquatic Sciences* **54**(12), 2907–2919.

- Jackson, G. D., O'Dor, R. K. and Andrade, Y. (2005), 'First tests of hybrid acoustic/archival tags on squid and cuttlefish', *Marine and Freshwater Research* **56**(4), 425–430.
- Jackson, G. and Moltschaniwskyj, N. (2002), 'Spatial and temporal variation in growth rates and maturity in the Indo-Pacific squid *Sepioteuthis lessoniana* (Cephalopoda: Loliginidae)', *Marine Biology* **140**(4), 747–754.
- Jacoby, D. M. P., Brooks, E. J., Croft, D. P. and Sims, D. W. (2012), 'Developing a deeper understanding of animal movements and spatial dynamics through novel application of network analyses', *Methods in Ecology and Evolution* .
- Jaynes, E. T. (1957), 'Information theory and statistical mechanics', *The Physical Review* **106**(4), 620–630.
- Jenks, K. E., Kitamura, S., Lynam, A. J., Ngoprasert, D., Chutipong, W., Steinmetz, R., Sukmasuang, R., Grassman, L. I., Cutter, P., Tantipisanuh, N., Bhumpakphan, N., Gale, G. A., Reed, D. H., Leimgruber, P. and Songsasen, N. (2012), 'Mapping the distribution of dholes, *Cuon alpinus* (Canidae, Carnivora), in Thailand', *Mammalia* **76**(2), 175–184.
- Jereb, P. and Roper, C. F. E. (2005), Cephalopods of the World: An annotated and illustrated catalogue of cephalopod species known to date, Technical Report Volume 1. Chambered nautilus and sepioids, FAO Species Catalogue for Fishery Purposes No.4 Volume 1.
- Johansen, K., Brix, O. and Lykkeboe, G. (1982), 'Blood gas transport in the cephalopod, *Sepia officinalis*', *Journal of Experimental Biology Letters* **99**, 331–338.
- Joyce, A. E. (2010), Sea temperature and salinity trends: Presentation of results, Science Series Data Report 43, Centre for Environment, Fisheries & Aquaculture Science (CEFAS).
- Karson, M. A., Boal, J. G. and Hanlon, R. T. (2003), 'Experimental evidence for spatial learning in cuttlefish (*Sepia officinalis*).', *Journal of Comparative Psychology* **117**(2), 149–155.
- King, A. J. and Adamo, S. A. (2006), 'The ventilatory, cardiac and behavioural responses of resting cuttlefish (*Sepia officinalis* L.) to sudden visual stimuli', *Journal of Experimental Biology* **209**(6), 1101.
- Kirkendall, L. R. and Stenseth, N. C. (1985), 'On defining "breeding once"', *American Naturalist* pp. 189–204.
- Klimley, A. P., Le Boeuf, B. J., Cantara, K. M., Richert, J. E., Davis, S. F. and Van Sommeran, S. (2001), 'Radio-acoustic positioning as a tool for studying site-specific behavior of the white shark and other large marine species', *Marine Biology* **138**(2), 429–446.
- Kotliar, N. B. and Wiens, J. A. (1990), 'Multiple scales of patchiness and patch structure: A hierarchical framework for the study of heterogeneity', *Oikos* **59**, 253–260.



- Koueta, N. and 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**(1), 93–109.
- Koueta, N. and 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**(1), 75–87.
- Koueta, N. and Boucaud-Camou, E. (2003), 'Combined effects of photoperiod and feeding frequency on survival and growth of juvenile cuttlefish *Sepia officinalis* L. in experimental rearing', *Journal of Experimental Marine Biology and Ecology* **296**(2), 215–226.
- Koueta, N., Boucaud-Camou, E. and Noel, B. (2002), 'Effect of enriched natural diet on survival and growth of juvenile cuttlefish *Sepia officinalis* l.', *Aquaculture* **203**(3-4), 293–310.
- Koueta, N., Camou-Boucaud, E. and Renou, A. M. (1995), 'Gonadotropic mitogenic activity of the optic gland of the cuttlefish, *Sepia officinalis*, during sexual maturation', *Journal of the Marine Biological Association of the United Kingdom* **75**(02), 391–404.
- Koueta, N., Castro, B. G. and Boucaud-Camou, E. (2000), 'Biochemical indices for instantaneous growth estimation in young cephalopod *Sepia officinalis* L.', *ICES Journal of Marine Science: Journal du Conseil* **57**(1), 1.
- Lacoue-Labarthe, T., Martin, S., Oberhansli, F., Teyssié, J. L., Markich, S., Jeffree, R. and Bustamante, P. (2009), 'Effects of increased pCO<sub>2</sub> and temperature on trace element (Ag, Cd and Zn) bioaccumulation in the eggs of the common cuttlefish, *Sepia officinalis*', *Biogeosciences* **6**(11), 2561–2573.
- Lacoue-Labarthe, T., Warnau, M., Oberhansli, F., Teyssié, J., Koueta, N. and Bustamante, P. (2008a), 'Differential bioaccumulation behaviour of Ag and Cd during the early development of the cuttlefish *Sepia officinalis*', *Aquatic Toxicology* **86**(3), 437–446.
- Lacoue-Labarthe, T., Warnau, M., Oberhansli, F., Teyssié, J. L. and Bustamante, P. (2010), 'Contrasting accumulation biokinetics and distribution of <sup>241</sup>Am, Co, Cs, Mn and Zn during the whole development time of the eggs of the common cuttlefish, *Sepia officinalis*', *Journal of Experimental Marine Biology and Ecology* **382**(3D2), 131–138.
- Lacoue-Labarthe, T., Warnau, M., Oberhansli, F., Teyssié, J. L., Jeffree, R. and Bustamante, P. (2008b), 'First experiments on the maternal transfer of metals in the cuttlefish *Sepia officinalis*', *Marine Pollution Bulletin* **57**(6), 826–831.
- Lamare, M. D. and Barker, M. F. (1999), 'In situ estimates of larval development and mortality in the New Zealand sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea)', *Marine Ecology Progress Series* **180**, 197–211.

## LIST OF REFERENCES.

---

- Langridge, K. V., Broom, M. and Osorio, D. (2007), 'Selective signalling by cuttlefish to predators', *Current Biology* **17**(24), R1044–R1045.
- Laptikhovsky, V., Salman, A., Onsoy, B. and Katagan, T. (2003), 'Fecundity of the common cuttlefish *Sepia officinalis* L. (cephalopoda, sepiida): a new look at the old problem', *Sceintia Marina* **67**(3), 279–284.
- Larsonneur, C., Bouysse, P. and Auffret, J. P. (1982), 'The superficial sediments of the English Channel and its western approaches', *Sedimentology* **29**(6), 851–864.
- Larsonneur, C., Vaslet, D. and Auffret, J. P. (1979), 'Les sédiments superficiels de la Manche, carte géologique de la marge continentale française', *Bureau des Recherches Géologiques et Minières, Ministère de Industrie, Service Géologique National* .
- Le Goff, R. and Daguzan, J. (1991), 'Growth and life cycles of the cuttlefish *Sepia officinalis* L. (Mollusca: Cephalopoda) in South Brittany (France)', *Bulletin of Marine Science* **49**, 341–348(8).
- Le Roy, R. and Simon, B. (2003), *Réalisation et validation d'un modèle de marée en Manche et dans le Golfe de Gascogne (application à la réalisation d'un nouveau programme de réduction des sondages bathymétriques)*, Brest: Service hydrographique et océanographique de la marine.
- Leathwick, J., Moilanen, A., Francis, M., Elith, J., Taylor, P., Julian, K., Hastie, T. and Duffy, C. (2008), 'Novel methods for the design and evaluation of marine protected areas in offshore waters', *Conservation Letters* **1**(2), 91–102.
- Lee, P. G. (1994), 'Nutrition of cephalopods: fueling the system', *Marine & Freshwater Behaviour & Physiology* **25**(1-3), 35–51.
- Lefebvre, S., Harma, C., Blin, J. L. et al. (2009), 'Trophic typology of coastal ecosystems based on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios in an opportunistic suspension feeder', *Marine Ecology Progress Series* **390**, 27–37.
- Lefkaditou, E., Politou, C. Y., Palialexis, A., Dokos, J., Cosmopoulos, P. and Valavanis, V. D. (2008), 'Influences of environmental variability on the population structure and distribution patterns of the short-fin squid *Illex coindetii* (Cephalopoda: Ommastrephidae) in the Eastern Ionian Sea', *Hydrobiologia* **612**(1), 71–90.
- Lek, S. and Guegan, J. F. (1999), 'Artificial neural networks as a tool in ecological modelling, an introduction', *Ecological Modelling* **120**, 65–73.
- Leporati, S. C., Pecl, G. T. and Semmens, J. M. (2007), 'Cephalopod hatchling growth: the effects of initial size and seasonal temperatures', *Marine Biology* **151**(4), 1375–1383.

## LIST OF REFERENCES.

---

- Levins, R. (1968), *Evolution in changing environments: some theoretical explorations*, Vol. 2, Princeton University Press.
- Lewbart, G. A. and Mosley, C. (2012), 'Clinical anesthesia and analgesia in invertebrates', *Journal of Exotic Pet Medicine* **21**(1), 59–70.
- Liu, C., Berry, P. M., Dawson, T. P. and Pearson, R. G. (2005), 'Selecting thresholds of occurrence in the prediction of species distributions', *Ecography* **28**(3), 385–393.
- Lohmann, K. J., Putman, N. F. and Lohmann, C. M. F. (2008), 'Geomagnetic imprinting: A unifying hypothesis of long-distance natal homing in salmon and sea turtles', *Proceedings of the National Academy of Sciences* **105**(49), 19096–19101.
- MacLeod, C. D., Mandleberg, L., Schweder, C., Bannon, S. M. and Pierce, G. J. (2008), 'A comparison of approaches for modelling the occurrence of marine animals', *Hydrobiologia* **612**(1), 21–32.
- Maddock, A. (2008), UK Biodiversity Action Plan; Priority habitat descriptions, Technical report, BRIG.
- Malgrange, B. (2009), Identification, analyse et mise en valeur des initiatives de gestion, de préservation et de valorisation des ressources mises en oeuvre par les pêcheurs en Bretagne, Technical report, Université Occidentale de Bretagne.
- Mangold, K. (1987), Reproduction, in P. R. Boyle, ed., 'Cephalopod Life Cycles', Vol. II, Academic Press, pp. 157–200.
- Mangold-Wirz, K. (1963), *Biologie des Cephalopodes benthiques et nectoniques de la Mer Catalane*, Hermann.
- Marine Management Organisation (MMO) (2010), 'The UK fishing industry in 2010 landings'.  
**URL:** [http://www.marinemanagement.org.uk/fisheries/statistics/annual\\_archive.htm](http://www.marinemanagement.org.uk/fisheries/statistics/annual_archive.htm)2010
- Mark, F. C., Jaeckel, G., Gutowska, M., Pörtner, H. O., Oellermann, M. and Ellington, C. (2007), 'Diurnal activity patterns and related changes in energy metabolism in the cephalopod *Sepia officinalis*', *Comparative Biochemistry and Physiology, Part A* **146**, S76.
- Marshall, D. J., Allen, R. M. and Crean, A. J. (2008), 'The ecological and evolutionary importance of maternal effects in the sea', *Oceanography and Marine Biology: An Annual Review* **46**, 203–250.
- Marshall, D. J., Bolton, T. F. and Keough, M. J. (2003), 'Offspring size affects the post-metamorphic performance of a colonial marine invertebrate', *Ecology* **84**(12), 3131–3137.

#### LIST OF REFERENCES.

---

- McBreen, F., Askew, N., Cameron, A., Connor, D., Ellwood, H. and Carter, A. (2011), UK-seamap 2010: Predictive mapping of seabed habitats in UK waters, Technical Report 446, Joint Nature Conservation Committee (JNCC).
- McCullagh, P. and Nelder, J. A. (1983), *Generalised linear modelling*, Vol. 134, Chapman & Hall.
- McLay, C. L. and Guinot, D. (1997), 'Ten arms meet ten legs: Decapoda (Mollusca: Cephalopoda: Sepioidea) spawn on Decapoda (Crustacea: Brachyura: Homolidae)', *Journal of Crustacean Biology* pp. 692–694.
- Mead, L. S. and Arnold, S. J. (2004), 'Quantitative genetic models of sexual selection', *Trends in Ecology & Evolution* **19**(5), 264–271.
- Meekan, M. G. and Fortier, L. (1996), 'Selection for fast growth during the larval life of Atlantic cod *Gadus morhua* on the Scotian Shelf', *Marine Ecology Progress Series* **137**(1), 25–37.
- Meekan, M. G., Vigliola, L., Hansen, A., Doherty, P. J., Halford, A. and Carleton, J. H. (2006), 'Bigger is better: size-selective mortality throughout the life history of a fast-growing clupeid, *Spratelloides gracilis*', *Marine Ecology Progress Series* **317**, 237.
- Melzner, F., Bock, C. and Pörtner, H. O. (2006a), 'Critical temperatures in the cephalopod *Sepia officinalis* investigated using *in vivo*  $^{31}\text{P}$  NMR spectroscopy', *The Journal of Experimental Biology* **209**, 891–906.
- Melzner, F., Bock, C. and Pörtner, H. O. (2006b), 'Temperature-dependent oxygen extraction from the ventilatory current and the costs of ventilation in the cephalopod *Sepia officinalis*', *Journal of Comparative Physiology B* **176**, 149–154.
- Melzner, F., Bock, C. and Pörtner, H. O. (2007), 'Allometry of thermal limitation in the cephalopod *Sepia officinalis*', *Comparative Biochemistry and Physiology, Part A* **146**, 149–154.
- Melzner, F., Mark, F. C. and Pörtner, H. O. (2007), 'Role of blood-oxygen transport in thermal tolerance of the cuttlefish, *Sepia officinalis*', *Integrative and Comparative Biology* **47**, 645–655.
- Messenger, J. B. (2001), 'Cephalopod chromatophores: neurobiology and natural history', *Biological Reviews* **76**(4), 473–528.
- Messenger, J. B., Nixon, M. and Ryan, K. P. (1985), 'Magnesium chloride as an anaesthetic for cephalopods', *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* **82**(1), 203–205.

## LIST OF REFERENCES.

---

- Miramand, P., Bustamante, P., Bentley, D. and Kouéta, N. (2006), 'Variation of heavy metal concentrations (Ag, Cd, Co, Cu, Fe, Pb, V, and Zn) during the life cycle of the common cuttlefish *Sepia officinalis*', *Science of The Total Environment* **361**(1-3), 132–143.
- Moltschaniwskyj, N. A. and Pecl, G. T. (2003), 'Small-scale spatial and temporal patterns of egg production by the temperate loliginid squid *Sepioteuthis australis*', *Marine Biology* **142**(3), 509–516.
- Moltschaniwskyj, N. A., Pecl, G. T., Lyle, J., Haddon, M. and Steer, M. (2003), 'Population dynamics and reproductive ecology of the southern calamary (*Sepioteuthis australis*) in Tasmania', *FRDC Final Report* .
- Moreno, A., Dos Santos, A., Piatkowski, U., Santos, A. M. P. and Cabral, H. (2009), 'Distribution of cephalopod paralarvae in relation to the regional oceanography of the western Iberia', *Journal of Plankton Research* **31**(1), 73–91.
- Morte, S., Redon, M. J. and Sanz-Brau, A. (1997), 'Feeding habits of juvenile *Mustelus mustelus* (Carcharhiniformes, Triakidae) in the western Mediterranean', *Cahiers de Biologie Marine* **38**(2), 103–107.
- Mousseau, T. A. and Dingle, H. (1991), 'Maternal effects in insect life histories', *Annual Review Entomology* **36**, 511–34.
- Mousseau, T. A. and Fox, C. W. (1998), 'The adaptive significance of maternal effects', *Trends in Ecology & Evolution* **13**(10), 403–407.
- Natural England (2010), Inshore special area of conservation (sac): Lyme bay and torbay, SAC Selection Assessment Version 2.5, Natural England.
- Naud, M. J., Shaw, P. W., Hanlon, R. T. and Havenhand, J. N. (2005), 'Evidence for biased use of sperm sources in wild female giant cuttlefish (*Sepia apama*)', *Proceedings of the Royal Society B: Biological Sciences* **272**(1567), 1047–1051.
- Neuenfeldt, S., Hinrichsen, H. H. and Nielsen, A. (2004), 'A method to geolocate eastern baltic cod by using data storage tags (dsts)', *ICES CM* **L06**, 1–14.
- Nixon, M. and Mangold, K. (1998), 'The early life of *Sepia officinalis*, and the contrast with that of *Octopus vulgaris* (cephalopoda)', *Journal of Zoology* **245**(4), 407–421.
- Odling-Smee, L. and Braithwaite, V. A. (2003), 'The role of learning in fish orientation', *Fish and Fisheries* **4**(3), 235–246.
- O'Dor, R. K. (2002), 'Telemetered cephalopod energetics: swimming, soaring, and blimping', *Integrative and Comparative Biology* **42**(5), 1065.

## LIST OF REFERENCES.

---

- O'Dor, R. K., Adamo, S., Aitken, J. P., Andrade, Y., Finn, J., Hanlon, R. T. and Jackson, G. D. (2002), 'Currents as environmental constraints on the behavior, energetics and distribution of squid and cuttlefish', *Bulletin of Marine Science* **71**(2), 601–617.
- O'Dor, R. K. and Webber, D. M. (1991), 'Invertebrate athletes: Trade-offs between transport efficiency and power density in cephalopod evolution', *Journal of Experimental Biology* **160**, 93–112.
- Olsen, B. and Sand-Jensen, K. (1994), 'Patch dynamics of eelgrass *Zostera marina*', *Marine Ecology Progress Series* **106**, 147–156.
- Ommen Kloeke, A. E. E., Douma, J. C., Ordoñez, J. C., Reich, P. B. and van Bodegom, P. M. (2012), 'Global quantification of contrasting leaf life span strategies for deciduous and evergreen species in response to environmental conditions', *Global Ecology and Biogeography* **21**(2), 224–235.
- Palmegiano, G. B. and d'Apote, M. P. (1983), 'Combined effects of temperature and salinity on cuttlefish (*Sepia officinalis* L.) hatching', *Aquaculture* **35**, 259–264.
- Pascual, E. (1978), 'Crecimiento y alimentacion de tres generaciones de *Sepia officinalis* en cultivo', *Investigacion Pesquera* **42**(2), 421–441.
- Paulij, W. P., Bogaards, R. H. and Denucé, J. M. (1990a), 'Influence of salinity on embryonic development and the distribution of *Sepia officinalis* in the Delta area (South Western part of the Netherlands)', *Marine Biology* **107**(1), 17–23.
- Paulij, W. P., Herman, P. M. J., Roozen, M. E. F. and Denucé, J. M. (1991), 'The influence of photoperiodicity on hatching of *Sepia officinalis*', *Journal of the Marine Biological Association of the United Kingdom* **71**(03), 665–678.
- Paulij, W. P., Herman, P. M. J., Van Hannen, E. J. and Denucé, J. M. (1990b), 'The impact of photoperiodicity on hatching of *Loligo vulgaris* and *Loligo forbesi*', *Journal of the Marine Biological Association of the United Kingdom* **70**(03), 597–610.
- Pawson, M. G. (1995), 'Biogeographical identification of English Channel fish and shellfish stocks', *MAFF Fisheries Research Technical Report* **99**.
- Pearce, J. L. and Boyce, M. S. (2005), 'Modelling distribution and abundance with presence-only data', *Journal of Applied Ecology* **43**(3), 405–412.
- Pearson, R. G. (2010), 'Species distribution modeling for conservation educators and practitioners', *Lessons in Conservation (LinC) Developing the capacity to sustain the Earth's diversity* p. 54.

- Pechenik, J. A., Eyster, L. S., Widdows, J. and Bayne, B. L. (1990), 'The influence of food concentration and temperature on growth and morphological differentiation of blue mussel *Mytilus edulis* l. larvae', *Journal of Experimental Marine Biology and Ecology* **136**(1), 47–64.
- Pecl, G. T. (2001), 'Flexible reproductive strategies in tropical and temperate *Sepioteuthis* squids', *Marine Biology* **138**(1), 93–101.
- Pecl, G. T. (2004), 'The *in situ* relationships between season of hatching, growth and condition in the southern calamary, *Sepioteuthis australis*', *Marine and freshwater research* **55**(4), 429–438.
- Pecl, G. T., Steer, M. A. and Hodgson, K. E. (2004), 'The role of hatchling size in generating the intrinsic size-at-age variability of cephalopods: extending the forsythe hypothesis', *Marine and Freshwater Research* **55**(4), 387–394.
- Pecl, G. T., Tracey, S. R., Semmens, J. M. and Jackson, G. D. (2006b), 'Use of acoustic telemetry for spatial management of southern calamary *Sepioteuthis australis*, a highly mobile in-shore squid species', *Marine Ecology Progress Series* **328**, 1–15.
- Pepin, P. (1988), 'Predation and starvation of larval fish: a numerical experiment of size-and growth-dependent survival', *Biological oceanography* **6**(1), 23–44.
- Perez-Losada, M., Guerra, A., Carvalho, G. R., Sanjuan, A. and Shaw, P. W. (2002), 'Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) around the Iberian Peninsula indicated by microsatellite DNA variation', *Heredity* **89**(6), 417–424.
- Perez-Losada, M., Guerra, A. and Sanjuan, A. (1999), 'Allozyme differentiation in the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) from the NE Atlantic and Mediterranean', *Heredity* **83**(3), 280–289.
- Phillips, S. (2010), 'A brief tutorial on maxent (species distribution modelling for conservation educators and practioners)', *Lessons in Conservation* pp. 107–134.
- Phillips, S. J. (2008), 'Transferability, sample selection bias and background data in presence-only modelling: a response to Peterson et al.(2007)', *Ecography* **31**(2), 272–278.
- Phillips, S. J., Anderson, R. P. and Schapire, R. E. (2006), 'Maximum entropy modeling of species geographic distributions', *Ecological modelling* **190**(3), 231–259.
- Phillips, S. J. and Dudík, M. (2008), 'Modeling of species distributions with maxent: new extensions and a comprehensive evaluation', *Ecography* **31**(2), 161–175.

- Phillips, S. J., Dudík, M., Elith, J., Graham, C. H., Lehmann, A., Leathwick, J. and Ferrier, S. (2009), 'Sample selection bias and presence-only distribution models: implications for background and pseudo-absence data', *Ecological Applications* **19**(1), 181–197.
- Phillips, S. J., Dudík, M. and Schapire, R. E. (2004), A maximum entropy approach to species distribution modeling, in 'Proceedings of the twenty-first international conference on Machine learning', p. 83.
- Piatkowski, U., Pierce, G. J. and Morais da Cunha, M. (2001), 'Impact of cephalopods in the food chain and their interaction with the environment and fisheries: an overview', *Fisheries Research* **52**(1), 5–10.
- Pierce, G. J., Allcock, L., Bruno, I., Bustamante, P., Gonzalez, A. F., Guerra, A., Jereb, P., Lefkaditou, E., Malham, S., Moreno, A., Pereira, J., Piatowski, U., Rasero, M., Sanchez, P., Begona Santos, M., Santurtun, M., Seixas, S., Sobrino, I. and Villanueva, R. (2010), 'Cephalopod biology and fisheries in Europe', *ICES Cooperative Research Report* **303**.
- 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., Santurtun, M., Boyle, P. R., Hastie, L. C., MacLeod, C. D., Smith, J. M., Viana, M., Gonzalez, A. F. and Zuur, A. F. (2008), 'A review of cephalopod environment interactions in European seas', *Essential Fish Habitat Mapping in the Mediterranean* pp. 49–70.
- Pierce, G. J., Wang, J. and Valavanis, V. (2002), 'Application of GIS to cephalopod fisheries: workshop report', *Bulletin of Marine Science* **71**(1), 35–46.
- Pinder, A. and Friet, S. (1994), 'Oxygen transport in egg masses of the amphibians *Rana sylvatica* and *Ambystoma maculatum*: convection, diffusion and oxygen production by algae', *Journal of Experimental Biology* **197**(1), 17.
- Pingree, R. D. and Maddock, L. (1977), 'Tidal eddies and coastal discharge', *Journal of the Marine Biological Association of the United Kingdom* **57**, 869–875.
- Pinnegar, J. K. and Platts, M. (2011), Dapstom- an integrated database & portal for fish stomach records. version 3.6. phase 3, final report., Technical report, Centre for Environment, Fisheries and Aquaculture Science, [www.cefas.defra.gov.uk/dapstom](http://www.cefas.defra.gov.uk/dapstom).
- Poirier, R., Chichery, R. and Dickel, L. (2004), 'Effects of rearing conditions on sand digging efficiency in juvenile cuttlefish', *Behavioural processes* **67**(2), 273–279.
- Pomerol, C. (1977), 'Dynamique comparée de trois bassins épicontinentaux: mer du nord, manche et bassin de paris', *Bulletin Centre Rech. Elf-Aquitaine* pp. 233–256.



- Poole, H. H. and Atkins, W. R. G. (1937), 'The penetration into the sea of light of various wavelengths as measured by emission or rectifier photo-electric cells', *Proceedings of the Royal Society of London. Series B, Biological Sciences* **123**(831), 151–165.
- Pörtner, H. O. (2008), 'Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view', *Marine Ecology Progress Series* **373**, 203–217.
- Pörtner, H. O., Langenbuch, M. and Michaelidis, B. (2005), 'Synergistic effects of temperature extremes, hypoxia, and increases in CO<sub>2</sub> on marine animals: From Earth history to global change', *Journal of Geophysical Research* **110**, 1978–2012.
- Pronk, R., Wilson, D. R. and Harcourt, R. (2010), 'Video playback demonstrates episodic personality in the gloomy octopus', *Journal of Experimental Biology* **213**(7), 1035.
- Reddy, S. and Dávalos, L. M. (2003), 'Geographical sampling bias and its implications for conservation priorities in Africa', *Journal of Biogeography* **30**(11), 1719–1727.
- Reid, A. and Jereb, P. (2005), *Cephalopods of the world. An annotated and illustrated catalogue of species known to date. Volume 1. Chambered nautilus and sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and spirulidae)*, Vol. 1, FAO Species Catalogue for Fishery purposes, Rome, chapter Family Sepiolidae, pp. 153–203.
- Reid, A., P., J. and Roper, C. (2005), *Cephalopods of the world. An annotated and illustrated catalogue of species known to date. Volume 1. Chambered nautilus and sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and spirulidae)*, Vol. 1, FOA Species Catalogue for Fishery Purposes, Rome, chapter Family Sepiidae, pp. 57–152.
- Replinger, S. E. and Wood, J. B. (2007), 'A preliminary investigation of the use of subcutaneous tagging in caribbean reef squid *Sepioteuthis sepioidea* (cephalopoda: Loliginidae)', *Fisheries Research* **84**(3), 308–313.
- Resetarits Jr, W. J. (1996), 'Oviposition site choice and life history evolution', *American Zoologist* **36**(2), 205–215.
- Richard, A. (1966a), 'Action de la température sur l'évolution genitale de *Sepia officinalis* l', *Compte rendu de la Académide des Sciences de Paris* **263**, 1998 – 2001.
- Richard, A. (1966b), 'La température, facteur externe essentiel de croissance pour le céphalopode *Sepia officinalis* l', *Compte rendu de la Académide des Sciences de Paris Série D* **263**, 1138 –1141.
- Richard, A. (1971), 'Contribution a l'étude de la biologie de la croissance et de la maturation sexuelle de *Sepia officinalis* l. (mollusque cephalopode).', *These Doct. Sc. Nat., University Lille*, 264p.

- Rigby, P. R. and Sakurai, Y. (2005), 'Multidimensional tracking of giant pacific octopuses in Northern Japan reveals unexpected foraging behaviour', *Marine Technology Society Journal* **39**(1), 64–67.
- Robbins, B. D. and Bell, S. S. (1994), 'Seagrass landscapes: a terrestrial approach to the marine subtidal environment', *Trends in ecology & evolution* **9**(8), 301–304.
- Roberts, M. J. and Sauer, W. H. H. (1994), 'Environment: the key to understanding the South African chokka squid (*Loligo vulgaris reynaudii*) life cycle and fishery?', *Antarctic Science* **6**(02), 249–258.
- Robin, J. P. and Denis, V. (1999), 'Squid stock fluctuations and water temperature: temporal analysis of English Channel loliginidae', *Journal of Applied Ecology* **36**, 101–110.
- Robinson, L. M., Elith, J., Hobday, A. J., Pearson, R. G., Kendall, B. E., Possingham, H. P. and Richardson, A. J. (2011), 'Pushing the limits in marine species distribution modelling: lessons from the land present challenges and opportunities', *Global Ecology and Biogeography* **20**(6), 789–802.
- Rocha, F., Guerra, A. and Gonzalez, A. F. (2001), 'A review of reproductive strategies in cephalopods', *Biological Reviews* **76**(3), 291–304.
- Rodhouse, P. G. (2001), 'Managing and forecasting squid fisheries in variable environments', *Fisheries Research* **54**(1), 3–8.
- Roper, C. F. E., Sweeney, M. J. and Nauen, C. (1984), 'Cephalopods of the world. an annotated and illustrated catalogue of species of interest to fisheries', *FAO Fisheries Synopsis* **3**(125).
- Rosenzweig, M. L. (1981), 'A theory of habitat selection', *Ecology* **62**(2), 327–335.
- Royer, J. (2002), Modélisation des stocks de céphalopodes de Manche, PhD thesis, University of Caen, p532.
- Royer, J., Pierce, G. J., Foucher, E. and Robin, J. P. (2006), 'The English Channel stock of *Sepia officinalis*: Modelling variability in abundance and impact of the fishery', *Cephalopod Stock Assessment Workshop, London, ENGLAND* (Cephstock,), 96–106. Elsevier Science Bv.
- Rumrill, S. S. (1990), 'Natural mortality of marine invertebrate larvae.', *Ophelia*. **32**(1-2).
- Salman, A., Bilecenoglu, M. and Guclusoy, H. (2001), 'Stomach contents of two Mediterranean monk seals (*Monachus monachus*) from the Aegean Sea, Turkey', *Journal of the Marine Biological Association of the United Kingdom* **81**(4), 719–720.
- Sanchez, P., Demestre, M., Recasens, L., Maynou, F. and Martin, P. (2008), 'Combining GIS and GAMs to identify potential habitats of squid *Loligo vulgaris* in the Northwestern Mediterranean', *Essential Fish Habitat Mapping in the Mediterranean* pp. 91–98.

- Sanvicente-Añorve, L., Leprêtre, A. and Davoult, D. (2002), 'Diversity of benthic macrofauna in the eastern English Channel: comparison among and within communities', *Biodiversity and Conservation* **11**(2), 265–282.
- Sauer, W. H. H., Lipinski, M. R. and Augustyn, C. J. (2000), 'Tag recapture studies of the chokka squid *Loligo vulgaris reynaudii* d'Orbigny, 1845 on inshore spawning grounds on the south-east coast of South Africa', *Fisheries Research* **45**(3), 283–289.
- Sauer, W. H. H., Smale, M. J. and Lipinski, M. R. (1992), 'The location of spawning grounds, spawning and schooling behaviour of the squid *Loligo vulgaris reynaudii* (Cephalopoda: Myopoda) off the Eastern Cape Coast, South Africa', *Marine Biology* **114**(1), 97–107.
- Scheel, D. and Bisson, L. (2012), 'Movement patterns of giant pacific octopuses, *Enteroctopus dofleini* (wülker, 1910)', *Journal of Experimental Marine Biology and Ecology* **416**, 21–31.
- Scimeca, J. M. (2006), *Invertebrate medicine*, Blackwell Publishing Ltd, chapter 6: Cephalopods, pp. 79–89.
- Segawa, S. (1990), 'Food consumption, food conversion and growth rates of the oval squid *Sepioteuthis lessoniana* by laboratory experiments', *Nippon Suisan Gakkaishi* **56**(2), 217–222.
- Segurado, P. and Araujo, M. B. (2004), 'An evaluation of methods for modelling species distributions', *Journal of Biogeography* **31**(10), 1555–1568.
- Semmens, J. M., Pecl, G. T., Gillanders, B. M., Waluda, C. M., Shea, E. K., Jouffre, D., Ichii, T., Zumholz, K., Katugin, O. N., Leporati, S. C. and Shaw, P. W. (2007), 'Approaches to resolving cephalopod movement and migration patterns', *Reviews in Fish Biology and Fisheries* **17**(2), 401–423.
- Şen, H. and Tanrikul, T. T. (2009), 'Efficacy of 2-phenoxyethanol as an anaesthetic for the musky octopus, *Eledone moschata* (Lamarck 1799), (Cephalopoda: Octopodidae)', *Turkish Journal of Veterinary and Animal Sciences* **33**(6), 463–467.
- Shashar, N., Rutledge, P. S. and Cronin, T. W. (1996), 'Polarization vision in cuttlefish—a concealed communication channel?', *Journal of Experimental Biology* **199**, 2077–2084.
- Shohet, A. J., Baddeley, R. J., Anderson, J. C., Kelman, E. J. and Osorio, D. (2006), 'Cuttlefish responses to visual orientation of substrates, water flow and a model of motion camouflage', *The Journal of Experimental Biology* **209**, 4717–4723.
- Sims, D. W., Genner, M. J., Southward, A. J. and Hawkins, S. J. (2001), 'Timing of squid migration reflects North Atlantic climate variability', *Proceedings of the Royal Society of London. Series B: Biological Sciences* **268**(1485), 2607.

## LIST OF REFERENCES.

---

- Smith, J. N., Grantham, H. S., Gales, N., Double, M. C., Noad, M. J. and Paton, D. (2012), 'Identification of humpback whale breeding and calving habitat in the Great Barrier Reef', *Marine Ecology Progress Series* **447**, 259–272.
- Smyth, T. J., Fishwick, J. R., Lisa, A. L. M., Cummings, D. G., Harris, C., Kitidis, V., Rees, A., Martinez-Vicente, V. and Woodward, E. M. S. (2010), 'A broad spatio-temporal view of the Western English Channel observatory', *Journal of Plankton Research* **32**(5), 585–601.
- Soberon, J. (2005), 'Interpretation of models of fundamental ecological niches and species' distributional areas', *Biodiversity Informatics* **2**, 1–10.
- Sobrino, I., Silva, L., Bellido, J. M. and Ramos, F. (2002), 'Rainfall, river discharges and sea temperature as factors affecting abundance of two coastal benthic cephalopod species in the gulf of cadiz (sw spain)', *Bulletin of Marine Science* **71**(2), 851–865.
- Sogard, S. M. (1997), 'Size-selective mortality in the juvenile stage of teleost fishes: a review', *Bulletin of Marine Science* **60**(3), 1129–1157.
- Sollberger, A. (1965), 'Biological rhythm research', *New York* .
- Stanford, R. and Pitcher, T. J. (2004), *Ecosystem simulations of the English Channel: climate and trade-offs*, Fisheries Centre, University of British Columbia.
- Staver, J. M. and Strathmann, R. R. (2002), 'Evolution of fast development of planktonic embryos to early swimming', *The Biological Bulletin* **203**(1), 58.
- Steer, M. A., Moltschaniwskyj, N. A., Nichols, D. S. and 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**(1), 73–89.
- Steer, M. A., Pecl, G. T. and 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.
- Steingrimsson, S. and Grant, J. W. A. (2003), 'Patterns and correlates of movement and site fidelity in individually tagged young-of-the-year atlantic salmon (*Salmo salar*)', *Canadian Journal of Fisheries and Aquatic Sciences* **60**(2), 193–202.
- Stockwell, D. (1999), 'The garp modelling system: problems and solutions to automated spatial prediction', *International Journal of Geographical Information Science* **13**(2), 143–158.
- Strathmann, R. R. (1985), 'Feeding and nonfeeding larval development and life-history evolution in marine invertebrates', *Annual Review of Ecology and Systematics* **16**, 339–361.

## LIST OF REFERENCES.

---

- Strathmann, R. R. (2007), 'Three functionally distinct kinds of pelagic development', *Bulletin of Marine Science* **81**(2), 167–179.
- Strathmann, R. R. and Chaffee, C. (1984), 'Constraints on egg masses. II. Effect of spacing, size, and number of eggs on ventilation of masses of embryos in jelly, adherent groups, or thin-walled capsules', *Journal of Experimental Marine Biology and Ecology* **84**(1), 85 – 93.
- Strathmann, R. R. and Strathmann, M. F. (1995), 'Oxygen supply and limits on aggregation of embryos', *Journal of the Marine Biological Association of the United Kingdom* **75**(3D02), 413–428.
- Swets, J. A. (1988), 'Measuring the accuracy of diagnostic systems', *Science* **240**(4857), 1285–1293.
- Sykes, A. V., Almansa, E., Lorenzo, A. and Andrade, J. P. (2009), 'Lipid characterization of both wild and cultured eggs of cuttlefish (*Sepia officinalis* L.) throughout the embryonic development', *Aquaculture Nutrition* **15**(1), 38–53.
- Sykes, A. V., Taipina, S., Goncalves, R. A., Bernardino, R. J. and Aragao, C. (2011), Efficiency of different anaesthetics as welfare promoters during human manipulation of European cuttlefish (*Sepia officinalis*) juveniles, in 'EuroCeph Poster no.1.10'.
- Taylor, R. G., Whittington, J. A., William III, E. and Pollock, K. H. (2006), 'Effect of different reward levels on tag reporting rates and behavior of common snook anglers in southeast Florida', *North American Journal of Fisheries Management* **26**(3), 645–651.
- Tingley, R. and Clements, G. R. (2011), Creating a bias grid for maximum entropy modelling (maxent). Accessed on 05/04/2012 [<http://myrimba.org/2011/04/18/>].
- Torbay Coast Countryside Trust '(TCCT) (2006), 'Torbay's seagrass beds. a hidden world'.
- Tsoar, A., Allouche, O., Steinitz, O., Rotem, D. and Kadmon, R. (2007), 'A comparative evaluation of presence-only methods for modelling species distribution', *Diversity and distributions* **13**(4), 397–405.
- Tsui, M. T. K. and Wang, W. X. (2007), 'Biokinetics and tolerance development of toxic metals in *Daphnia magna*', *Environmental Toxicology and Chemistry* **26**(5), 1023–1032.
- Tullrot, A. (2009), Background document for *Zostera* beds, seagrass beds., Technical report, OSPAR Convention.
- Turner, S. J., Hewitt, J. E., Wilkinson, M. R., Morrissey, D. J., Thrush, S. F., Cummings, V. J. and Funnell, G. (1999), 'Seagrass patches and landscapes: the influence of wind-wave dynamics and hierarchical arrangements of spatial structure on macrofaunal seagrass communities', *Estuaries and Coasts* **22**(4), 1016–1032.

- UK Meteorological Office (UKMO) (2012), 'Midas land surface stations data (1853-current), [internet].', Available from <http://badc.nerc.ac.uk/view/badc.nerc.ac.uk>.
- Unrine, J. M., Jackson, B. P., Hopkins, W. A. and Romanek, C. (2006), 'Isolation and partial characterization of proteins involved in maternal transfer of Selenium in the western fence lizard (*Sceloporus occidentalis*)', *Environmental toxicology and chemistry* **25**(7), 1864–1867.
- Valavanis, V. D., Georgakarakos, S., Kapantagakis, A., Palialexis, A. and Katara, I. (2004b), 'A GIS environmental modelling approach to essential fish habitat designation', *Ecological Modelling* **178**(3), 417–427.
- Valavanis, V. D., Georgakarakos, S., Koutsoubas, D., Arvanitidis, C. and Haralabous, J. (2002), 'Development of a marine information system for cephalopod fisheries in Eastern Mediterranean', *Bulletin of Marine Science* **71**(2), 867–882.
- Valavanis, V. D., Pierce, G. J., Zuur, A. F., Palialexis, A., Saveliev, A., Katara, I. and Wang, J. (2008), 'Modelling of Essential Fish Habitat based on remote sensing, spatial analysis and GIS', *Hydrobiologia* **612**(1), 5–20.
- VEMCO (2003), *VRAP Hardware Manual*, VEMCO Limited, Shad Bay, NS.
- Vézina, A. F. and Hoegh-Guldberg, O. (2008), 'Effects of ocean acidification on marine ecosystems', *Marine Ecology Progress Series* **373**, 199–201.
- Vidal, E. A. G., DiMarco, F. P., Wormuth, J. H. and Lee, P. G. (2002), 'Influence of temperature and food availability on survival, growth and yolk utilization in hatchling squid', *Bulletin of Marine Science* **71**(2), 915–931.
- Vidal, E. A. G., Haimovici, M. and Hackbart, V. C. S. (2010), 'Distribution of paralarvae and small juvenile cephalopods in relation to primary production in an upwelling area off southern Brazil', *ICES Journal of Marine Science* **67**(7), 1346–1352.
- Villanueva, R., Moltschaniwskyj, N. A. and Bozzano, A. (2007), 'Abiotic influences on embryo growth: statoliths as experimental tools in the squid early life history', *Reviews in Fish Biology and Fisheries* **17**(2), 101–110.
- Voss, G. L. (1983), 'A review of cephalopod fisheries biology', *Memoirs of the National Museum Victoria* **44**, 229–241.
- Walsh, L. S., Turk, P. E., Forsythe, J. W. and Lee, P. G. (2002), 'Mariculture of the loliginid squid *Sepioteuthis lessoniana* through seven successive generations', *Aquaculture* **212**(1-4), 245–262.

## LIST OF REFERENCES.

---

- Waluda, C. M. and Pierce, G. J. (1998), 'Temporal and spatial patterns in the distribution of squid *Loligo* spp. in United Kingdom waters', *South African Journal of Marine Science* **20**(1), 323–336.
- Waluda, C. M., Trathan, P. N. and Rodhouse, P. G. (1999), 'Influence of oceanographic variability on recruitment in the *Illex argentinus* (cephalopoda: Ommastrephidae) fishery in the south atlantic', *Marine Ecology Progress Series* **183**, 159–167.
- Wang, J., Pierce, G. J., Boyle, P. R., Denis, V., Robin, J. P. and Bellido, J. M. (2003), 'Spatial and temporal patterns of cuttlefish (*Sepia officinalis*) abundance and environmental influences-a case study using trawl fishery data in French Atlantic coastal, English Channel, and adjacent waters', *ICES Journal of Marine Science* **60**(5), 1149.
- Ward, G. (2007), Statistics in Ecological Modeling; Presence-Only Data and Boosted MARS, PhD thesis, Department of Statistics, Stanford University.
- Ward, P. D. and Boletzky, S. V. (1984), 'Shell implosion depth and implosion morphologies in three species of *Sepia* (Cephalopoda) from the Mediterranean Sea', *Journal of the Marine Biological Association of the United Kingdom* **64**(04), 955–966.
- Warnau, M., Temara, A., Jangoux, M., Dubois, P., Iaccarino, M., De Biase, A. and Pagano, G. (1996), 'Spermioxicity and embryotoxicity of heavy metals in the echinoid *Paracentrotus lividus*', *Environmental toxicology and chemistry* **15**(11), 1931–1936.
- Watanuki, N. A. and Hirayama, I. Z. (2000), 'Why do cuttlefish *Sepia esculenta* enter basket traps? space occupation habit hypothesis', *Fisheries Science* **66**(2), 190–197.
- Watanuki, N. and Iwashita, T. (1993), 'Tags for cuttlefish *Sepia esculenta*', *Recent Advances in Fisheries Biology* pp. 619–625.
- Wearmouth, V. J., Durkin, O. C., Bloor, I., McHugh, M., Rundle, J. and Sims, D. (2012), 'A method for long-term electronic tagging and tracking of juvenile and adult common cuttlefish *Sepia officinalis*', *Journal of Experimental Marine Biology and Ecology* .
- Weatherhead, P. J. and Robertson, R. J. (1979), 'Offspring quality and the polygyny threshold:" the sexy son hypothesis"', *American Naturalist* pp. 201–208.
- Wells, M. J. (1958), 'Factors affecting reactions to mysis by newly hatched sepia', *Behaviour* pp. 96–111.
- Wells, M. J. and Wells, J. (1970), 'Observations on the feeding, growth rate and habits of newly settled *Octopus cyanea*', *Journal of Zoology* **161**(1), 65–74.
- Williamson, R. (1995), 'A sensory basis for orientation in cephalopods', *Journal of the Marine Biological Association of the United Kingdom* **75**(01), 83–92.

#### LIST OF REFERENCES.

---

- Wilson, P. D., Downey, P. O., Leishman, M., Gallagher, R., Hughes, L. and O'Donnell, J. (2009), 'Weeds in a warmer world: Predicting the impact of climate change on Australia's alien plant species using MaxEnt', *Plant Protection Quarterly* **24**(3), 84–87.
- Wolfram, K., Mark, F. C., John, U., Lucassen, M. and Pörtner, H. O. (2006), 'Microsatellite DNA variation indicates low levels of genetic differentiation among cuttlefish (*Sepia officinalis* L.) populations in the English Channel and the Bay of Biscay', *Comparative Biochemistry and Physiology D-Genomics & Proteomics* **1**(3), 375–383.
- Wood, C. (2007), *Seasearch Observer's Guide to Marine Life of Britain and Ireland*, Marine Conservation Society.
- Woodroffe, C. D. (2003), *Coasts: Form, process and evolution*, Cambridge University Press.
- Yesson, C., Taylor, M. L., Tittensor, D. P., Davies, A. J., Guinotte, J., Baco, A., Black, J., Hall-Spencer, J. M. and Rogers, A. D. (2012), 'Global habitat suitability of cold-water octocorals', *Journal of Biogeography* **39**(7), 1278–1292.
- Zeebe, R. E. and Wolf-Gladrow, D. (2001), 'CO<sub>2</sub> in seawater: Equilibrium, kinetics, isotopes', *Elsevier Oceanography Series* **65**, 1–346.
- Zouhiri, S., Vallet, C., Mouny, P. and Dauvin, J. C. (1998), 'Spatial distribution and biological rhythms of suprabenthic mysids from the English Channel', *Journal of the Marine Biological Association of the United Kingdom* **78**(04), 1181–1202.